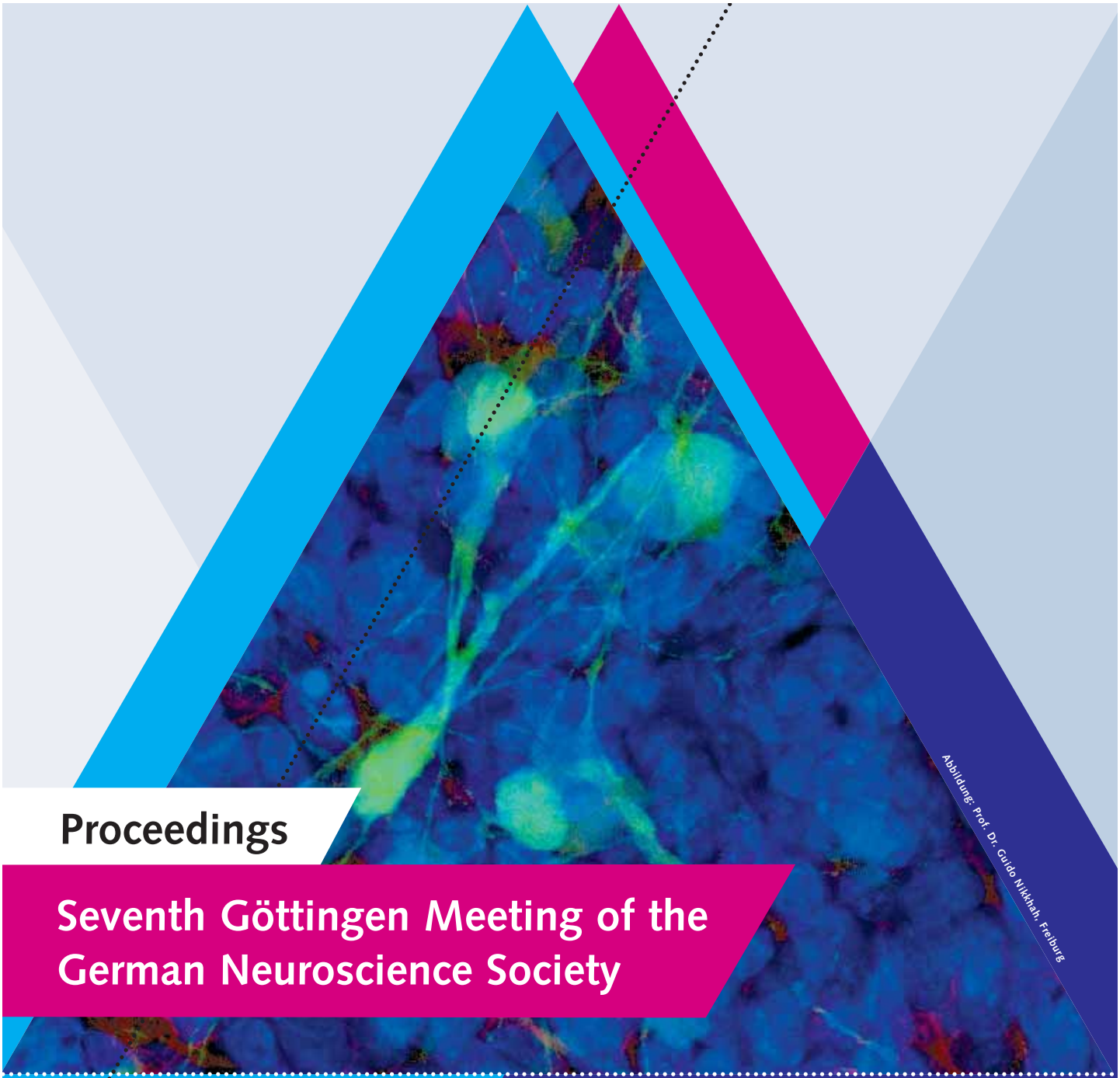

Abbildung: Prof. Dr. Guido Nikkhah, Freiburg

# Proceedings

# Seventh Göttingen Meeting of the German Neuroscience Society

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# Proceedings of the 7th Meeting of the German Neuroscience Society / 31th Göttingen Neurobiology Conference 2007

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# Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction

Christof Niehrs

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Wnt signaling plays diverse roles during embryonic development and in adults and is implicated in human disease, including cancer. LDL-receptor-related protein 5 and 6 (LRP5/6) are key receptors required for transmission of Wnt/β-catenin signaling in metazoa. Although the role of these receptors in Wnt signaling is well established, their coupling with the cytoplasmic signaling apparatus remains poorly defined. In a protein modification screen for regulators of LRP6 we have identified Casein kinase 1 γ (CK1γ), a membrane bound member of the CK1 family. In *Xenopus* embryos, CK1γ is required during antero-posterior patterning CNS to promote posteriorizing Wnt/β-catenin signaling. LRP6 is associated with CK1γ and harbors multiple, modular phosphorylation sites. Wnt treatment induces rapid phosphorylation of CK1γ sites, which promotes Axin recruitment. The results reveal an evolutionarily conserved mechanism that couples Wnt receptor activation to the cytoplasmic transduction apparatus in the regulation of CNS patterning.

# Success and Faillure of Translational Research: The Example of Multiple Sclerosis

Hans Lassmann

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Multiple sclerosis (MS) is widely seen as a chronic inflammatory demyelinating disease of the central nervous system, driven by autoimmunity. Thus, classical models of experimental autoimmune encephalomyelitis (EAE) are commonly regarded as suitable tools to study pathogenesis of MS and to evaluate new therapeutic strategies. Although certain similarities between MS and EAE are obvious and some therapeutic interventions are beneficial in both diseases, many treatments, which are highly effective in EAE, failed the test in MS patients. Only recently, systematic studies in MS revealed major differences between MS and EAE pathogenesis, which may in part explain this unsatisfactory situation. These differences relate to the nature of the inflammatory response, the mechanisms of demyelination and tissue injury and the factors, which determine chronicity and progression of the disease. In contrast to EAE, which is basically driven by an inflammatory response mediated through MHC Class II restricted Th-1 cells, T-cell mediated inflammation in MS is regularly dominated by Class I restricted T-cells. Adaptive immune responses in MS patients are in part directed against antigens, so far not identified as targets for autoimmunity in EAE, which is best exemplified by the antibody reaction against the astrocytic antigen Aquaporin 4 in patients with Neuromyelitis optica. Initial demyelination in a subset of MS patients occurs in the near absence of T-cell infiltrates and follows a pathway of tissue injury, which suggests a major involvement of innate immunity. While chronicity in EAE is mainly driven by the continuous presence of a peripheral depot of the sensitising antigen, progression of disease in MS appears to be driven by a compartmentalization of the immune response within the central nervous system. In addition, MS lesions have a surprisingly high capacity for remyelination, which seems to be mainly counteracted by the active demyelinating disease process. Finally, EAE models by themselves are based on heterogeneous pathogenetic mechanisms, depending upon the genetic background of the animal and the mode of sensitisation. These new results imply that translational MS research requires, to be more successful than now, much more careful selection and use of a much wider spectrum of experimental models.

# Breaking the silence and move your paralysed limb: Brain-Computer-Interfaces (BCI)

Niels Birbaumer

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A critical review of invasive and non-invasive BCIs and its clinical applications. Direct brain communication in locked-in syndrome and other conditions leading to paralysis and restoration of movement after stroke and high spinal cord injury are the focus of the review. Non-invasive EEG based BCI have reached the stage of practical daily use in patients with amyotrophic lateral sclerosis(ALS), particularly with the P 300 event related brain potential and sensorimotor rhythm as communication signals. Effective communication with completely locked in patients trained in BCI-use after entering the locked-in state was not reliably achieved. Data on completely locked-in patients and reasons for failure to learn are given. Invasive BCIs for communication and movement restoration in humans had only limited effects despite wide public interest. Animal data show extremely long training times to control single cell responding but excellent transformations into movement patterns.A new MEG-based BCI for movement restoration in chronic stroke is presented and results on cortical reorganisation in stroke after BCI training presented. Ethical questions concerning end-of life decisions, quality of life in paralysis and clinical issues in BC -use need to be addressed and empirically investigated to assure effective and ethically acceptable BCI application.Data on quality of life and emotional changes are documented with brain imaging data in ALS. A BCI using BOLD response from cicumscribed brain regions is introduced and results of regulation of emotional brain areas in healthy and criminal psychopath populations presented. Supported by the Deutsche Forschungsgemeinschaft (DFG) and the National Institutes of Health, NINDS.

# *- Schilling Research Award Lecture -*
# *In vivo* imaging of axon development and degeneration

Thomas Misgeld

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The loss of axonal connections is a shared feature of neuronal development and of neurological disease. During development, dismantling of aberrant axons is necessary to establish precise connectivity. Recurrence of axon loss in adult life, in contrast, can lead to severe disability. The mechanisms that underlie physiological and pathological axon loss are not well understood, but recent data support the notion that nerve cells possess active machinery that allows them to selectively dismantle parts of their axon. Analyzing this intrinsic axon dismantling machinery might be important to find ways to ameliorate neurodegeneration.

In our work, we have taken advantage of *in vivo* microscopy of transgenic mouse lines with fluorescently labeled neurons to visualize how axons dismantle during development and after injury. In my talk, I will show how we have used this approach (1) to reveal a novel form of axon dismantling in development (which we dubbed 'axosome shedding'), (2) to analyze posttraumatic axon degeneration after spinal cord injury, and (3) to visualize axonal transport in intact nerves and entire axonal arbors.

Our studies suggest that multiple pathways of axon dismantling exist, and that glial cells might play a central role in some forms of axon loss. Thus, *in vivo* imaging emerges as a versatile tool to study the cell biology and pathology of peripheral and central axons in living organisms.

# Imaging cellular network dynamics in three dimensions using fast 3D laser scanning

Werner Göbel

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Spatiotemporal activity patterns in three-dimensionally organized cellular networks are fundamental to the function of the nervous system. Despite advances in functional imaging of cell populations, a method to resolve local network activity in three dimensions has been lacking. Here a three-dimensional (3D) line-scan technology for two-photon microscopy is introduced that permits fast fluorescence measurements from several hundred cells distributed in 3D space. Sinusoidal vibration of the microscope objective at 10 Hz is combined with 'smart' movements of galvanometric x-y scanners to repeatedly scan the laser focus along a closed 3D trajectory. More than 90% of cell somata were sampled by the scan line within volumes of 250 µm side length. Using bulk-loading of calcium indicator, this method was applied to reveal spatiotemporal activity patterns in neuronal and astrocytic networks in the rat neocortex in vivo. Two-photon population imaging using 3D scanning opens the field for comprehensive studies of local network dynamics in intact tissue.

# THE NEURONAL BASIS FOR COGNITION

Rodolfo R. Llinas

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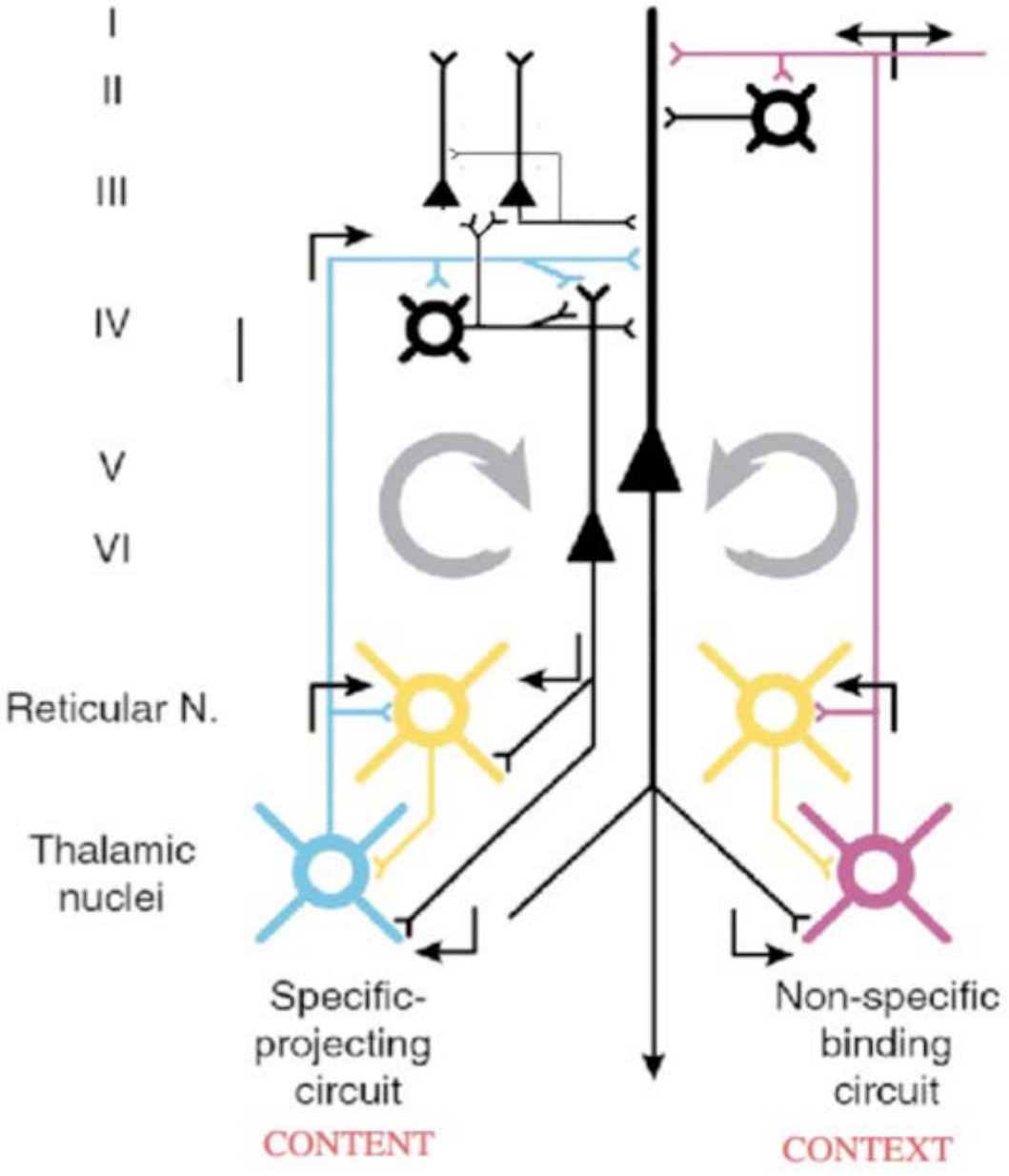
Attempting to understand how the brain, as a whole, might be organized seems, for the first time, to be a serious topic of inquiry. One aspect of its neuronal organizational that seems particularly central to global function is the rich thalamocortical interconnectivity, and the most particularly the reciprocal nature of the thalamocortical neuronal loop function. Moreover, the interaction between the specific and non-specific thalamic loops suggests that rather than a gate into the brain, the thalamus represents a hub from which any site in the cortex can communicate with any other such site or sites. The main impetus of this talk is to explore the hypothesis that large-scale, temporal coincidence of specific and non- specific thalamic activity generates the functional states that characterize cognition and the evolutionary origin of such crucial functional property. The neuropsychiatric implications of this functional perspective will also be considered.



# Pattern Learning and Recognition: Lessons from Small Olfactory Systems.

Gilles Laurent

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The olfactory system offers a good opportunity to study systems and computational issues of general relevance with relative ease. For example, odors usually are very complex chemical objects and yet, they are perceived as singular entities (e.g., rose, coffee, burnt rubber). Our brains must thus bind specific sets of input features into singular percepts and presumably, also into singular representations. At the same time, olfactory circuits are relatively shallow: olfactory cortex, where such transformations and representations may occur, is only two synapses away from odor transduction. The complexity of the underlying processing may thus be smaller than for other senses such as vision and hearing. Using insects and zebrafish nervous systems as experimental models, we have derived some possible principles for olfactory processing in particular, and sensory processing in general. Our results may shed some light on issues such as brain oscillations, synchronization, pattern learning and recognition, brain circuit dynamics and neural coding. I will attempt to summarize these ideas.

# Cellular mechanisms of memory consolidation in the hippocampal formation

Uwe Heinemann

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Freely moving rodents develop in the hippocampal formation sharp wave ripple (SPW-R)complexes which last für about 80 ms during which time ripples recur with frequencies of up to 200 Hz. The ripples replace oscillatory events in the theta and Gamma range once an animal has received a reward. They occur also during sleep. During such events neurons are reactivated which were active during exploratory behaviour. Hence SPW-R are events during which stored information is replayed fascilitating information transfer from the working memory in the hippocampus into more permanent memory stores in the cortical mantle. We have tested the hypothesis that LTP protocols can also induce SPW-R complexes. Indeed activation of associational fibers in the hippocampus can induce SPW-R in hippocampal area CA3. The induction is facilitated when the LTP protocol is applied after induction of theta and gamma oscillations which are within seconds replaced by sharp wave ripple complexes. The induction depends on NMDA receptor activation. Preliminary data suggest that SPW-R once induced can cause LTP in target neurons in the subiculum and possibly also in the entorhinal cortex. The SPW-R complexes are immediately replaced by theta and gamma osciullation when acetylcholine or carbachole are applied suggesting that the SPW-R working mode and the theta-gamma oscillatory mode represent functionally different working modes in the hippocampus.

# New Concepts in Sound Localization - Inhibition Matters

Benedikt Grothe

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Recent studies provoked reconsideration of the neuronal representation of space in the mammalian auditory system mammals. Key to these new concepts was an unanticipated role of inhibition in shaping auditory perceptions. Low frequencies, which are most relevant for us, are localized by differences in time-of-arrival at the two ears (interaural time difference, ITD). The traditional scenario assumes that ITDs are encoded by the peak activity within an array of neurons each tuned to a different best ITD leading to a neuronal map of azimuthal space. In contradiction to this model, studies in mammalian species rarely show best ITDs near 0 azimuth and commonly observe best ITDs far off the midline, often outside the range of ITDs caused by natural, single- source sounds. An important quality of the ITD functions of single neurons is that their steepest slopes are close to, or at, 0 ITD. These revelations provoke consideration of a different space-encoding strategy, that may explain why mammalian studies have heretofore failed to reveal an ITD-based space map. The alternative explanation for ITD processing invokes a new role for temporally accurate synaptic inhibition within ITD circuitry. ITD detector neurons in the mammalian medial superior olive (MSO) receive strong glycinergic inhibition from the medial nucleus of the trapezoid boy, which is highly specialized for temporal acuity. This inhibition is responsible for tuning of the steep slopes of ITD functions to the physiologically relevant range. Blockade of the glycinergic inhibition in the MSO caused the steep slopes of the ITD functions to shift away from the midline. The most likely explanation of this effect is that contralateral, phase-locked inhibition from the MNTB is causing the delay of the contralateral excitation in a manner that precisely tunes the ITD detector. Another example of an inhibitory connection with a specific temporal tuning is the GABAergic inhibition from one dorsal nucleus of the lateral lemniscus (DNLL) to its contralateral counterpart. DNLL neurons are part of the binaural system and prefer stimuli located in the contralateral hemisphere. Our recent in vitro studies revealed that GABAergic inhibition in DNLL neurons lasts more than 10 ms, beyond the end of a signal, an unusually long-lasting effect on the scale of auditory brainstem kinetics ('persistent inhibition', PI). This is likely due to presynaptic processes since glycinergic inputs to the same cell do not elicit persistent inhibition. Our in vivo recordings indicate that PI is an essential feature for distinguishing direct sounds from echoes, allowing faithful sound localization and segregation even in reverberant environments. Taken together, the recent findings dedicate a key role in temporal processing to accurate inhibitory connections in auditory processing. Since auditory systems are operating at exceptional speed, these findings raise the possibility that inhibition in other systems is also much more specific than previously believed. Literature Grothe (2003) Nat Rev Neurosci 4:540 Hancock & Delgutte (2004) J Neurosci 24:7110 McAlpine et al (2001) A neural code for low-frequency sound localization in mammals. Nature Neurosci 4:396 Pollak et al. (2003) TINS 26:33 Siveke et al. (2006) J Neurophys 96:1425

# Symposia

S1 Gene Silencing by RNA Interference in Models of De- and Regeneration
*Paul Lingor and Nicole Déglon, Göttingen and Orsay (F)*

S2 Experience-Induced Plasticity in the Olfactory Pathway: From Single Neurons to Neural Odor Representation
*Jean-Christophe Sandoz and C. Giovanni Galizia, Toulouse (F) and Konstanz*

S3 Neuronal Dendrites: Synaptic Function, Plasticity and Information Processing
*Knut Holthoff and Arthur Konnerth, Munich*

S4 Structure and Function of the Vertebrate Retina
*Oliver Biehlmaier and Stephan C. F. Neuhauss, Zürich (CH)*

S5 Cannabinoids and the Nervous System: Different Views on Multiple Actions
*Dirk Czesnik, Goettingen*

S6 The Cortical Nerve Impulse
*Fred Wolf and Maxim Volgushev, Göttingen and Bochum*

S7 Molecular Aspects of Synapse Function and Dysfunction in the Mammalian Brain
*Matthias Kneussel, Hans-Jürgen Kreienkamp and Stefan Kindler, Hamburg*

S8 Olfactory Development: Common Principles and Differences across Phyla
*Joachim Schachtner and Wolfgang Rössler, Marburg and Würzburg*

S9 Recent Advances in the Use of Cell Penetrating Peptides
*Gunnar P.H. Dietz, Goettingen*

S10 Generating Rhythmic Movement: From Microcircuits to Complex Motor Programs
*Ansgar Büschges and Hans-Joachim Pflüger, Köln and Berlin*

S11 Brain Tumors
*Rainer Glass and Michael Synowitz, Berlin*

S12 Computational Models of Vision
*Laurenz Wiskott and Gustavo Deco, Berlin and Barcelona (E)*

S13 Functional Role of Nucleotide Signaling in the Nervous System
*Peter Illes and Herbert Zimmermann, Leipzig and Frankfurt/Main*

S14 Cell Intrinsic Mechanisms in the Regulation of Neural Development
*Dorothea Schulte and Dieter Engelkamp, Frankfurt/Main*

S15 Microglia: Role in Neurodegeneration and Repair
*Harald Neumann and Marco Prinz, Bonn and Göttingen*

S16 Active Sensing: How Nervous Systems Explore the External World
*Martin C. Göpfert and Harald Luksch, Cologne and Aachen*

# Symposia

S17 Genetics and Molecular Mechanisms of Parkinson's Disease
*Marius Ueffing and Thomas Gasser, Neuherberg and Tübingen*

S18 Compositionality: Neuronal Basis of Complex Behavior
*Theo Geisel and Moshe Abeles, Göttingen and Ramat Gan (Israel)*

S19 Spatial Cognition: From Rodents to Humans
*Hanspeter A. Mallot and Johannes Thiele, Tübingen*

S20 The Drosophila NMJ: Unravelling Principal Mechanisms of Synapse Formation, Function and Plasticity
*Andreas Prokop and Stephan S. Sigrist, Manchester (UK) and Göttingen*

S21 Glia Development: Molecular Control of Specification, Migration, Differentiation and Myelination of Oligodendrocytes and SchwannCells
*Michael Wegner, Erlangen*

S22 Real-Time Voltage-Sensitive Dye Imaging of Cortical Network Activities across Sensory Modalities
*Dirk Jancke and Hartwig Spors, Bochum and Heidelberg*

S23 Synchronization of Circadian and Neuronal Oscillators
*Monika Stengl, Marburg*

S24 Do We Know What the Early Visual System Computes?
*Matthias Bethge and Christoph Kayser, Tübingen*

# Symposium

## S1: Gene Silencing by RNA Interference in Models of De- and Regeneration

***Paul Lingor and Nicole Déglon, Göttingen and Orsay (F)***

### Slide

**S1-1** Opening remarks for Symposium 1
*Paul Lingor and Nicole Déglon, Göttingen and Orsay (F)*

**S1-2** Mechanisms of small-RNA-mediated gene regulation in mammals
*Tom Tuschl, New York, NY (USA)*

**S1-3** Disinhibition of regenerating retinal ganglion cells by gene silencing
*Ann Logan and Martin Berry, Birmingham (UK)*

**S1-4** Targeting apoptosis and regeneration of CNS neurons by RNAi *in vivo* and *in vitro*
*Paul Lingor, Veronique Planchamp, Uwe Michel, Lars Tönges, Sebastian Kügler and Mathias Bähr, Göttingen*

**S1-5** Lentiviral-mediated silencing of mutated and endogenous wild-type huntingtin in rodent models of Huntington's disease
*Nicole Déglon, Orsay (F)*

**S1-6** Lentiviral Vector and Adeno-associated Vector-based therapy for Motoneuron disease through RNA interference
*Cedric Raoul, Lausanne (CH)*

**S1-7** Conserved genetic interactions in membrane trafficking identified by synthetic lethality analysis
*Stefan Eimer, Göttingen*

### Poster

**TS1-1A** Reduction in tyrosine hydroxylase positive cells in SNpc is achieved upon glutathione depletion: implication in PD.
*M. Garrido, U. Michel, Z. Shevtsova, U. Schöll, M. Bähr and S. Kügler, Göttingen*

**TS1-2A** Granulocyte-colony stimulating factor (G-CSF) as a neuroprotective therapy in models of Parkinson's disease
*K. Meuer, T. Frank, C. Pfitzer, P. Teismann, C. Krüger, B. Göricke, R. Laage, P. Lingor, JCM. Schlachetzki, GPH. Dietz, D. Weber, B. Ferger, A. Bach, JB. Schulz, M. Bähr, A. Schneider and JH. Weishaupt, Göttingen and Heidelberg*

**TS1-3A** TRANSFERRIN-ASSOCIATED LIPOPLEXES FOR siRNA DELIVERY IN CULTURED NEURONS AND BRAIN TISSUE
*C. Culmsee, AL. Cardoso, S. Simoes, N. Plesnila, M. de Lima and E. Wagner, München and Coimbra (P)*

Introductory Remarks to Symposium 1

# Gene Silencing by RNA Interference in Models of De- and Regeneration

Paul Lingor and Nicole Déglon, Göttingen and Orsay (F)

The symposium is intended to give an overview on the implementation of RNA interference in the context of neurode- and -regeneration. An introductory talk will summarize the discovery and the principles of RNA interference. The following contributions will give examples of in vitro and in vivo implementation of the technique in models of neurodegenerative diseases and regenerative paradigms. The use of viral vectors and different transfection techniques will be highlighted as well as the utilization of animal models including nematodes, rodents and primates.

Sponsored by DFG Forschungszentrum Molekularphysiologie des Gehirns (ZMPG), INVITROGEN GmbH and QIAGEN GmbH

# Targeting apoptosis and regeneration of CNS neurons by RNAi *in vivo* and *in vitro*

Paul Lingor, Veronique Planchamp, Uwe Michel, Lars Tönges, Sebastian Kügler and Mathias Bähr

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Gene silencing by RNA interference has now been widely established as a tool for the functional evaluation of target genes in neuronal cells and as an experimental therapeutic method in models of neurological disease.
The talk will give examples of siRNA/shRNA delivery to CNS neurons, including AAV-mediated transduction, chemical transfection and electroporation. We then show that siRNA-mediated downregulation of the immediate early gene c-Jun and shRNA-induced downregulation of the small GTPase RhoA is protective in a rat optic nerve axotomy model *in vivo*. Using siRNA-mediated knock-down in an *in vitro* regeneration model of midbrain dopaminergic neurons we show a differential role for c-Jun-N-terminal kinase isoforms for survival and regeneration of this neuron population. We thus demonstrate that RNAi can effectively be employed to selectively elucidate the function of proteins in CNS neurons *in vivo* and *in vitro*, especially when genetic models or specific pharmacological inhibitors are not available.

# Lentiviral-mediated silencing of mutated and endogenous wild-type huntingtin in rodent models of Huntington's disease

Nicole Déglon

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Huntington's disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion in the huntingtin (htt) protein. No cure or preventive treatments are available to alleviate the neurodegeneration in this disease. Gene silencing using RNA interference (RNAi) represents a powerful tool with high therapeutic potential for the treatment of dominantly inherited diseases. In the present project, we showed that three shRNAs targeting wild type human mutated htt transcript significantly diminished HD-like pathology in mice and rat models of HD. Doxycycline-regulated htt vectors were produced to control sihtt treatment and reversibility of the system. Initiation of shRNA expression after the onset of HD symptoms significantly reduced the accumulation of htt aggregates and loss of the striatal marker, DARPP-32. To investigate the impact of the silencing of endogenous wild type htt, shRNA with various species specificities were designed. The expression of these shRNA in the striatum of mice and rats induced a downregulation of endogenous htt in accordance with the predicted selectivity and the downregulation of endogenous and/or mutated htt in rodent was not associated with a decreased therapeutic efficacy or increased striatal vulnerability. Long-term expression of the shRNAs in rodent brains did not show overt toxicity. Taken together, these results establish the potential of lentiviral-mediated expression of shRNA as therapeutic tools to counteract HD.

# Reduction in tyrosine hydroxylase positive cells in SNpc is achieved upon glutathione depletion: implication in PD.

Manuel Garrido[1,2], Uwe Michel[1,2], Zinayida Shevtsova[1,2], Ulrike Schöll[1], Mathias Bähr[1,2] and Sebastian Kügler[1,2]

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Parkinson's disease (PD) is the second most common neurodegenerative disorder. The loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of the midbrain is the major pathological feature of this disease. The most widely used animal models of PD are based on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA), both toxic to nigrostriatal pathways and acting as mitochondrial complex I and I / IV inhibitors, respectively. These toxins lead to an increase in reactive oxygen species (ROS) and subsequent cellular degeneration. The degeneration in these chemical lesion models is fast and acute (1 to 2 weeks) and thus does not adequately represent the human idiopathic disease that develops over decades.

Mitochondrial dysfunction and ROS accumulation have been associated with SNpc neurodegeneration. Glutathione (GSH) is the major antioxidant molecule in cells responsible for ROS detoxification. A reduction of GSH levels in SNpc of PD patients has been shown. The GSH decrease appears to occur before the dysfunction of the mitochondria electron transport system and neurodegeneration in presymptomatic PD. The purpose of this work was to establish a novel animal model of the idiopathic PD with a slow onset and progressive neuronal cell loss in SNpc following the decrease of GSH in the SNpc dopaminergic cells.
We used recombinant adeno-associated virus serotype 2 (rAAV2) viral vectors expressing shRNAs targeting the catalytic subunit of glutamate cysteine ligase and glutathione reductase (GCL/GR; the key enzymes in GSH synthesis and recycling). By this way we attempted to create an alternative oxidative stress PD model by RNA interference and investigate the hypothesis that the oxidative stress can be the cause of dopaminergic cell loss.

Using this technique, a progressive decrease in viability of primary neurons and in total glutathione (GSX) content were observed 6, 8 and 10 days after rAAV2 infection *in vitro*. Next, we have stereotaxically injected rAAV2 expressing shRNA into rat SNpc and observed a progressive reduction of tyrosine hydroxylase (TH) positive cells in SNpc *in vivo*.
We conclude that viral vectors mediated gene transfer can be used efficiently to create novel animal model systems of neurodegenerative diseases.

Acknowledgements
This work was funded by Deutsche Forschungsgemeinschaft through the DFG-Research Center 'Molecular Physiology of the Brain'. MG holds a studentship from EC Marie Curie Actions RTN-NSR #504636.

# Granulocyte-colony stimulating factor (G-CSF) as a neuroprotective therapy in models of Parkinson's disease

Katrin Meuer[1], Tobias Frank[1], Claudia Pfitzer[3], Peter Teismann[2], Carola Krüger[3], Bettina Göricke[1], Rico Laage[3], Paul Lingor[1], Johannes C.M. Schlachetzki[1], Gunnar P.H. Dietz[1], Daniela Weber[3], Boris Ferger[4], Alfred Bach[3], Jörg B. Schulz[2], Mathias Bähr[1], Armin Schneider[3] and Jochen H. Weishaupt[1]

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The hematopoietic Granulocyte-Colony Stimulating Factor (G-CSF) plays a crucial role in controlling the number of neutrophil progenitor cells. Its function is mediated via the G-CSF receptor (G-CSFR), which was shown to be expressed not only on hematopoietic cells but also by dopaminergic neurons of the substantia nigra (SN). Thus, we investigated the protective effect of G-CSF in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of Parkinson's disease (PD). Substantial protection was found against $MPP^+$-induced death of primary dopaminergic cell neurons *in vitro*. Moreover, daily application of 40 µg/kg G-CSF s.c. significantly reduced the MPTP-induced dopaminergic cell loss in the SN as shown by quantification of tyrosine hydroxylase-positive cells in mice. Using HPLC, a corresponding reduction in striatal dopamine depletion after MPTP application was observed in G-CSF-treated mice. Hence, our data suggest that G-CSF is a novel therapeutic opportunity for the treatment of PD, because it is well-tolerated and already approved for the treatment of neutropenic conditions in man. Consequently, after providing prove-of-principle that G-CSF could be a valuable therapy for Parkinson's disease, we are currently testing pharmacokinetically improved G-CSF variants, e.g. pegylated G-CSF with an extended serum half-life time compared to conventional G-CSF.

# TRANSFERRIN-ASSOCIATED LIPOPLEXES FOR siRNA DELIVERY IN CULTURED NEURONS AND BRAIN TISSUE

Carsten Culmsee[1], Ana Luisa Cardoso[2,3], Sergio Simoes[2,4], Nikolaus Plesnila[5], Maria de Lima[2,3] and Ernst Wagner[1]

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siRNA application in models of CNS diseases is hampered by obstacles against efficient extra- and intracellular delivery. Here we tested lipid-based vectors for efficient siRNA delivery in cultured neurons and in mouse brain tissue. Cationic liposomes composed of DOTAP/cholesterol associated with Transferrin (Tf) complexed siRNA at an optimal lipid/siRNA charge ratio of 3/2 as evaluated in the PicoGreen exclusion assay. Our results further demonstrate efficient cellular uptake of fluorescent-labeled siRNA delivered in Tf-lipoplexes and up to 50% specific target gene knockdown in primary neurons stably expressing luciferase. The Tf-lipoplexes did not cause any toxic effects as evaluated in trypan blue and MTT assays. Most promising, significant reduction of luciferase activity was also achieved in brain tissue as evaluated 48 h after local stereotactic injection of the siRNA complexes into the striatum, cortex or hippocampus of transgenic luciferase expressing mice. Tf-lipoplexes transferring siRNA silencing AIF, Bid or c-Jun protected HT-22 immortalized neuronal cells and primary embryonic neurons against glutamate toxicity and oxygen glucose deprivation, respectively. Overall, our results suggest that Tf-lipoplexes are stable and efficient delivery systems for specific gene silencing in cultured neurons and in brain tissue and hence useful tools for future studies on therapeutic targets in models of neurodegenerative diseases.

# Symposium

## S2: Experience-Induced Plasticity in the Olfactory Pathway: From Single Neurons to Neural Odor Representation

***Jean-Christophe Sandoz and C. Giovanni Galizia, Toulouse (F) and Konstanz***

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Introductory Remarks to Symposium 2

# Experience-Induced Plasticity in the Olfactory Pathway: From Single Neurons to Neural Odor Representation

Jean-Christophe Sandoz and C. Giovanni Galizia, Toulouse (F) and Konstanz

Olfaction plays of major role in the organization of feeding, mating or social behaviours in most animal species. Some of these behaviours involve genetically predetermined odour signals that rely on highly-specialized olfactory sub-systems. But most behaviours rely on complex olfactory signals that are permanently changing, and therefore survival often depends on the ability to adapt quickly to the environment, for instance by learning and memorizing the relevant odours. How the general olfactory system is able to cope with this second possibility is the subject of this Symposium.

Across phyla, the organization of the peripheral olfactory nervous system is strikingly similar. Odours are detected peripherally by numerous olfactory receptors neurons (ORNs) that each carry a particular receptor protein tuned more or less broadly to some odour molecule characteristics. These neurons relay odour information to a first olfactory center, the olfactory bulb (OB) in vertebrates and the antennal lobe (AL) in insects. Both structures are organized in a similar modular way, with each of its subunits, the glomeruli, receiving input from OSNs expressing the same olfactory receptor type. The glomeruli are a site of intensive synaptic contacts between several neuron types, in particular inhibitory local neurons providing local between-glomerulus computation, and second-order neurons that relay processed information to higher brain centers. Neural computation within the first olfactory center is thought to mediate better discrimination between similar olfactory inputs, allowing more segregated odour representations to be conveyed to higher brain centers.

In recent years, the strong development of optical imaging techniques has allowed, together with electrophysiology, molecular genetics and behavioural analyses, a significant progression in our understanding of olfactory processing and of neural odour representation in the brain of several model systems among which mice, zebrafish, Drosophila and honeybees. On the basis of this developing knowledge, research has increasingly turned to the question of the olfactory system's plasticity. Thus, several studies have already pointed out remarkable changes that take place within primary olfactory centers - both anatomically or in their odour-evoked activity - after olfactory experience. However, a crucial question remains concerning the interpretation of such results: are these changes related to an olfactory memory engramm, being by-products of storage mechanisms, or are they related to modifications of local computations, for instance modulating the neural representation of learned odours to make them more easily discernible from other stimuli?

The Symposium will bring together key neuroscientists in this quickly-evolving research field, who use electrophysiological and optical imaging methods both on single neurons and on whole neuronal networks, in the search for experience-induced changes in the structure and/or activity of olfactory centers. The symposium will attempt to give an overview of results obtained on the plasticity of olfactory centers after experiences ranging from passive olfactory exposure to appetitive and aversive associative conditioning. In some systems that will be presented, plasticity induces the genesis of new neurons that integrate into the network, while in others the numbers and/or strengths of synapses are modified. In still other systems, changes in calcium responses of olfactory neurons are recorded in vivo in learning animals. A balance of presentations from scientists working on vertebrate and invertebrate models will provide across-phyla exchanges in order to discuss the commonalities and/or differences between models of olfactory plasticity.

# Visualizing olfactory memories in Drosophila by optical imaging

Ronald L. Davis, Dinghui Yu and David Akalal

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Recent advances in optical imaging have made possible the visualization of neural activity in the *Drosophila* brain in response to sensory stimuli. We have used transgenically supplied reporters for synaptic transmission and calcium influx to visualize these processes and the formation of cellular memory traces in the olfactory nervous system of *Drosophila* after olfactory classical conditioning. The first memory trace detected occurs in the projection neurons of the antennal lobe. This trace forms within a few minutes after training, lasts only a few minutes, and occurs as the recruitment of new sets of projection neurons into the representation of the odor used for training. A second memory trace forms in the dorsal paired medial neurons beginning at 30 minutes after training. This memory trace is detectable as both increased synaptic transmission and calcium influx and persists for about 2 hours after training. A third memory trace was recently discovered in certain types of mushroom body neurons and forms in response to spaced training. Spaced training employs multiple training trials with a rest between each trial, and is effective at producing long-term behavioral memory. The long-term memory trace forms after the memory trace in the dorsal paired medial neurons. These data suggest that multiple memory traces are formed in different parts of the olfactory nervous system in response to olfactory classical conditioning and that each trace may guide behavior over different windows of time after conditioning.

# Olfactory learning effects measured in the honeybee mushroom bodies

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The neural basis of associative olfactory learning in the honeybee has been studied in an effort to monitor learning behavior and correlating neural activity. Opto- and electrophysiological recordings were applied to study associative plasticity at the level of the single neuron and neural circuits. The report shall focus on recent data collected from the mushroom body. Special emphasis will be given to the question, how memory contents about rewarded odors are encoded in the network properties of the input and the output of the mushroom body. Imaging both the pre- and postsynaptic compartments at the olfactory input region of the mushroom bodies (the lip region) reveals that odors are represented in specific patterns of microglomerular activation at the input sides and columnar activations at the output sides. A comparison of the properties of the odor responses shows that mushroom body intrinsic Kenyon cells code odors in a sparse way both in the time domain and at the level of the population of activated neurons. Odor learning leads to small changes at the postsynaptic sides of the Kenyon cells with a dominance of a reduction of synaptic efficiency to the non trained odor (CS-). Electrophysiological recordings indicate three forms of learning related plasticity at the output side of the mushroom body, recruitment of neurons, enhancement and reduction of responses to the learned odor. A single identified alpha lobe extrinsic neuron, the PE1, could be identified in extracellularly multielectrode recordings of neural activity on the basis of its specific double and triple spikes during spontaneous activity. This neuron reduces its spike frequency in the phasic part of its responses to the CS+ during the course of conditioning. The responses to the CS- did not change, and those to a control odor (Ctr) showed a non-significant decrease. No learning-related changes were seen in the none-PE1 neurons. Intracellular recordings of the PE1 reveal long-lasting potentiation (LTP) induced by the pairing of tetanic electric stimulation of the Kenyon cells with intracellular depolarization.

A model will be presented that integrates associative plasticity at the input and the output sides of the mushroom body and predicts that at least part of learning related changes of neural wiring is caused by associative enhancement of inhibitory neurons acting directly on to out put neurons and feeding back to the input side.

# Integrating new neurons into adult olfactory system

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In the adult olfactory bulb, newly born neurons are constitutively generated throughout life and form an integral part of normal functional circuitry. This process of late neurogenesis is subject, at various stages, to modulation and control by external influences, suggesting strongly that it represents a plastic mechanism by which the brain's performance can be optimized according to the environment in which it finds itself. But optimized how? And why?

This presentation will concentrate on such functional questions regarding neurogenic plasticity. After outlining the processes of adult neurogenesis in the olfactory system, and after discussing their regulation by internal and environmental influences, we shall ask how existing neuronal circuits can continue to work in the face of constant cell arrivals and departures, and explore the possible functional roles that newborn neurons might subserve in the adult olfactory system. In particular, we shall report the degree of sensitivity of the bulbar neurogenesis to the level of sensory inputs and, in turn, how the adult neurogenesis adjusts the neural network functioning to optimize sensory information processing. We will bring together recently described properties and emerging principles of adult neurogenesis that support a much more complex role for the adult-generated cells than just providers of replaceable units. Throughout, and concentrating exclusively on mammalian systems, we shall stress that adult neurogenesis constitutes another weapon in the brain's armory for dealing with a constantly changing world.

# Synaptic plasticity in the olfactory bulb.

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The synapses formed by the olfactory nerve (ON) convey sensory information to olfactory glomeruli, the first stage of central odor processing. The convergence of axons of sensory neurons expressing the same receptor onto topographically-defined glomeruli creates a spatial map of the molecular features of the odor in the glomerular layer of the olfactory bulb. Glomerular "odor images" are reproducible within and across animals, and are similar for structurally similar odorant molecules, but they are plastic not static. We derived this conclusion from electrophysiogical and optical imaging studies in mouse olfactory bulb slices. In addition to short-term plasticity via presynaptic dopamine and $GABA_B$ receptors, ON-mitral cell (MC) synapses express long-term depression (LTD) after brief low-frequency ON stimulation. Induction of ON-MC LTD requires the activation of metabotropic glutamate receptors but does not require ionotropic glutamatergic and GABAergic synaptic transmission. ON-MC LTD is expressed presynaptically and associated with LTD of presynaptic calcium transients. Long-lasting plasticity at the ON-MC synapse is likely to prove relevant for the establishment, maintenance and experience-dependent refinement of odor maps in the olfactory bulb.

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# Visualization of odour and reinforcer representations in the *Drosophila* brain: an imaging approach towards olfactory memory traces

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During associative learning a neutral stimulus is temporally paired with a relevant stimulus, ultimately changing its significance for behaviour. For example, when an odour is associated with a reward it can become attractive. Conversely, if an odour is associated with a punishment, it can become repulsive. We address the question of which neurons mediate the reinforcing properties of punishing or rewarding stimuli during associative learning in Drosophila. To this end, we use two methods: Optical imaging of calcium activity and light-induced activation of genetically defined neuronal populations. In a comparative study we have determined that FRET-based, ratiometric calcium sensors display a better signal-to-noise ratio in comparison with other calcium sensors in the central brain of Drosophila in vivo. Using cameleon 2.1 as a sensor we visualize the representations of odour identities and intensities at various levels of olfactory processing, from receptor neurons over projection neurons to intrinsic mushroom body neurons. But how are odour representations associated with relevance, a positive or negative value? Besides monitoring odour representations optical calcium imaging enabled us to show that dopaminergic neurons innervating the mushroom body lobes do not only respond specifically to a punishing electric shock stimulus, but predict a punishment after an associative olfactory learning procedure. As a second method, we report the use of channelrhodopsin-2 (Nagel et al. 2003, PNAS 100:13940) as a tool to directly activate distinct neurons non-invasively by illumination with blue light in Drosophila larvae. In another learning paradigm (Hendel et al. 2005, J Comp Physiol A 191:265) Drosophila larvae were trained to avoid or approach an odour that was paired with a punishing or rewarding reinforcing stimulus. The light-induced activation of modulatory neurons substitutes for these reinforcing stimuli. If an odour is paired with light-induced activation of dopaminergic neurons, the odour becomes repulsive. Conversely, if an odour is paired with light-induced activation of octopaminergic/tyraminergic neurons, the odour becomes attractive. Therefore, distinct modulatory neurons are sufficient to mediate the reinforcing properties of punishing or rewarding stimuli, respectively.

# Carbon dioxide mediates specific synaptic and behavioral adaptation in *Drosophila*

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Carbon dioxide ($CO_2$) is an important chemosensory stimulus with different ethological meaning for many different insects. In fruit flies, $CO_2$ is a major component of the fear pheromone dSO, which mediates potent avoidance behavior (Suh et al., 2004). In another context, $CO_2$ affects attraction to food sources in female *Drosophila* (Faucher et al., 2006). In most insects, a dedicated and anatomically distinct sensory circuit exists to detect $CO_2$ at concentrations one million-fold below that of humans. Given the extreme sensitivity of insects to this compound and the apparent labeled-line by which olfactory sensory neurons tuned to $CO_2$ communicate with the central nervous system, we asked whether prolonged exposure in supra-ambient $CO_2$ concentrations would lead to functional and behavioral adaptation.
In fruit flies, $CO_2$ activates a population of approximately 25 olfactory sensory neurons that project to a single glomerulus in the antennal lobe, which represents the first olfactory neuropil (De Bruyne et al., 2003; Suh et al. 2004). Using the GAL4/UAS system to visualize the different olfactory neurons innervating this specific glomerulus, anatomical changes on separate processing levels due to long-term $CO_2$ exposure were investigated. The results show an enlargement of the $CO_2$-glomerulus in a concentration-dependent manner. This effect is stimulus- and glomerulus-specific. On the functional level, we find that $CO_2$-tuned neurons in the *Drosophila* antenna faithfully report $CO_2$ concentrations in the new environment, but that a class of local interneurons becomes significantly increased to this stimulus, producing reduced behavioral responses to this compound. This sensory-specific adaptation may allow animals to measure absolute $CO_2$ concentrations and relate this to the baseline of ambient $CO_2$, thus retaining maximum sensitivity to this ethologically important stimulus.

Reference: De Bruyne et al. (2003). XXV. AChemS meeting, .no. 379; Faucher et al. (2006). J.Exp.Biol. 209:2739-48; Suh et al. (2004). Nature 431:854-9.

# Plasticity of odour-evoked activity in the honeybee antennal lobe: coupled aversive conditioning and calcium imaging

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Intense previous work has studied the neurobiological bases of olfactory learning in two insect models, the fruitfly *Drosophila melanogaster* and the honeybee *Apis mellifera*. Until now, however, direct comparison between results in the two species has been difficult because most of the fruitfly work was based on aversive conditioning (reinforcer: electric shock), while the bee work was based on appetitive conditioning (reinforcer: sucrose solution). To allow more direct comparisons between the two models, we have developed a novel aversive conditioning paradigm in the honeybee. The Sting Extension Response (SER) is the reflex extension of the bee sting in response to a mild electric shock. By presenting an odour (CS) in temporal association with the electric shock (US), we show that bees are able to form odour-shock associations. This conditioning is clearly associative, as shown in differential conditioning experiments, in which bees respond to the odour associated to the electric shock (CS+) but not to another non-reinforced odour (CS-). Using a pharmacological approach, we show that SER conditioning depends on the dopamine system in honeybees, as in fruitflies. To study plasticity in the first relay of the insect olfactory pathway - the antennal lobe - related to aversive conditioning, we have developed a coupled SER conditioning / calcium imaging preparation. We can at the same time monitor the behavioural responses of a live bee learning to differentiate between two odours (CS+ and CS-), and record calcium signals evoked by these odours during conditioning. We thus image the honeybee brain while it learns aversive associations, and relate brain activity changes directly to learning success.

# Learning in the insect antennal lobe: how much plasticity can bees bear?

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The primary olfactory center of honeybees, the antennal lobe, is a complex neural network: receptor neuron axons converge onto olfactory glomeruli, local neurons branch within and between glomeruli, and output neurons send information to higher order brain centers. Some output neurons are multiglomerular, and therefore collect information from many glomeruli, having access to the combinatorial activity pattern in the antennal lobe. Other output neurons are uniglomerular, and have access to the combinatorial pattern only via local neurons. These neurons are spontaneously active in the absence of olfactory stimulation. They can be recorded using calcium imaging by selectively backfilling them with calcium sensitive dyes. We studied the effect of single odor pulses onto spontaneous activity patterns of the uniglomerular projection neurons of a particular tract, the lACT tract, with a temporal resolution of 200 ms. We found that during the two minutes after an odor pulse, spontaneous activity did not increase, but the spontaneous activity patterns were modified, with an increased probability of activity patterns that resemble the previously smelled odor. Next, we studied the effect that appetitive training has onto odor representation. We used absolute training, differential training, and backward training controls. We found that olfactory learning did not modify odor-responses in lACT neurons during the 15 minutes after appetitive training. The results will be discussed in a model of how plasticity and reliability both contribute to efficient brain functioning.

# β-arrestin2 mediated internalization of mammalian odorant receptors

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Odorant receptors comprise the biggest subfamily of G-protein-coupled receptors. While the endocytic mechanisms of other G-protein-coupled receptors have been characterized extensively, almost nothing is known about the intracellular trafficking of odorant receptors. The present study describes the endocytic pathway of mammalian odorant receptors, which bind β-arrestin2 with high affinity and are internalized via a clathrin-dependent mechanism. After prolonged odorant exposure receptors are not targeted to lysosomal degradation but accumulate in recycling endosomes. Odorant-induced odorant receptor desensitization is promoted by PKA phosphorylation and is dependent on serine and threonine residues within the third intracellular loop of the receptor. Moreover, β-arrestin2 is redistributed into the dendritic knobs of mouse olfactory receptor neurons after treatment with a complex odorant mixture. Prolonged odorant exposure resulted in accumulation of β-arrestin2 in intracellular vesicles. Adaptation of olfactory receptor neurons to odorants can be abolished by the inhibition of clathrin mediated endocytosis, showing the physiological relevance of the here described mechanism of odorant receptor desensitization. A better understanding of odorant receptor trafficking and further insight into the molecular determinants underlying the interactions of odorant receptors with β-arrestin2 and other trafficking proteins will therefore be important to fully understand the mechanisms of adaptation and sensitization in the olfactory epithelium.

# The Virtual Antennal Lobe: Encoding odorant molecular structure and predicting scent using computational principles of insect olfaction

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The chemical sense of insects has evolved to encode and classify odorants. Thus, the neural circuits in their olfactory system are likely to implement an efficient method for coding, processing and classification of chemical information. Most olfactory systems show a three-step organization:[1] In the first step olfactory receptors encode the odorants to neural signals, in the second step those signals are decorrelated, while in the third step a perceptual quality is assigned to the stimulus. Based on this organization, we derived a novel method for encoding odorants and predicting their scent.

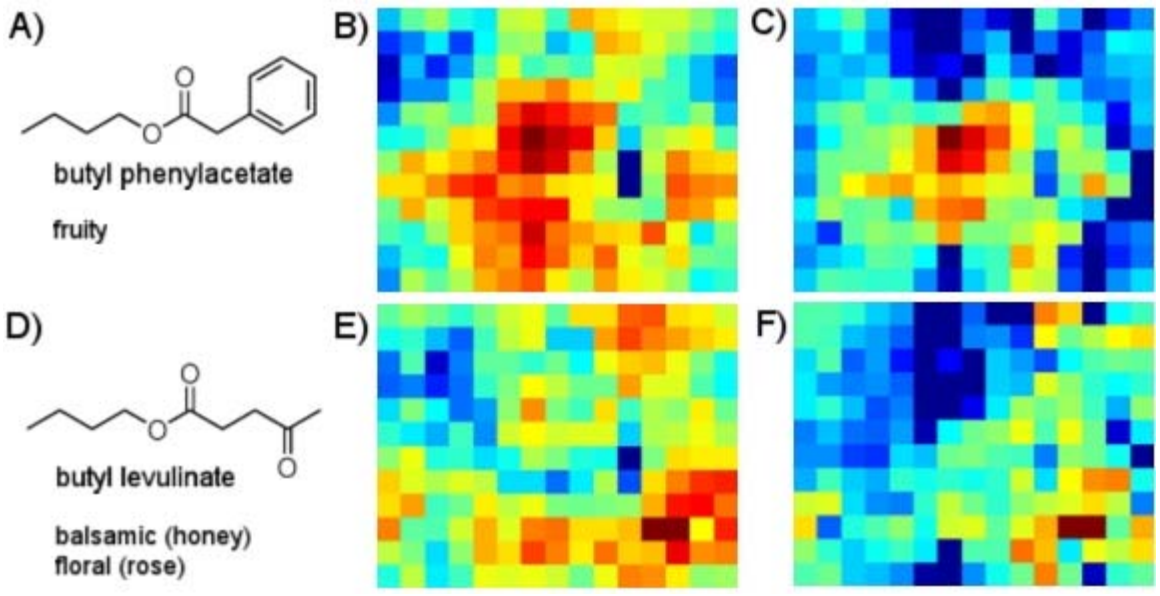
We modeled the receptor stage by training a self-organizing map (SOM) on an odorant dataset, where each odorant was represented by 184 calculated molecular properties (*descriptors*). Each SOM-unit acts as a 'virtual receptor': It responds strongest if the distance to the stimulus compound is minimal, and vice versa. This yielded activation patterns reminiscent of those observed in the antennal lobe of insects. Fig. 1 shows two example odorants (A: butyl phenylacetate, D: butyl levulinate) and their corresponding patterns (B and E).

To decorrelate the receptor signals we implemented correlation-based lateral inhibition[2]: The more correlated the response patterns of two receptors are, the stronger they inhibit each other. Mathematically, the response from one receptor is decreased by a function of the weighted average of all other responses, where the weight is given by the Pearson correlation coefficient. Fig. 1 C) and F) show the patterns after decorrelation.

Using the preprocessed signals as input, we trained a Naive Bayes classifier to predict odorant scents, corresponding to the assignment of a perceptual value. In a machine-learning experiment for 66 scents, we could achieve an $F_1$-measure of up to 0.6 ('fruity' odorants). In some cases, the processed data led to better classification than the original descriptors. Moreover, we found that the decorrelation step increased classification performance. In addition, were able to reduce dimensionality by a factor of up to 18 compared to the original descriptor set, while only barely affecting odorant scent prediction performance, indicating that this representation efficiently captures structural information relevant for odorant coding.

**References**

1. Firestein S, *Nature* 413:211-218 (2001)
2. Linster C, Sachse S and Galizia CG, *J. Neurophysiol.* 93:3410-3417 (2005)



# Transition from sea to land: adaptations of the central olfactory pathway in the terrestrial giant robber crab.

Steffen Harzsch, Marcus Stensmayr and Bill Hansson

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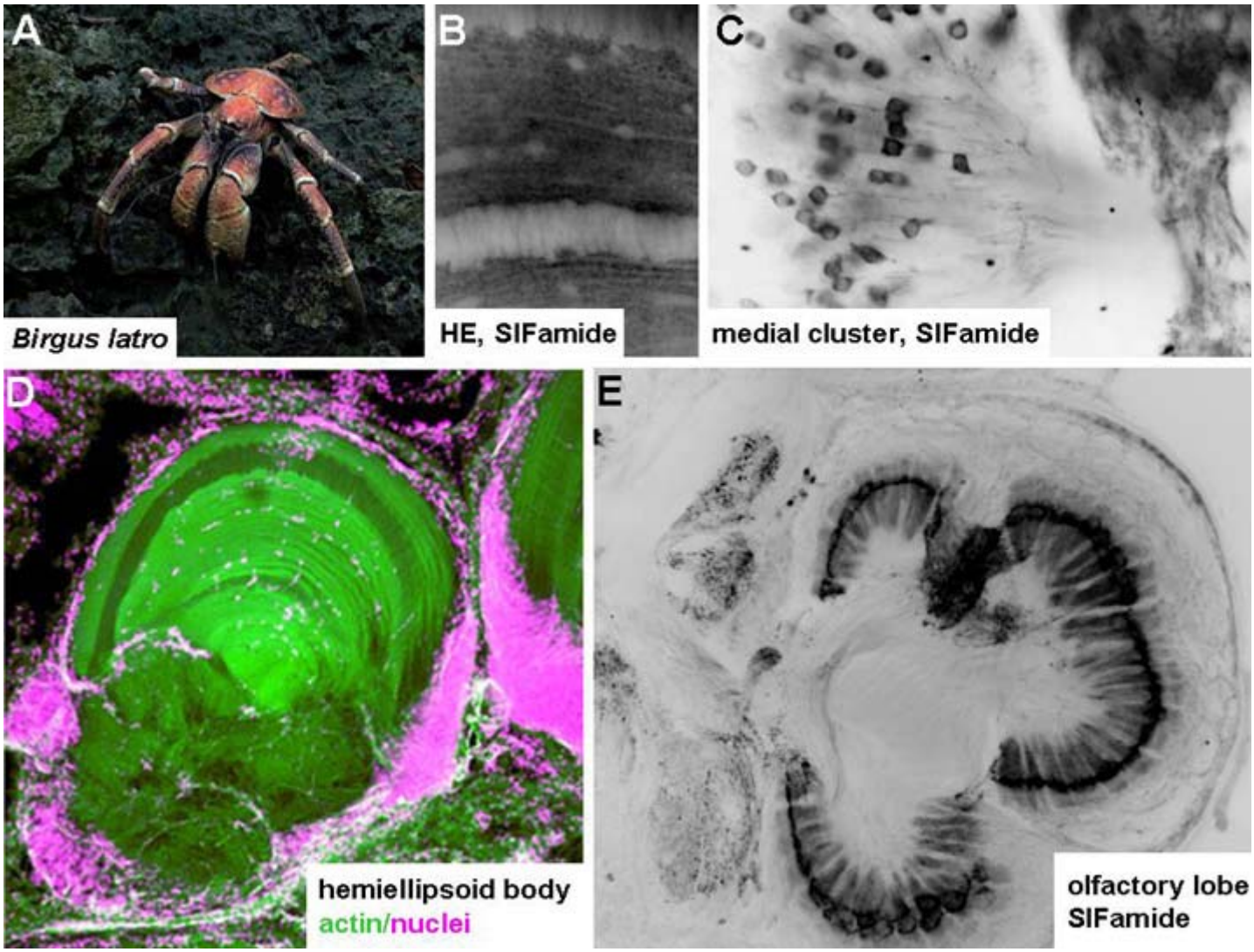
The robber crab Birgus latro (A) displays a fully terrestrial life style. Recent studies have indicated that this transition from sea to land has coincided with distinct changes both of physiological and behavioural aspects of olfaction in this animal. In their peripheral olfactory pathway, robber crabs have evolved an olfactory sense with a high degree of resemblance to the insect system (Stensmyr et al. 2005. Curr. Biol. 15:116). In this contribution we explored changes in the central olfactory pathway that may be related to the terrestrial life style. Section series of the brain were processed histochemically and the immunolocalisation of SIFamide was analysed. The olfactory lobe is hypertrophic in comparison to other crustaceans and forms 3 sublobes (E). Many local olfactory interneurons cluster show strong SIFamide immunoreactivity (C). The hemiellipsoid body (HE) as the protocerebral target of olfactory projection neurons has undergone most dramatic changes in that it displays an organization that is yet undescribed for Crustacea. It is organized into what seems to be densely stacked shells of parallel fiber layers. In comparison, the HE of crayfish is much smaller and is subdivided into hundreds of microglomeruli. We will compare these architectural features across several other terrestrial crustaceans to understand the evolution of the olfactory pathway during the transition from sea to land.



# *Delphinium norhegnii* against Strychnin induced seizures in mice.

Muhammad Liaquat Raza and Shabana U Simjee

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*Dephinium* specie traditonaly employed in treating epilepsy by the herbal practitioners in the Eastern system of medicine. One of the *Delphinium* specie has been evaluated for anticonvulsant actions against the strychnin induced seizured. Using three doses of *D. nordhegnii* suppressed the seizures after strychnin administration in mice. The results are comparible with standard AEDs.

# Symposium

## S3: Neuronal Dendrites: Synaptic Function, Plasticity and Information Processing

***Knut Holthoff and Arthur Konnerth, Munich***

### Slide

**S3-1** Opening remarks for Symposium 3
*Knut Holthoff and Arthur Konnerth, Munich*

**S3-2** Plasticity compartments in basal dendrites of neocortical pyramidal neurons.
*Jackie Schiller, Haifa (Israel)*

**S3-3** Spatio-temporally Graded NMDA Spike/Plateau Potentials in Basal Dendrites of Neocortical Pyramidal Neurons.
*Guy Major, Alon Polsky, Jackie Schiller, Winfried Denk and David Tank, Cardiff (UK), Haifa (Israel), Heidelberg and Princeton (USA)*

**S3-4** Dendritic and axonal properties for high-frequency temporal coding in auditory neurons
*Nace L. Golding, Paul J. Mathews and Luisa L. Scott, Austin (USA)*

**S3-5** Bidirectional single-shock synaptic plasticity in cortical neurons induced by dendritic spikes
*Knut Holthoff, Yury Kovalchuk and Arthur Konnerth, Munich*

### Poster

**TS3-1A** Detailed passive cable models of hippocampal granule cells obtained with two-photon microscopy
*C. Schmidt-Hieber, P. Jonas and J. Bischofberger, Freiburg*

**TS3-2A** Possibility of peripheral nerve lesion related to the transient nitric oxide mobilization. The neurohistochemical study in rabbit.
*M. Lackova, A. Schreiberova, D. Kolesar, N. Lukacova and J. Marsala, Kosice (Slovakia)*

**TS3-3A** Mobile endogenous calcium buffers promote the impact of spine neck geometry on spino-dendritic cross-talk
*H. Schmidt and J. Eilers, Leipzig*

**TS3-4A** Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons
*UV. Nägerl, G. Köstinger, JC. Anderson, KAC. Martin and T. Bonhoeffer, München and Zürich (CH)*

**TS3-5A** RhoSAP, a novel protein of vesicle cycling at the PSD
*J. Dahl, J. Bockmann and TM. Boeckers, Ulm*

**TS3-6A** Characterization of SerSAP3: A Rap-GAP containing protein of postsynaptic densities (PSDs)
*A. Dolnik, J. Bockmann, TV. Wuttke, H. Lerche and TM. Böckers, Ulm*

**TS3-7A** Characterization of *Drosophila* ProSAP (DProSAP) and its possible connection to Wnt signaling pathways
*AM. Grabrucker, U. Thomas, A. Schmitt, S. Glaschick, ED. Gundelfinger, U. Nienhaus and TM. Böckers, Ulm and Magdeburg*

**TS3-8A** Influence of Abi-1 on dendritogenesis and synaptic morphology of hippocampal neurons
*S. Johannsen, C. Proepper and TM. Boeckers, Ulm*

**TS3-9A** Morphological and quantitative study of nerve cells in the Anterior Ventral Nucleus of the Human Thalamus
*MM. Sabubeh, SM. Bani Hani and MS. AL-Haidari, Nablus (Palestinian Authority), Irbid (Jordan) and Baghdad (Iraq)*

**TS3-10A** Different density of the $GABA_{B1}$ subunit in subcellular compartments of CCK- and PV- containing hippocampal interneurons

*A. Gross, R. Shigemoto, M. Frotscher, I. Vida and A. Kulik, Freiburg and Okazaki (J)*

Introductory Remarks to Symposium 3

# Neuronal Dendrites: Synaptic Function, Plasticity and Information Processing

Knut Holthoff and Arthur Konnerth, Munich

During the last decade the active properties of mammalian dendrites have become an important scientific focus. Active conductances in dendrites of pyramidal neurons can shape the integration of synaptic signals and are even capable to induce regenerative events. Besides sodium-based action potentials, which can propagate throughout the dendritic tree, neocortical pyramidal neurons can also sustain dendritic spikes that are spatially restricted. We want to highlight newly discovered functions of the dendritic tree of neurons for the integration of synaptic signals and its involvement in synaptic plasticity and signal integration.

# Plasticity compartments in basal dendrites of neocortical pyramidal neurons.

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Synaptic plasticity rules largely determine how cortical networks develop and store information. Using confocal imaging and dual site focal synaptic stimulation we show that basal dendrites, which receive the majority of synapses innervating neocortical pyramidal neurons, contain two compartments with respect to plasticity rules. Synapses innervating the proximal basal tree are easily modified when paired with the global activity of the neuron. In contrast synapses innervating the distal basal tree fail to change in response to global supra-threshold activity or local dendritic spikes. These synapses can undergo long-term potentiation (LTP) under unusual conditions when local NMDA-spikes, which evoke large calcium transients, are paired with a "gating-molecule": BDNF. Moreover, these synapses use a new temporal plasticity rule which is an order of magnitude longer than spike timing dependent plasticity (STDP) and prefers reversed pre/postsynaptic activation order. The newly described plasticity compartmentalization of basal dendrites expands the networks plasticity rules and may support different learning and developmental functions.

# Spatio-temporally Graded NMDA Spike/Plateau Potentials in Basal Dendrites of Neocortical Pyramidal Neurons.

Guy Major[1,4], Alon Polsky[2], Jackie Schiller[2], Winfried Denk[3] and David Tank[4]

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There is considerable controversy over the ionic basis and computational implications of local dendritic spike/plateau potentials in thin dendrites of cortical pyramidal neurons. We stimulated basal dendrites of layer 5 pyramidal neurons in vitro with brief focal pulses of glutamate delivered by iontotophoresis or uncaging. There was a graded 7-fold increase in dendritic spike/plateau amplitude, measured at the soma, from 3 mV for inputs near the dendritic tip (240 μm from soma) to 23 mV for proximal inputs (50 μm from soma), on average, at baseline membrane potentials near -72 mV. Spike/plateaus were accompanied by a large calcium transient over a 20 to 40 μm long 'hot spot' at the input site, with a smaller calcium transient extending to the dendritic tip. Stronger inputs produced longer plateaus with larger calcium transients and more negative voltage thresholds. These findings together with pharmacological experiments confirm that local spike/plateaus are dominated by NMDA conductances along the entire length of terminal basal dendrites, endowing them with versatile computational capabilities, strongly dependent on the cortical network state. We show how graded NMDA plateau potentials may contribute to persistent firing during working memory and to directional selectivity.

# Dendritic and axonal properties for high-frequency temporal coding in auditory neurons

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Coincidence detection of excitatory inputs in dendrites is a fundamental process in synaptic integration that dictates the probability and timing of action potential signaling. In neurons concerned with temporal coding, a major question is how the precision of coincidence detection is maintained in the face of temporal distortions imposed on excitatory synaptic potentials by dendritic cable properties. We have addressed this issue in the principal neurons of the medial superior olive (MSO), which detect submillisecond disparities in synaptic activity driven by the two ears, cues used for azimuthal sound localization. Using somatic and dendritic patch-clamp recordings we have shown that both real and simulated EPSPs propagating from the dendrites to the soma undergo a voltage-dependent decline in both rise time and duration. These effects are largely eliminated in the presence of 100 nM alpha-dendrotoxin, a blocker of Kv1 family potassium channel subunits. This EPSP sharpening appears progressively more robust at distal dendritic locations, providing a distance-dependent compensation for the increase in synaptic rise time and duration expected from cable filtering. Parallel experiments using voltage-clamp recordings in nucleated patches at 35°C revealed that dendrotoxin-sensitive potassium currents are active over the entire subthreshold voltage range and exhibit the appropriate kinetics to provide a rapid and transient repolarization less than 400 µs following the peak of excitation.

The high rate of action potential output in MSO principal neurons, which under normal conditions can reach hundreds of hertz, has the potential to disrupt binaural coincidence detection through their propagation from the somatically-located axon back into the dendritic compartment receiving concurrent synaptic excitation and inhibition. However, paired somatic and dendritic recordings reveal that the dendrites are electrically insulated from backpropagating action potentials, which can be attenuated up to 98% during responses to synaptic excitation. Furthermore, even at the soma, action potentials appeared small and graded in amplitude, a phenomenon dependent on the rate of rise of excitation. Paired somatic whole-cell recordings and axonal loose-patch recordings reveal that all putative spikes at the soma represent all-or-none action potential output in the axon, and that the axon has the capability of entraining at unusually high frequencies (up to 1400 Hz).

Taken together, our studies of dendritic integration in the principal cells of the MSO show that the subthreshold activation of low voltage-activated potassium channels simultaneously sharpens the precision of excitation via active EPSP repolarization, while simultaneously providing a steady leak that electrically segregates the site of action potential initiation in the axon from the site of synaptic integration in the soma and dendrites. These features contribute to the ability of these neurons to encode sound localization cues at a temporal resolution two to three orders of magnitude finer than most neurons of the central nervous system

# Bidirectional single-shock synaptic plasticity in cortical neurons induced by dendritic spikes

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Mammalian dendrites are active structures capable of regenerative electrical activity. Using fast confocal imaging in combination with low affinity calcium indicator dyes we have investigated the characteristics and physiological function of local dendritic spikes in layer 5 pyramidal neurons of mouse visual cortex.
We found that these dendritic spikes initiate a fast calcium transient in a small spine-dendritic compartment. Furthermore, a single of these dendritic spikes induced long-term synaptic depression (sLTD). This form of activity-dependent synaptic plasticity did not require somatic spiking. The induction of sLTD is input-specific and dependent on activation of NMDA-receptors. However, the co-incident activation of a single synaptically-induced local dendritic spike and a back-propagating action potential often produced long-term potentiation (sLTP). We found a tight correlation between the amplitude of the dendritic calcium transient during induction and the direction of the resulting synaptic plasticity. A low amplitude calcium transient in the spine-dendritic compartment induced sLTD, whereas a high amplitude calcium transient induced sLTP. Remarkably, these local dendritic calcium transients had a similar time-course. Experiments using moderate intracellular BAPTA concentrations revealed that the calcium transient in activated spines is crucial for the induction of single shock synaptic plasticity. Under these conditions, the dendritic calcium transients during induction are negligible, but activated individual spines still respond with calcium transients of different amplitude. Under low intracellular BAPTA concentration, there was a strong correlation between the amplitude of the calcium transient in activated spines and the direction of the resulting synaptic plasticity. A low amplitude calcium transient in the activated spines induced sLTD, whereas a high amplitude calcium transient induced sLTP.
In conclusion, we identified a new form of rapidly induced bidirectional synaptic plasticity. We propose that sLTP and sLTD underlie the rapid acquisition of information in cortical circuits.

# Detailed passive cable models of hippocampal granule cells obtained with two-photon microscopy

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The electrotonic structure of a neuron largely shapes the propagation of synaptic potentials along its dendrites. To obtain detailed passive cable models of adult dentate gyrus granule cells, we combined patch-clamp recordings with morphological 3-dimensional reconstructions. Simultaneous two-pipette somatic recordings were performed in acute slices from adult 2- to 4-months old mice at 22°C. Short current pulses (0.5 ms, 80-200 pA) were injected via one pipette while voltage responses were recorded with the other pipette. The voltage transient following the current pulse showed a pronounced initial rapid decay ($t < 2$ ms) that accounted for 79.9 ± 2.9 % of the total amplitude, as estimated by peeling the slow component ($t0 = 35.3 \pm 5.7$ ms, mean ± SEM, n=4). The morphology of the biocytin-filled FITC-avidin-labeled cells, including soma, dendrites and part of the axon, was reconstructed using two-photon microscopy (Zeiss LSM 510, Coherent Chameleon-XR tuned to 790 nm). After deconvolution of the 3-dimensional image stacks, an automated filament-tracing software was used to obtain unbiased detailed compartmental models. Specific membrane capacitance ($Cm = 0.98 \pm 0.03$ µF/cm2), specific membrane resistance ($Rm = 41.2 \pm 2.0$ kΩ cm2) and intracellular resistivity ($Ri = 180 \pm 30$ Ω cm) were obtained by direct least-squares fitting of the model's response to the experimental data assuming homogenous membrane properties. Spine density was counted on representative dendritic segments after deconvolution, and spines were implemented into the model by scaling Rm and Cm of spine-bearing compartments appropriately. Both the rise as well as the fast and slow components of the decay in the voltage response could be precisely reproduced by the model. However, when spines were omitted, an appropriate fit of the fast components could not be achieved.

In conclusion, two-photon microscopy was used to obtain a detailed 3-dimensional geometry and a corresponding cable model of hippocampal granule cells. The results suggest that the detailed morphology appears to be critically important for the shape of transient voltage responses as they occur during brief excitatory synaptic inputs.

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# Possibility of peripheral nerve lesion related to the transient nitric oxide mobilization. The neurohistochemical study in rabbit.

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During high-frequency synaptic activity the effectiveness and reliability of synaptic transmission significantly changes- this phenomenon called synaptic plasticity. Nitric oxide (NO) is the neurotransmitter to which many physiological and pathological functions in the peripheral nervous system have been attributed. Enzyme responsible for the synthesis of NO, nitric oxide synthase, was visualised in the peripheral nerves by applying histochemistry to its specific histochemical marker- NADPH diaphorase.

The distribution of diaphorase-exhibiting axons was examined in peripheral nerves of rabbit using longitudinal sections. The morphologically distinct kinds of diaphorase exhibiting axons were identified in saphenous, femoral, sciatic, mediane and radial nerve. The prominent diaphorase exhibiting bundles were detected in sciatic nerve separated from hindlimb and radial nerve isolated from forelimb. Axonal diaphorase positivity was detected in the whole course of examined nerves. The considerably tapered diaphorase labeled fibers were seen in the nerve bundles of nerve fibers corresponding to the femoral and sciatic nerve afflicted by paraplegia as the outgrowth of lumbosacral spinal cord ischemia with NO-mobilization. Reduction of diameter of diaphorase positive fibers in femoral nerve corresponded to the region near the femoral nerve ramify on saphenous nerve. In addition, histologic and histomorphometric analysis proved that the process of axonal regeneration after transient ischemia took places despite ischemic lesion in the femoral nerve earlier than in sciatic nerve. The nerve degeneration in terms of experimental evaluation was evident in the following week after ischemic injury indicating that the detected nerve dysfunction was attributable to the increased level of NO-synthase a thus resulted in neurodegeneration effect of NO suspenseless during ischemia.

In coincidence with our previous observations, the recent research has raised the possibility that NO may also be released at a greater distance from neuronal cell body and, therefore, anterograde axoplasmatic transport of NO-synthase along with other synaptically acting enzymes and neurotransmitters may serve as a mechanism allowing for remote synaptic activity of NO.

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# Mobile endogenous calcium buffers promote the impact of spine neck geometry on spino-dendritic cross-talk

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Dendritic spines convey most excitatory input in the brain. One of their presumed functions is to compartmentalize calcium, thereby, constituting a separated signaling domain for this potent second messenger. The size of spines is dynamically adjusted during synaptic activation, yet, functional consequences of these movements in the adult brain are largely unclear. Only recently we challenged the notion of isolated spine calcium-signaling by showing that mobile endogenous calcium binding proteins (CaBPs) can break the spine limit for calcium. In the case of neighboring co-active spines that process slow synaptic calcium signals, substantial amounts of calcium were shuttled into the dendritic shaft where calcium summed and activated dendritic downstream signaling. We hypothesized that adjusting the spine morphology could provide a powerful tool to regulate this form of spatial biochemical integration in dendrites. We tested this hypothesis by using a kinetic computer model in which spines of cerebellar Purkinje neurons were coupled to their parent dendrite by a neck of variable length and diameter. Our results show that, in the presence of the native CaBPs Calbindin-D28k and Parvalbumin, the spine neck morphology exerts a substantial influence on spino-dendritc coupling and is a major determinant of dendritic calcium summation and concomitant calmodulin activation.

# Protracted synaptogenesis after activity-dependent spinogenesis in hippocampal neurons

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Activity-dependent morphological plasticity of neurons is central to understanding how the synaptic network of the central nervous system becomes reconfigured in response to experience. In recent years several studies have shown that synaptic activation that leads to the induction of long-term potentiation also drives the growth of new dendritic spines, raising the possibility that new synapses are made. We examine this directly by correlating timelapse two-photon microscopy of newly-formed spines on CA1 pyramidal neurons in organotypic hippocampal slices with electron microscopy. Our results show that while spines up to eight hours in age fail to form synapses, older spines, ranging from 15 to 19 hours, have ultrastructural characteristics typical of synapses. Our data support the view that spinogenesis leads rapidly - within tens of minutes - to the formation of contacts with presynaptic boutons, which slowly - over the course of many hours - mature into new synapses.

# RhoSAP, a novel protein of vesicle cycling at the PSD

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RhoSAP, a novel protein of vesicle cycling at the PSD

Glutamatergic synapses in the central nervous system are characterized by an electron dense network of cell adhesion proteins, cytoskeletal proteins, scaffolding and adaptor proteins, membrane bound receptors and channels, G-proteins and a wide range of different signalling modulators and effectors underneath the postsynaptic membrane. This so called postsynaptic density (PSD) ressembles a complex signaling machinery. We performed a Yeast-Two-Hybrid screen with the PDZ domain of the PSD scaffolding molecule ProSAP2/Shank3 as bait and identified a novel interacting protein without any known functions. This molecule was named after its Rho-GTPase domain: RhoSAP (Rho GTPase Synapse Associated Protein). Performing database analysis yield to the human analogue RICH-2 and displayed a close relation with Nadrin. Interestingly, RhoSAP also contains a N-terminal situated BAR domain known that could facilitate membrane curvature and a Pro-rich domain at its C-terminus that could possibly act as a SH3 binding motif. Due to the finding of three splice variants of the C-terminal region, a second screen was carried out with RhoSAP's Pro-rich C-Terminus as bait to discover interacting proteins. Surprisingly, Syndapin I, a molecule involved in vessicle endocytosis via direct interaction with Dynamin I was found. Furthermore, Syndapin I contains a C-terminal SH3 domain that suggests to be the binding motif for RhoSAP's Pro-rich Domain. Providing new insight into RhoSAP's function, this interaction as well as the interaction with ProSAP2's PDZ domain was verified by Pull Down Assays and Co-Immunprecipitations. Taken together, RhoSAP is a novel ProSAP2/Shank3 interacting PSD protein, that displays several protein/protein interaction domains. Due to the N-terminal BAR domain, and the interaction with Syndapin I, RhoSAP might act within endocytic processes of the postsynaptic membrane. - Project supported by SFP 497/ B8

# Characterization of SerSAP3: A Rap-GAP containing protein of postsynaptic densities (PSDs)

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In a yeast two hybrid screen using the PSD protein ProSAP interacting protein 1 (ProSAPiP1) as bait, we isolated partial clones of a novel Rap-GAP domain containing protein. This protein, termed SerSAP3, is characterized by several serine rich stretches, a PDZ domain and a C-terminal coiled coil region. The SerSAP3 - ProSAPiP1 interaction is mediated by leucine zipper containing coiled-coil regions of SerSAP3 and ProSAPiP1 as revealed by blot-overlay assay and co-transfection experiments. SerSAP3 mRNA and protein is widely expressed in neurons of the brain and is found in dendrites, spines and PSDs. High protein levels are especially detected in the hippocampus and cerebellum, where SerSAP3 is predominantly localized in cerebellar Purkinje cells. SerSAP3 binds to actin and codes for two distinct regions that are responsible for the targeting to dendritic spines and PSDs, where it colocalizes with proteins of ProSAP/Shank family. SerSAP3 shows some structural similarity to the spine associated Rap-GAP, SPAR, which is able to alter size and complexity of dendritic spines. This effect is not seen after transfection of the full length SerSAP3 protein, an N-terminal SerSAP3 construct, however, leads to the formation of numerous longer and thinner spines paralleled by a significant reduction of the dendritic arbor. The cloning of SerSAP3 adds a new member to the growing list of proteins that are able to regulate small GTPases at spines and synapses. This new molecule might therefore well be important for the structural remodeling of spines and PSDs.

# Characterization of *Drosophila* ProSAP (DProSAP) and its possible connection to Wnt signaling pathways

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Chemical synapses serve as specialized contact sites between neurons and enable communication within the central nervous system. An important feature of the synapse is the ability to reorganize the molecular setup and morphology in response to synaptic activation. This so called "synaptic plasticity" may be the underlying mechanism of learning and memory formation.

Mammalian ProSAP/Shank proteins serve as scaffolding proteins within postsynaptic densities of dendritic spines. A single ProSAP homologue, DProSAP, has been identified in *Drosophila*. Both overexpression and RNAi-mediated knockdown of DProSAP cause a planar cell polarity (PCP) phenotype, most notably in the eye. It is known that mutations affecting the Wnt pathway, e.g. the loss of Frizzled receptors, also cause a PCP phenotype. Wnt proteins play an important role in the development of neurons. We reasoned that the similarity of phenotypes in flies might reflect a conserved functional interplay between ProSAPs and Wnt in hippocampal neurons. Therefore the DProSAP protein was investigated closer in different attempts. A yeast-two-hybrid assay was performed to identify the interaction partners of DProSAP.

To further substantiate a variety of interactions for their in vivo significance we performed a genetic modifier approach, in which mutants for potential interaction partners of DProSAP were crossed to flies, which either overexpressed or suppressed DProSAP in restricted areas of wing imaginal disc epithelia. 14 mutants were identified to either suppress or enhance the wing vein phenotypes associated with altered levels of DProSAP, among them mutants in DHomer and WASP, a downstream effector of DAbi, and Dishevelled (Dsh), a component of Wnt signalling cascades. Imaginal discs and pupate wings were prepared and the overexpression of DProSAP was shown. DProSAP constructs were cloned into eGFP vectors and transfected into HeLa cells. Co-transfected Abi-1 that has a homologue (DAbi) in Drosophila and DProSAP colocalize.

To discover the role of WNT proteins or other stimulatory effects, synapses were investigated closer using 4Pi microscopy. Either GFP fusion proteins transfected into hippocampal neurons or fluorescence immunohistochemistry with antibodies directed against synaptic proteins were used to visualize synaptic contacts.

In a further approach to investigate the connection between Wnt and ProSAPs, *Xenopus leavis* full size dishevelled and four constructs, each lacking one of the dsh domains and the C-term were transfected into hippocampal cells. In future experiments the influence of dsh on synaptogenesis and synapse development will be investigated.

# Influence of Abi-1 on dendritogenesis and synaptic morphology of hippocampal neurons

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The rearrangement of the actin cytoskeleton plays an essential role for the outgrowth of dendrites and the development as well as the maturation of synapses. The regulation of these processes, however, is not known in detail. In different model systems it was shown, that Abl-interacting protein 1 (Abi-1) and its interaction partners WAVE2, Rac, Eps8, SOS1 and Abl tyrosine kinase are very important for the formation and reorganisation of the actin cytoskeleton.

To investigate, if Abi-1 has a similar role in neurons, down regulation of Abi-1 by a vector based RNAi method was established in primary hippocampal neurons from rat.

Transfection of neurons with Abi-1 RNAi led to a highly branched dendritic arbor. Overexpression of Abi-1 resulted in an opposite phenotyp with a simplification of the dendritic tree. Additionally, Abi-1-down regulation by RNAi in neurons decreased the density of synapses and reduced the number of mature synapses, while Abi-1 overexpression increased the number of synapses and led to more mature synapses.

These results point out the importance of Abi-1 in actin cytoskeleton rearrangement processes in neurons. Abi-1 protein concentration was shown to be essential for regulated outgrowth of dendrites as well as development and maturation of synapses.

This project is supported by the DFG and the Land Baden-Württemberg (1423/74).

# Morphological and quantitative study of nerve cells in the Anterior Ventral Nucleus of the Human Thalamus

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In this study the Golgi method of silver impregnation of neurons was used for the study of qualitative and quantitative features of neurons in the anterior ventral (AV) thalamic nucleus of adult human. Two types of cells were found in the human AV thalamic nucleus: Golgi-type I neurons and Golgi-type II neurons. Golgi-type I cells were found to be of medium to large (mean diameter=26 µm ± 2.4) and have varying shapes (multipolar, round or fusiform). They were found to have many (3-10) primary dendrites with many branches. They had more branching points and free dendrite tips than Golgi-type II cells.Golgi-type II cells were small to medium ( mean diameter= 15.3 µm ± 2.3). Their shape varied between multipolar, oval and triangular. They had few (1-6) primary dendrites which had few or relatively rich arborizations. The dendrites of Golgi-type II cells may have spines and/or grape-like appendages on their shafts or at their tips. Golgi-type I and Golgi-type II neurons in the human AV nucleus were compared with their counterparts in non-human and human thalamic nuclei other than the AV nucleus. Hopefully, this study will improve our understanding of the structure of the AV thalamic nucleus in the human and represent background of knowledge for further studies about possible morphological and/or quantitative changes that may occur in some neuronal types during certain diseases.

| | **Mean cell body diameter** | **Mean B.P number** | **Mean F.D.T number** |
|---|---|---|---|
| Golgi-Type I cells | 26um (n=42)<br>(S.D=2.4) | 19.2(n=24)<br>(S.D=2.99) | 25.96(n=24)<br>(S.D=7.25) |
| Golgi-Type II cells | 15.3um (n=32)<br>(S.D=2.3) | 8.69(n=32)<br>(S.D=3.75) | 11.84(n=32)<br>(S.D=5.63) |

# Different density of the $GABA_{B1}$ subunit in subcellular compartments of CCK- and PV- containing hippocampal interneurons

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$GABA_B$ receptors mediate metabotropic effects of the inhibitory neurotransmitter GABA in the brain. Presynaptic receptors regulate neurotransmitter release, whereas the activation of postsynaptic ones activate a K+conductance resulting in slow IPSPs. In the hippocampus, high levels of the $GABA_{B1}$ subunit have been observed in somata of a subset of GABAergic interneurons. To investigate the cellular and subcellular distribution of the $GABA_{B1}$ subunit in interneurons, we preformed double immunofluorescent labelling and high-resolution pre-embedding electronmicroscopy.In the present study we focused on cholecystokinin (CCK)- and parvalbumin (PV)- containing interneurons. Light microscopic investigations revealed that CCK+ cells contain a large intracellular reserve pool of the $GABA_{B1}$ subunit, whereas PV+ cells contain less protein in their somata. Electron microscopic analysis showed, that the $GABA_{B1}$ protein was found along the extrasynaptic plasma membrane of dendritic shafts and to a lesser extent on axon terminals of both types of interneurons. Quantitive analysis revealed, that the density of immunogold particles is higher on dendrites and axons of CCK+ interneurons, than on those of PV+ interneurons.

These results suggest a differential regulation of $GABA_B$ receptor expression in the two types of interneurons and indicate stronger modulation of CCK interneurons by GABAergic system.

# Symposium

## S4: Structure and Function of the Vertebrate Retina

*Oliver Biehlmaier and Stephan C. F. Neuhauss, Zürich (CH)*

### Slide

**S4-1** Dissecting the structure and function of retinal ribbon synapses
*Johann Helmut Brandstätter, Hanna Regus-Leidig, Dana Specht and Susanne tom Dieck, Erlangen*

**S4-2** Inhibition in the outer retina: mechanism and function
*Maarten Kamermans, Amsterdam (NL)*

**S4-3** The ionic basis of horizontal cell function
*Andreas Feigenspan and Reto Weiler, Oldenburg*

**S4-4** Bipolar Cells of the Mouse Retina: Types and Functions
*Silke Haverkamp, Frankfurt/Main*

**S4-5** Dendritic processing in the direction-selective circuitry of the retina
*Susanne E Hausselt, Thomas Euler, Peter B. Detwiler and Winfried Denk, Heidelberg and Seattle (USA)*

**S4-6** Genetic approach to study structure and function of the zebrafish retina
*Oliver Biehlmaier, Zürich (CH)*

### Poster

**TS4-1A** A model for spontaneous, propagating waves in the developing vertebrate retina
*MH. Hennig, Edinburgh (UK)*

**TS4-2A** The developing photoreceptor ribbon
*H. Regus-Leidig, D. Specht, S. tom Dieck and JH. Brandstätter, Frankfurt*

**TS4-3A** Molecular aspects of cytomatrix dynamics at the photoreceptor ribbon synapse
*D. Specht, S. tom Dieck, H. Regus-Leidig and JH. Brandstätter, Frankfurt/Main and Erlangen*

**TS4-4A** OFF midget bipolar cells of the primate retina express AMPA receptors.
*C. Puller, S. Haverkamp and U. Grünert, Frankfurt/Main and Melbourne (AUS)*

**TS4-5A** Altered retinal cone opsin expression in postnatally hypothyroid Pax8-deficient mice
*A. Glaschke, M. Glösmann and L. Peichl, Frankfurt/Main*

**TS4-6A** Postsynaptic expression of a putative L-type calcium channel at photoreceptor ribbon synapses in the mouse retina
*S. tom Dieck, D. Specht, H. Regus-Leidig and JH. Brandstätter, Frankfurt/Main and Erlangen*

**TS4-7A** Cone photoreceptors and ultraviolet vision in the flower bat Glossophaga soricina (Microchiroptera, Phyllostomidae)
*B. Müller, L. Peichl, Y. Winter, O. von Helversen and M. Glösmann, Frankfurt/Main, Bielefeld and Erlangen*

**TS4-8A** Glycogen phosphorylase immunoreactive amacrine cells in the macaque monkey retina
*S. Haverkamp, S. Majumdar and H. Wässle, Frankfurt/Main*

**TS4-9A** Individual variation in rod absorbance spectra correlated with opsin gene polymorphism in sand goby *(Pomatoschistus minutus)*
*MJ. Jokela-Määttä, A. Vartio, L. Paulin and K. Donner, Helsinki (FIN)*

**TS4-10A** Müller cell gliosis in response to light-evoked photoreceptor apoptosis
*T. Pannicke, A. Wurm, I. Iandiev, A. Reichenbach, P. Wiedemann and A. Bringmann, Leipzig*

**TS4-11A** Alterations in Müller glia cell physiology in a porcine model of retinal detachment
*A. Wurm, T. Pannicke, I. Iandiev, A. Reichenbach, P. Wiedemann, S. Uhlmann and A. Bringmann, Leipzig*

**TS4-12A** Two faces of calcium activation after optic nerve trauma: life or death of retinal ganglion cells in vivo depends on calcium dynamics
*S. Prilloff, I. Noblejas, V. Chedhomme and BA. Sabel, Magdeburg*

**TS4-13A** Wavelength discrimination in goldfish: Inhibitory mechanisms in the retina
*C. Albrecht, B. Weirich and C. Mora-Ferrer, Mainz*

**TS4-14A** EFFECT OF GLYCINE- AND $GABA_a$-RECEPTOR BLOCKADE ON GOLDFISH FLICKER PERCEPTION
*B. Benkner and C. Mora-Ferrer, Mainz*

**TS4-15A** Retinal expression and integration of the USH1G protein SANS in the USH protein interactom
*N. Overlack, T. Märker, E. van Wijk, B. Reidel, T. Goldmann, R. Roepman, H. Kremer and U. Wolfrum, Mainz and Nijmegen (NL)*

**TS4-16A** The pharmacological profile of the contrast-dependent optomotor response in Goldfish
*RB. Schmidt-Hoffmann and C. Mora-Ferrer, Mainz*

**TS4-17A** GOLDFISH FULL-FIELD MOTION PERCEPTION IS ELIMINATED AFTER BLOCKADE OF THE ROD ON-CHANNEL UNDER SCOTOPIC ILLUMINATION CONDITIONS AND IMPAIRED UNDER MESOPIC ILLUMINATION CONDITIONS
*VM. Vergin and C. Mora-Ferrer, Mainz*

**TS4-18A** APPLICATION OF ATR-FTIR MICROSPECTROSCOPY TO THE INVESTIGATION OF THE PHOTOTRANSDUCTION PROCESS
*S. Massaro, T. Zlateva, L. Quaroni and V. Torre, Mogliano Veneto (Treviso) (I) and Trieste (I)*

**TS4-19A** Expression of erg1 potassium channels in horizontal cell bodies but not axon terminals of the mouse retina
*A. Feigenspan and R. Weiler, Oldenburg*

**TS4-20A** Localization of connexin57 in dendro-dendritic and axo-axonal gap junctions of mouse horizontal cells
*K. Schultz, U. Janssen-Bienhold, P. Dirks, J. Shelley, G. Hilgen, S. Hombach, K. Willecke and R. Weiler, Oldenburg and Bonn*

**TS4-21A** Rod and cone contributions to horizontal cell responses in the mouse retina
*J. Shelley, T. Schubert, K. Dedek, M. Seeliger and R. Weiler, Oldenburg and Tübingen*

**TS4-22A** Turtle retinal ganglion cells encode stimulus speed, direction and acceleration
*A. Thiel, M. Greschner, J. Ammermüller and J. Kretzberg, Oldenburg*

**TS4-23A** Retinal gene expression in mice and chicks with visual experience that induces refractive errors.
*R. Schippert, C. Brand, M. Feldkaemper and F. Schaeffel, Tübingen*

**TS4-24A** Visual pigment regeneration mechanisms in the *zebrafish*
*VC. Fleisch, H. Schoenthaler, J. von Lintig and S. Neuhauss, Zürich (CH), Vienna (A) and Freiburg*

**TS4-25A** Zebrafish Model for Human Usher Syndrome Shows Light Adaptation Defects and Retinal Degeneration
*C. Hodel, SCF. Neuhauss and O. Biehlmaier, Zürich (CH)*

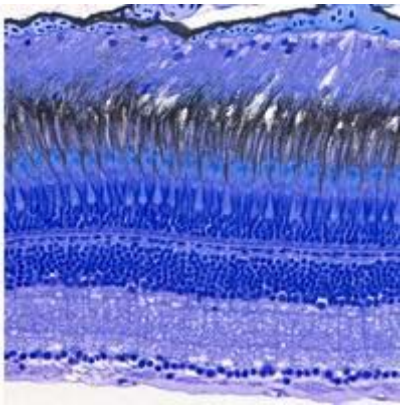
**TS4-26A** Glutamate transport in the zebrafish retina
*A. Lesslauer and SCF. Neuhauss, Zürich (CH)*

**TS4-27A** The Zebrafish Mutant *noir* is a Model for Inner Retinal Dystrophies
*CM. Maurer, HB. Schönthaler, Y. Makhankov and SCF. Neuhauss, Zürich (CH) and Wien (A)*

Introductory Remarks to Symposium 4

# Structure and Function of the Vertebrate Retina

Oliver Biehlmaier and Stephan C. F. Neuhauss, Zürich (CH)



After the seminal work by Ramon y Cajal at the end of the 19th and especially since the late sixties of the last century our understanding of the retina as an approachable part of the brain (Dowling JE, 1987) has largely improved. Many cell types and a lot of pathways have been described in many different species and thus contributed to our impressive amount of information on the retina. One of the big advantages of the retina is its accessibility as a peripheral part of the CNS where complex structures and circuits can be studied.

However, many details on synaptic structure and function of specific cell populations of the vertebrate retina, like photoreceptors, bipolar cells, or ganglion cells still remain unsolved. Recent research has fostered our understanding of retinal structure and function to a large extent. The symposium will start by a talk by Helmut Brandstätter and coworkers who will provide new insights into the structure and function of the very first synapse in the visual system: the photoreceptor ribbon synapse. The following talks will then tackle the signal transmission and especially the feedback mechanism between photoreceptors and horizontal cells. Maarten Kamermans and colleagues will explain the complex negative feedback mechanism from horizontal cells to cones which is influenced by glutamate-gated channels, and Andreas Feigenspan and colleagues will focus on the the ionic basis of horizontal cell function. Next, Silke Haverkamp and her colleagues will introduce us to new morphological types of bipolar cells in the mouse retina and their synaptic plasticity, thus providing us with new information about one of the largest cell populations in the retina. New functional insights into dendritic processing in the direction-selective circuitry of the retina will then be given by Susanne Hausselt and colleagues. Finally, Oliver Biehlmaier and coworkers will provide new data on retinal structure and function found in the zebrafish by genetic means.

# Dissecting the structure and function of retinal ribbon synapses

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Photoreceptors in the retina transmit light signals over a dynamic range of several orders of magnitude in intensity. They continuously adjust their synaptic output to changing inputs thus, optimizing the information transfer. This is different from conventional neurons which encode information by the rate of action potentials limiting the amount of information transfer. Graded synaptic output by photoreceptors requires a powerful chemical synapse, the ribbon synapse, which allows the release of several hundreds of synaptic vesicles per second. To understand ribbon synapse function, we need to know more about the molecular components that organize this highly complex synapse.

In recent studies we have shown that the mature ribbon complex is composed of at least two separate molecular compartments which are defined by the differential distribution of proteins at the cytomatrix at the active zone (CAZ): a ribbon-associated compartment and an active zone compartment. The CAZ protein Bassoon, which localizes at the border between the two compartments, interacts directly with the ribbon protein RIBEYE, is involved in linking the two compartments and contributes to the physical integrity of the ribbon complex (1,2).

Currently, our group investigates the early steps in photoreceptor synaptogenesis and the concept of ribbon synapse assembly from "active zone precursors". Furthermore, we try to break down the molecular components of the ribbon complex into proteins that undergo activity dependent plastic changes and stable proteins that maintain the synapse over a longer period of time.

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(2) tom Dieck S, Altrock WD, Kessels M, Qualmann B, Regus H, Brauner D, Fejtová A, Bracko O, Gundelfinger ED, and Brandstätter JH (2005) J Cell Biol 168:825-836.

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# Inhibition in the outer retina: mechanism and function

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Retinal horizontal cells feed back negatively to cone and in that way create the center surround organization of bipolar cells. Such center surround organization remains present throughout the neurons in the visual system. It has been suggested by many authors that the lateral inhibition in the outer retina is involved in contrast enhancement. One of the places where such lateral interaction takes place is the feedback pathway from horizontal cells to cones. Although, this system has been studied extensively over the last decades, the exact nature of the feedback pathway remains an issue of intense debate. In this presentation I will present data that support a rather unconventional feedback mechanism, an electrical feedback mechanism mediated by hemichannels.

Since the knowledge about the communication between cones and horizontal cells has increased immensely in the last decade, I will revisit the role of horizontal cells. Given the special nature of the feedback pathway from horizontal cells to cones and the vast difference in response dynamics between inner retinal neurons and outer retinal neurons, a purely inhibitory role of horizontal cells becomes unlikely. I will present data that show that horizontal cells function as a general gain control mechanism in the outer retina. Negative feedback enhances the sensitivity of ganglion cells to center stimulation instead of decreasing it as is predicted by the classical center/surround organization.

# The ionic basis of horizontal cell function

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Horizontal cells are second-order GABAergic neurons in the vertebrate retina that provide a lateral pathway responsible for the antagonistic surround properties of inner retinal neurons. The axon-bearing type of horizontal cell is characterized by a cell body receiving input exclusively from cones, and an elaborate axon terminal system, which is postsynaptic to rods. Using immunocytochemistry, single-cell RT-PCR and patch-clamp recordings from isolated cell bodies and axon terminals, we determined the expression of voltage- and ligand-gated ion channels as a prerequisite for horizontal cell function within the retinal network.

Horizontal cell bodies do not generate sodium-based action potentials, and the density of voltage-gated sodium channels is low (8.5 ± 1.1 pA/pF). In contrast, the sodium current density is significantly higher in axon terminals (23.4 ± 4.5 pA/pF), leading to the generation of single small-amplitude spikes after depolarizing current injection. Therefore, in horizontal cell bodies calcium is likely to play an important role for graded potential changes and of course for intracellular events involved in the modulatory role of horizontal cells. Extracellular calcium can enter the cell following voltage-dependent opening of L- and N-type calcium channels, whereas T-type channels are apparently not expressed. In addition, activation of ionotropic glutamate receptors of the AMPA and kainate subtype, but not NMDA receptors, provide an additional route for calcium entry from the extracellular space. Finally, release of calcium from internal stores following activation of ryanodine receptors is most likely involved in the regulation of the intracellular calcium concentration.

Inward rectifier potassium channels of the Kir family and *ether-à-go-go*-related gene isoform 1 (erg1) potassium channels are simultaneously expressed in isolated horizontal cells of the mouse retina. Whereas inward rectifier channels are present in both cell bodies and axon terminals, expression of erg1 is confined to cell bodies and primary dendrites. Erg1-mediated currents are characterized by a negative current-voltage relation at positive membrane potentials and delayed onset kinetics due to C-type inactivation, and they are selectively and entirely abolished by haloperidol. Injection of depolarizing current steps in current-clamp experiments reveal the presence of oscillatory potentials, centered at around 100 Hz. Finally, horizontal cells show a bimodal voltage response, with open erg channels clamping the voltage change to 25 mV. Inactivation of erg channels by large depolarizing currents generates a sharp switch in the voltage response to 142 mV. Our results suggest a cone-specific modulation by erg channels of the glutamate-induced voltage response of horizontal cells.

Finally, horizontal cells express chloride channels of the $GABA_A$ receptor subtype with moderate affinity for GABA($EC_{50}$ = 30 μM) and a main (29.8 pS) and two conductance states (20.2 and 10.8 pS), but not $GABA_C$ or glycine receptors. A putative depolarizing action of GABA is currently under debate; however, the presence of the chloride transporter KCC2 in horizontal cells is at least questionable.

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# Bipolar Cells of the Mouse Retina: Types and Functions

Silke Haverkamp

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Bipolar cells transfer the visual signals from the photoreceptors in the outer retina to the ganglion cells in the inner retina and thus represent a key element of retinal organization. At least 10 different types of bipolar cells have been described and it is surprising how similar the types are, when different mammals are compared. The axons of the bipolar cells terminate at distinct levels within the inner plexiform layer (IPL). ON-bipolar cells show depolarizing light responses and terminate in the inner half of the IPL, OFF-bipolar cells hyperpolarize in response to light and terminate in the outer half of the IPL. The subdivision into ON and OFF bipolar cells is based on the expression of ionotropic glutamate receptors in OFF bipolar cells and the metabotrophic glutamate receptor mGluR6 in ON bipolar cells. OFF bipolar cells can be further subdivided according to the specific expression of AMPA or kainate receptors. The physiological consequences of this molecular diversity are different temporal resolution and possibly different threshold sensitivity.

There are 10-15 types of ganglion cells in most mammalian retinae. However, so far we know only little about which type of bipolar cell is connected to which type of ganglion cell. Because of the increasing availability of mutant mice, the mouse retina has become an important tool to study the synaptic and molecular details of the numerous circuits within the mammalian retina. Transgenic mouse lines expressing GFP in certain cell types are extremely helpful to determine how retinal circuits are assembled and how retinal neurons alter their structure and form the precise synaptic connections within the plexiform layers. In addition, different bipolar cell types can be labeled by immunocytochemical markers: type 3, type 5 and rod bipolar cells express the calcium-binding protein CaB5, whereas type 1/2 bipolar cells express the neurokinin 3 receptor (NK3R) (Ghosh et al., 2004). Type 7 and type 9 bipolar cells are labeled in transgenic mouse lines (Huang et al., 2003; Haverkamp et al., 2005).

Most of the cone bipolar cells of the mouse retina contact all the cone pedicles (between 5-10) within their dendritic field - with one exception: the type 9 bipolar cell has a wide dendritic tree that selectively contacts S-opsin expressing cones. The S-cone selective or blue cone bipolar cells comprise only 1-2% of the bipolar cell population, and in the ventral mouse retina, where most cones express both L- and S-opsin, blue cone bipolar cells contact only those cones, which express S-opsin only. We suggest that these are the genuine blue cones of the mouse retina. The blue cone bipolar cells of the mouse retina are closely similar to the primate blue cone bipolar cells, and they most likely represent the phylogenetically ancient color system of the mammalian retina.

Literature:

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Haverkamp, Wässle, Duebel, Kuner, Augustine, Feng, Euler (2005) J Neurosci 25:5438-45.

# Dendritic processing in the direction-selective circuitry of the retina

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The visual system dedicates substantial resources to the detection of image motion and its direction. In the retina direction selectivity (DS) has been intensely studied for over 40 years. Nevertheless, the mechanism underlying the initial computation of the direction signal is still not fully understood. Direction-selective signals are first generated presynaptic to the DS ganglion cells in interneurons called starburst amacrine cells (SACs). Two-photon $Ca^{2+}$ imaging (*Denk & Detwiler, 1999, PNAS 96, 7035-40*) showed that SACs generate larger dendritic $Ca^{2+}$ signals for motion from their somata towards their dendritic tips (outward) than for motion in the opposite direction (inward) (*Euler et al., 2002, Nature 418:845-852*). This dendritic DS persists in the presence of GABA-receptor antagonists, suggesting that inhibitory network interactions are not essential.

To study the mechanisms underlying the computation of DS in SAC dendrites, electrical responses to expanding and contracting circular wave visual stimuli were measured via somatic whole-cell recordings in whole-mount rabbit retina and quantified using Fourier analysis. Fundamental and, especially, harmonic frequency components were larger in response to expanding (outward motion) than to contracting stimuli (inward motion). The presence of harmonics indicates non-linearity, which, as the relationship between harmonic amplitudes and holding potential indicates, might be due to the activation of voltage-gated channels. $[Ca^{2+}]$ changes in SAC dendrites evoked by voltage steps and monitored by two-photon microscopy suggest that the distal dendrite is tonically depolarized relative to the soma, due in part to resting currents mediated by tonic glutamatergic synaptic input, and that high-voltage $Ca^{2+}$-channels are active at rest.

Supported by compartmental modeling, we conclude that DS computation in SACs is a dendrite-autonomous process, relying on voltage-gated channels and a dendritic voltage gradient, which provides the spatial asymmetry necessary for direction discrimination.

# Genetic approach to study structure and function of the zebrafish retina

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The era of zebrafish research was launched in 1981 when George Streisinger published the seminal paper on the zebrafish as an animal model for developmental and genetic analyses. He already highlighted the main advantages of this animal model: small size, high fecundity, short lifecycle, and ease of care. First studies on zebrafish retina and vision emerged in the mid-eighties. Until today, we learned a lot about the adult and developing zebrafish retina and the completion of several 'large scale' mutagenesis screens provided us with a vast number of mutant strains with retinal defects. In addition to this forward genetic approach, reverse genetic approaches emerged during recent years and offer the possibility to generate gene knock-downs by injecting specific antisense-oligonucleotides into fertilized eggs. Both, zebrafish mutants and morphants open up the possibility to dissect genetic factors that influence structure and function of the vertebrate retina. Here we present two studies describing a forward and a reverse genetics approach, respectively.

*Forward genetics:* The zebrafish *mariner* mutant carries a mutation in the gene encoding for Myosin VIIa, a protein known to be involved in Usher syndrome 1B in human patients. We examined light and dark adapted mutant larvae of 3 different alleles at different developmental stages. Morphological and behavioral analyses show a defect in light/dark adaptation processes in these mutants. Therefore, we conclude that the primary defect in the visual system of Myosin VIIa mutants is due to a defect in light adaptation.

*Reverse genetics:* In humans, mutations in the nyctalopin (*nyx*) gene lead to congenital stationary night blindness (CSNB1), affecting synaptic transmission between both types of photoreceptors and ON-bipolar cells. We cloned the zebafish *nyx* ortholog and raised a polyclonal antibody to study both its expression pattern and protein localization. The protein was localized postynaptically in both synaptic layers, and morpholino injections revealed a defect in synaptic transmission of the ON-pathway and impaired contrast sensitivity in visual performance assays. Thus, our data show that nyctalopin plays an important role in retinal synapse function in both cone and rod pathway.

# A model for spontaneous, propagating waves in the developing vertebrate retina

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The immature retina spontaneously generates propagating waves of activity (retinal waves), which appear before the onset of normal vision and are thought to play a role in visual system development (Wong, 1999; Sernagor et al., 2001). Early retinal waves are caused by spontaneous activity in cholinergic amacrine cells, which form a coupled network that supports the propagation of wave-like events. In addition, a voltage-dependent potassium current in amacrine cells is thought to prevent runaway excitation and regulate wave properties (Zheng et al., 2006). A previous model based on these basic assumptions was able to reproduce the main features of retinal waves (Feller et al., 1997), but did not attempt to relate wave properties to biophysical cellular processes. In addition, it is very sensitive to parameter variations and produces an uneven retinal coverage (cf. Elliott and Shadbolt, 1999).

In this study, a biophysically plausible model of retinal waves is presented and compared to calcium imaging data from the embryonic turtle retina (*pseydemys scripta elegans*, Stages 22-23, provided by E. Sernagor, Newcastle University). The main assumptions of the model are:

1.Spontaneous activity is generated by small random depolarisations in amacrine cells (which not always initiate a propagating wave).
2.Amacrine cells are coupled by *weak* cholinergic synapses.
3.Due to low-threshold voltage gated calcium channels, cells are intrinsically highly excitable.
4.Depolarisation activates a long-lasting hyperpolarising potassium conductance, which reduces cellular excitability.
5.The decay time of the potassium conductance increases as a function of the level of depolarisation.

These mechanisms are consistent with recent data from the developing rabbit retina (Zheng et al., 2006) and allow the model to reproduce non-overlapping random waves with typical velocity, size and long-tailed inter-wave interval distributions. In particular, the level of spontaneous activity strongly self-influences wave properties: realistic waves are only generated only at an intermediate levels. At low levels, the cellular excitability is only little affected, and strong periodic oscillations appear. These become non-periodic and diverse and resemble typical retinal waves as the level of spontaneous activity increases, which leads to a concomitant decrease of excitability. Even higher levels of spontaneous activity lead to a disappearance of waves because cellular excitability is reduced below the threshold required for patterned activity. In conclusion, it appears that spontaneous activity in the developing retina may be specifically regulated into a parameter regime where they become irregular and non-periodic. It will therefore be interesting to study how regulation of waves and the gradual maturation of retinal circuits are associated during development.

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# The developing photoreceptor ribbon

Hanna Regus-Leidig[1,2], Dana Specht[1,2], Susanne tom Dieck[1,2] and Johann Helmut Brandstätter[1,2]

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The current concept of synapse assembly at conventional chemical synapses postulates "active zone precursor vesicles" that deliver active zone protein complexes to nascent synapses, leading to the rapid and efficient formation of the presynapse (1). A specialization of the cytomatrix at the active zone (CAZ) is the photoreceptor ribbon in the retina. It is an electron-dense plate-like organelle that extends from the site of transmitter release into the presynaptic terminal tethering a large number of synaptic vesicles. In our group we are interested in elucidating the way that ribbon synapses are assembled.

In the mouse retina immunofluorescent aggregates of the presynaptic proteins Bassoon, Piccolo, RIBEYE and RIM1 are present in the neuroblast layer very early in postnatal development. These proteins are transported in a complex along the growing photoreceptor axon to the future ribbon synaptic site. Ultrastructurally the aggregates appear as round, electron-dense matrix. These profiles themselves are non-membranous but surrounded by attached synaptic vesicle-like structures. Thus, they are clearly different from the 80 nm "precursor vesicles" at conventional synapses. 3D-reconstruction shows that most of these profiles are spheres with up to 200 nm in diameter. These "precursor spheres" accumulate around postnatal day 4 in the developing photoreceptor terminals, the time the first ribbons have been reported (2). With increasing age a continuous decrease in the number of precursor spheres is accompanied by an increase in the number of ribbons.

To investigate the specific contribution of the various presynaptic proteins to ribbon formation, we plan to knockdown every single one during synaptogenesis by RNA interference (RNAi). So far, the limiting factor in RNAi experiments in photoreceptors was the efficient transfer of the DNA and its stable and long term expression in the photoreceptors. Recently, we established a lentiviral transfection method in organotypic retinal culture which allows the efficient and stable integration of DNA into photoreceptors during development. Transfecting mouse retina at embryonic day 17 results in a high transfection rate with almost 60% of the transfected cells being photoreceptors. We are confident that RNAi knockdown experiments and subsequent immunocytochemical and biochemical analysis of the photoreceptors will enable us to unravel the assembly of the photoreceptor ribbon synapse.

(1) Zhai et al. 2001. Neuron 29:131-143.
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# Molecular aspects of cytomatrix dynamics at the photoreceptor ribbon synapse

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Ribbon synapses are specialized glutamatergic synapses present in sensory neurons such as retinal photoreceptors and auditory and vestibular hair cells. They are designed to dynamically modulate transmitter release in response to changes in stimulus intensity. Retinal photoreceptors transmit light signals over a dynamic range of several orders of magnitude in intensity, i.e. from star light to bright day light. The characteristic hallmark of photoreceptor ribbon synapses is an electron-dense presynaptic organelle that tethers arrays of synaptic vesicles at the site of transmitter release.

Dark-adapted rod photoreceptors usually contain a single large ribbon which is clearly visible as a horseshoe-shaped structure in the photoreceptor terminals already at the light microscopical level. During the light phase, ribbons are significantly smaller and, in addition, electron-dense material pinches off the ribbon resulting in club-shaped and spherical profiles surrounded by synaptic vesicles (1). As these activity-driven structural changes may alter synaptic function and thus, the release properties of the synapse, it is of fundamental interest to find out which of the proteins of the cytomatrix at the active zone (CAZ) undergo dynamic changes and which are stable.

We developed an in vitro assay applying EGTA (low calcium at the synapse) or a calcium-ionophore (high calcium at the synapse) to enrich club-shaped / spherical ribbon profiles and large, rod-like ribbon profiles, respectively. With light and electron microscopic immunocytochemistry we screened for proteins that belong to one of the two recently identified molecular compartments of the ribbon complex (2) to evaluate their contribution to ribbon dynamics. While the CAZ protein Bassoon and a calcium channel alpha1 subunit seem to be stable components of the ribbon complex other CAZ proteins, e.g. Piccolo, RIBEYE and KIF3A associate with club-shaped and spherical profiles under EGTA conditions and thus, undergo highly dynamic changes in their localization. In summary, the proteins of the two molecular compartments of the ribbon complex undergo dynamic changes independently, suggesting activity-driven fine-tuning of photoreceptor ribbon structure and function.

(1) Spiwocks-Becker et al., 2004 Eur J Neurosci 19:1559-1571
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# OFF midget bipolar cells of the primate retina express AMPA receptors.

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At least ten types of bipolar cells are present in the primate retina. Bipolar cells receive input from cones, which use glutamate as their neurotransmitter. Recent studies suggested that different types of OFF bipolar cells express specific types of ionotropic (AMPA or kainate) glutamate receptors (GluRs) at their contacts with cone pedicles. The differences in the kind of expressed GluR subtypes as well as in the site of GluR expression at the cone pedicle are thought to influence the physiological behaviour of OFF bipolar cells. However, the question of which GluR type is expressed by which type of OFF bipolar cell in primate retina is still open.

In this study, the expression of AMPA and kainate receptor subunits at the dendritic tips of flat (OFF) midget bipolar (FMB) cells was analyzed in the retina of the common marmoset, *Callithrix jacchus*. We used pre-embedding electron microscopy and double immunofluorescence with subunit specific antibodies. The FMB cells were labeled with antibodies against the carbohydrate epitope CD15. Cone pedicles were identified with peanut agglutinin or with antibodies to the short wavelength sensitive (S) cone opsin.

Immunoreactivity for the GluR1 subunit and for CD15 is preferentially located at triad-associated flat contacts. Furthermore, the large majority of GluR1 immunoreactive puncta is localized at the dendritic tips of FMB cells. These results suggest that FMB cells express the AMPA receptor subunit GluR1. In contrast, the kainate receptor subunit GluR5 is not colocalized with the dendritic tips of FMB cells, or with the GluR1 subunit. Immunoreactive puncta for the GluR1 subunit are only rarely associated with S-cone pedicles. This is consistent with our recent findings in marmoset retina that FMB cells do not contact S-cone pedicles. The expression of AMPA receptor (GluR2/3 and GluR4) and kainate receptor (GluR6/7) subunits is reduced at S-cone pedicles in comparison to medium/long wavelength sensitive cone pedicles.

# Altered retinal cone opsin expression in postnatally hypothyroid Pax8-deficient mice

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We studied the effects of postnatal hypothyroidism on retinal development and spatial patterning of cone opsin expression in Pax8-deficient mice. Pax8-/- mice, due to the absence of thyroid follicular cells, are incapable of synthesizing thyroxine/triiodothyronine and deplete thyroid hormones after cessation of maternal supply.

The retinae of Pax8-/-, Pax8+/-, and wild-type (WT) littermates were studied. Gross retinal anatomy was evaluated on toluidine blue-stained vertical semithin sections. Immunocytochemistry with retinal cell type-specific markers was used on frozen sections to examine the postnatal development of the major retinal neuron classes. The spatio-temporal expression of shortwave-sensitive (S) and middle-to longwave-sensitive (L) cone opsins was assessed from birth to postnatal day 21 on frozen sections and on isolated whole retinae using opsin antibodies. The level of S and L cone opsin expression was quantified using RT-PCR and Western blotting.

Pax8-/- mice showed no overt mutant phenotype in eye size, gross retinal anatomy, and in the time course of structural differentiation of retinal photoreceptors, horizontal cells, bipolars, amacrines, and ganglion cells. In contrast, WT dorso-ventral gradients of mouse S and L cone opsin expression were significantly altered in Pax8-/- mice. Cone opsin labeling identified upregulation of S opsin in all cones, concomitant with a reduction of L opsin expression throughout the retina. Altered S and L opsin transcript and protein levels in the mutant retinae were confirmed by semiquantitative RT-PCR and Western blotting, respectively. S and L cone opsin expression in heterozygous mice also differed from that in WT littermates, but to a lesser extent.

In conclusion, hypothyroid Pax8-/- mice show no apparent alterations in general retinal development. Our finding that cone opsin regulation is thyroid hormone dependent within the first three weeks postnatally is consistent with a ligand-dependent role of the thyroid receptor beta 2, which has previously been demonstrated to function in cone opsin spatial patterning by repressing S opsin and activating L opsin.

# Postsynaptic expression of a putative L-type calcium channel at photoreceptor ribbon synapses in the mouse retina

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L-type voltage dependent calcium channels (VDCCs) allow changes in membrane potential to control the rate of neurotransmitter release and thus play an important role in presynaptic transmission at retinal photoreceptor ribbon synapses. Different pore-forming alpha1-subunit isoforms, e.g. alpha1F, alpha1D, are responsible for the kinetics and amplitude of voltage-gated calcium influx at these synapses. Mutations in the human CACNA1F gene which codes for the alpha1F subunit of VDCCs, cause incomplete congenital stationary night blindness type-2.

Mouse models with loss-of-function mutations in the murine cacna1f gene (1,2) show a similar functional phenotype - impaired photoreceptor ribbon synaptic transmission - as described for loss-of-function mutations in the presynaptic cytomatrix protein Bassoon (3) and the presynaptic calcium channel interactor CaBP4 (4). Recently we could show that the localization of the presynaptic photoreceptor VDCC is altered in Bassoon mutant mice (5).

Here we provide evidence for the specific postsynaptic expression of an alpha1S-like epitope at photoreceptor ribbon synapses by immunocytochemistry at the light and electron microscopical level, complemented by RT-PCR and Western blotting experiments. ON bipolar cells express this putative L-type calcium channel subunit in their invaginating dendrites at rod and cone photoreceptor ribbon synapses. Alpha1S-like subunit labeling clearly differs from alpha1C subunit-labeling which is present along the entire bipolar cell. Furthermore, the alpha1S-like subunit and the metabotropic glutamate receptor 6 (mGluR6) are co-expressed in the ON bipolar cell dendritic tips. Surprisingly, at Bassoon deficient photoreceptor ribbon synapses both the presynaptic alpha1F subunit and the postsynaptic alpha1S-like subunit are highly reduced in their expression. This is in contrast to mGluR6 which is still present at Bassoon mutant photoreceptor synapses.

The distinct postsynaptic expression of a putative L-type calcium channel at photoreceptor ribbon synapses adds another level of sophistication in synaptic transmission at this most complex chemical synapse in the CNS. Given the possible involvement of different pre- and postsynaptic L-type calcium channels in photoreceptor to bipolar cell synaptic transmission, interpretation of older morphological and physiological data should be carefully re-examined in the face of dissecting pre- from postsynaptic effects.

Literature cited

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# Cone photoreceptors and ultraviolet vision in the flower bat Glossophaga soricina (Microchiroptera, Phyllostomidae)

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The echolocating microchiropteran bats are strongly nocturnal. Their eyes are commonly small and the retinae are heavily rod dominated. However, in a comparative study we could demonstrate that microchiroptera also possess significant cone populations and hence the basis for daylight vision and colour vision (Müller & Peichl, Invest. Ophthalmol. Vis. Sci. 46: E-Abstract 2259, 2005). The present study has analysed the cone types and cone densities, and assessed the spectral tuning of the shortwave sensitive (S) cone opsin, in the nocturnal flower bat Glossophaga soricina (Phyllostomidae). In behavioural experiments, G. soricina was able to detect ultraviolet stimuli in dark-adapted (rod-stimulating) conditions (Winter et al., Nature 425: 612-614, 2003). We were interested to see whether the cones could also confer UV sensitivity.

Adult animals came from our breeding colony. Eyes were enucleated directly post mortem and processed for opsin immunocytochemistry. In paraformaldehyde-fixed retinae, middle-to-long-wave sensitive (L) cones and S-cones were assessed using cone opsin specific antisera. Retinal cDNA was used to PCR-amplify and sequence the tuning-relevant part of the S opsin gene.

We find that Glossophaga soricina possesses L cones and S cones. Across the retina, cone densities range from 4000/mm² to 7000/mm². The L cones form a regionally varying majority of 60-80%, and the S cones a minority of 20-40% (relatively high compared to other mammals). G. soricina is identical to mouse in the amino acids that have been shown to tune the mouse S opsin to UV rather than blue.

The cones may be useful for visual orientation at dusk and dawn. The presence of two spectral cone types provides the basis for dichromatic colour vision, including the detection of UV light, in cone-stimulating light conditions. Opsin gene analysis indicated the presence of an L opsin and a UV-tuned S opsin also in the vespertilionid bat Myotis velifer (Wang et al., Mol. Biol. Evol. 21: 295-302, 2004). This is different from most eutherian mammals which have the S opsin tuned to blue. So far, UV sensitivity of the S cones has only been found among rodents and bats.

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# Glycogen phosphorylase immunoreactive amacrine cells in the macaque monkey retina

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Cholinergic amacrine cells are found in every vertebrate retina and are easily recognised by their distinct morphology. They have been called 'starburst' amacrine cells and occur as mirror symmetric pairs of cells. The OFF-type stratifies in the outer portion of the inner plexiform layer (IPL) and has its soma in the amacrine cell layer; the ON-type stratifies in the inner IPL and has its soma in the ganglion cell layer. Cholinergic amacrine cells are involved in the generation of direction selective light responses of certain ganglion cells. The coverage factor of their dendritic fields is extremely high: about 10 in primates and up to 70 in rabbits, and the dendrites cofasciculate with each other and with the dendritic branches of ON/OFF direction-selective ganglion cells and ON- and OFF parasol ganglion cells.

We now found an additional pair of mirror symmetric amacrine cell types in the macaque monkey retina which can be labelled with an antiserum against glycogen phosphorylase (glypho). These cells are similar to the cholinergic ones: the density is comparable, the dendrites costratify in the same layers and they are also GABAergic. The cells are good candidates to be involved in the generation of direction selectivity and/or the triggering of parasol ganglion cells and, therefore, we are interested in the synaptic circuitry of these cells.

We did double labelling experiments with bipolar and amacrine cell markers (CaB5, vGlut3) to get information about the pre- and postsynaptic partners, and synaptic markers were used to label ribbon synapses (CtBP2) and inhibitory synapses (bassoon). The calcium-binding protein CaB5 stains at least three types of bipolar cells in the primate retina (DB3, DB4 and rod bipolar cells), and our results showed that the glypho-OFF cells get input from DB3 and the glypho-ON cells from DB4 bipolar cells. The vesicular glutamate transporter vGluT3 stains a population of amacrine cells with dendrites that stratify in a broad band in the middle of the IPL, and we found that the vGluT3- and the glypho-immunoreactive amacrine cells are extensively associated to each other. So far it is not clear if the vGluT3 cells are glutamatergic and/or glycinergic neurons. The localisation of the corresponding transmitter receptors at these cells will help to answer this question.

# Individual variation in rod absorbance spectra correlated with opsin gene polymorphism in sand goby *(Pomatoschistus minutus)*

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In aquatic animals, evolutionary adaptation of rod spectral sensitivity to the ambient light is particularly necessary and striking (Lythgoe 1972, Bowmaker 1994, Yokoyama and Tada 2000). Many studies have focused on the spectral adaptation between different fish species (Hunt et al 1996; Hope et al 1997). However, little work has been done on evolutionary adaptation between separated populations. In studying this, our objective is to illuminate the constraints under which the evolutionary tuning of visual pigments works. We measured the rod absorbance spectra (λmax) of four sand goby populations (Baltic, Swedish, English, and Adriatic) by microspectrophotometry (MSP). After MSP, the rod opsins of individual fishes were sequenced to reveal potential amino acid substitutions. We detected differences in λmax values between populations (Jokela et al. 2003). The greatest λmax shift was between the Baltic (508.3 nm) and the Adriatic (503.0 nm) populations. The variation of the Baltic λmax values was significant (506-511 nm), but the spectral differences correlated well with the opsin gene substitutions. Fishes with 506-507 nm had "normal" F261 while redshifted fishes (510-511 nm) had F261F/Y. Baltic common gobies (515 nm) have also this substitution and it is known to redshift human cone spectra (Merbs and Nathans 1993). Evidently, the polymorphism of the Baltic sand goby population appears to indicate ambiguous selection pressures in the Baltic. This is also the first time that spectral and sequence differences are correlated in individual animals other than Primates.

# Müller cell gliosis in response to light-evoked photoreceptor apoptosis

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Apoptosis of photoreceptor cells occurs in important blinding diseases such as retinitis pigmentosa and age-related macula degeneration, as well as after exposure to excessive blue light. Whereas the mechanisms of light-induced photoreceptor cell degeneration are well investigated, much less is known regarding the response of retinal glial (Müller) cells to photoreceptor apoptosis. Therefore, we investigated the gliotic alterations of Müller cells in response to light-evoked retinal degeneration by immunocytochemistry, cell swelling experiments, and patch-clamp recording. One eye of pigmented rats was exposed to blue light (405 nm) for 30 min. Three days after light exposure, immunostaining of retinal tissue revealed an upregulation of glial fibrillary acidic protein, a marker of gliosis in Müller cells, especially in the transition zone between the injured and uninjured retinal areas. The expression of the glial water channel, aquaporin-4, was increased in the outer nuclear layer of the degenerating region. The immunostaining for the inwardly rectifying potassium channel, Kir4.1, was decreased and showed a uniform distribution in the injured retina, whereas this protein was concentrated around blood vessels and at the limiting membranes in control retinas. The alterations in retinal Kir4.1 staining corresponded well with a decrease in the amplitude of inward currents of isolated Müller cells (to ~20%; Fig. 1) which was associated with a positive shift of the membrane potential. Most of the Müller cells from the light-injured areas displayed fast transient (A-type) outward potassium currents, which were not observed in control cells. Moreover, we found that the osmotic cell volume regulation of Müller cells alters after light exposure. Application of a hypotonic solution to acutely isolated retinal slices had no effect on the size of Müller cell bodies in control tissues, whereas Müller cells in slices from light-damaged retinas displayed a swelling of their somata. The data indicate that alterations in the expression of potassium and water channels are parts of the Müller cell response to the ionic and osmotic imbalances in the outer retina caused by light-induced photoreceptor apoptosis.

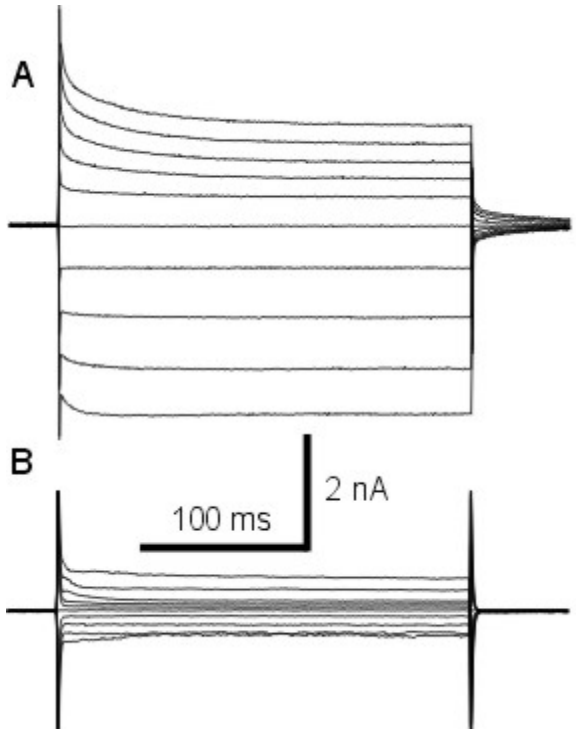


**Fig. 1.** The $K^+$ conductance of Müller cells decreases within 3 days after exposure to excessive blue light.
Examples of whole-cell currents recorded in a cell from a control retina (A) and from a light-exposed retina (B).
The currents were evoked by 20-mV incremental voltage steps between -160 and +20 mV, from a holding potential of -80 mV.

# Alterations in Müller glia cell physiology in a porcine model of retinal detachment

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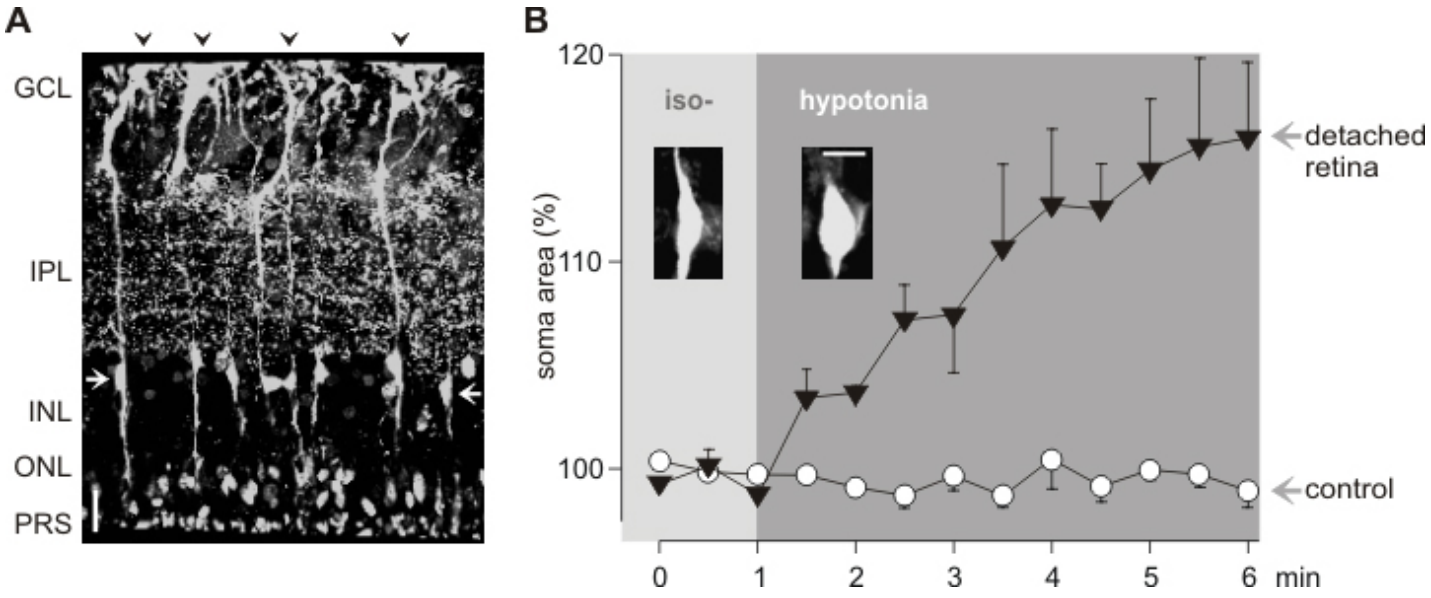
Retinal detachment (RD) causes degeneration of retinal neurons and glial cell reactivity. We used the porcine eye because of its similarities with the human eye to investigate the gliotic alterations of retinal Müller glial cells (MC) during RD by patch-clamp recording, cell swelling experiments, and immunohistochemistry. Seven days after RD, MC displayed a reactive gliosis as indicated by an increased expression of the filament proteins vimentin and glial fibrillary acidic protein. The K+ conductance was decreased in MC isolated from detached and attached areas of the operated eyes. Because RD may be associated with edema formation, we studied the osmotic swelling characteristics of MC evoked by hypotonic stress (Fig. 1). There was a significant negative correlation between the amplitude of the inward K+ currents and the extent of cellular swelling. We propose that altered water and ion transport through MC membranes impair volume regulation in the retina.

Fig.1: Osmotic swelling of MC in detached retinas. A: MC in a slice of a control retina were selectively stained with the fluorescent dye, Mitotracker Orange. Arrows mark MC somata in the inner nuclear layer (INL). Scale bar, 10 µm. B: Exposure to hypotonic solution caused swelling of MC somata in detached and attached retinal tissues of the operated eyes. MC of control retinas only swell in the presence of Ba2+ (1 mM). Original records of a MC soma in a slice of a detached retina are shown. Scale bar, 5 µm.



# Two faces of calcium activation after optic nerve trauma: life or death of retinal ganglion cells in vivo depends on calcium dynamics

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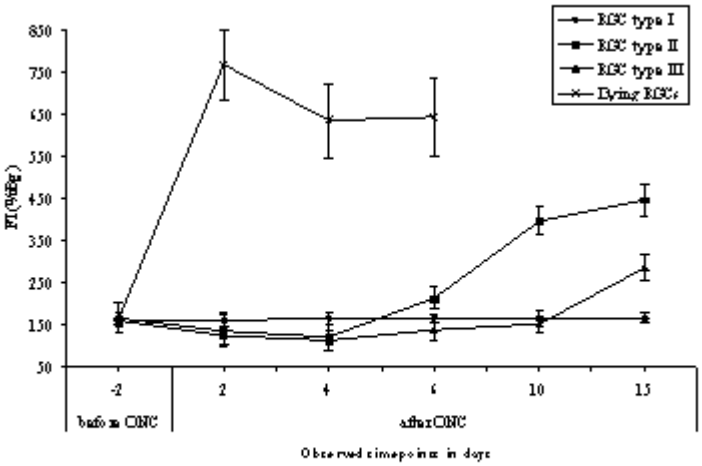
Calcium elevations after neurotrauma are not only implicated in cell death but were also shown to contribute to adaptive plasticity. We now wished to resolve this contradiction by following calcium dynamics after optic nerve crush in vivo. Methods: Adult rats received no injury (n=5), unilateral mild (n=10) or moderate optic nerve crush (n=10) (ONC), or axotomy (n=5). Before surgery, retinal ganglion cells (RGCs) were retrogradely labeled with Oregon Green BAPTA-dextran, a fluorescent calcium marker. Calcium-related fluorescence intensity (FI) was repeatedly measured in individual RGC in vivo using the ICON method. Results: Four different RGC types were found: Type I cells without FI change were found in sham rats and also in both ONC groups. Type II cells were found only after mild ONC, showing an initial calcium depression of 26% at day 4 followed by an increase of 169% 15 days after ONC. Type III RGCs were found only after moderate ONC and were also characterized by calcium hypoactivation followed by a slower return toward baseline and a delayed calcium increase of 72% above baseline. 60-65% of the RGCs in both ONC groups and all RGCs in the axotomy group died within 6 days following a fast and massive calcium increase of 287% with a concomitant 138% soma size increase. Conclusions: Whereas a rapid calcium elevation leads to cell death, an initial calcium depression followed by a delayed calcium hyperactivation predicts cell survival. We propose that hyperactivation of surviving cells contributes to recovery of electrophysiological activity and visual functions in deafferented brain regions.




**RGC fluorescence intensity modifications after ONC**

# Wavelength discrimination in goldfish: Inhibitory mechanisms in the retina

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**Purpose:** "Red-green"-wavelength discrimination in goldfish depends upon the ambient illumination level, GABAa-and D1-dopamine-receptor mediated actions within the retina (Neumeyer & Arnold, 1989; Wietsma & Spekreijse, 1991; Mora-Ferrer & Neumeyer, 1996). If retinal coding is important for wavelength discrimination and inhibitory mechanisms are involved, it is unclear how wavelengths in the range between 400-540 nm are encoded. To address this question behavioral wavelength experiments were performed in which either the glycine or the glycine and the GABAa-receptors were blocked. **Methods:** Goldfish wavelength discrimination ability was investigated using a two-choice forced procedure (Neumeyer, 1986) in three wavelength ranges: 404-470nm, 470-540 nm, and 540-660 nm. Animals were trained to approach a test field illuminated with light of a given wavelength (training stimulus) regardless of a second test field illuminated with light of a second wavelength (test stimulus). Once training was complete and animals exhibited a stable choice behavior, injections of either strychnine (glycine-receptor antagonist, calculated intra-ocular concentration: 1 mikroM) or of strychnine and bicuculline (GABAa-receptor-antagonist, 1/60-100 mikroM) was injected into both eyes of photopically adapted animals and wavelength discrimination in the respective wavelength ranges tested for maximally 105 minutes. **Results:** Three animals were tested pre- and post-injection of strychnine in the 404-660 nm range and four pre-/post-injection of strychnine/bicuculline in the 404-547 nm range. Blockade of glycine receptors had no significant effect on goldfish wavelength discrimination ability in the whole spectral range tested, i.e. 404-660 nm. For animals injected with strychnine and bicuculline, wavelength discrimination in the 404-450 nm range was not affected at all. In the 471-547 nm range, a significant reduction of the relative choice frequency for the training wavelength 495 nm was observed when presented with the test stimulus of 520 nm wavelength. However, the change in delta-lambda was maximally about 8 nm considering the range at threshold level determined by the size of the standard error of the mean post-injection. For test stimulus 471 nm choice frequency was reduced from 93 % to 77.5 % but in a non-significant manner. **Conclusions:** Goldfish wavelength discrimination exclusively depends upon a retinal GABAa-receptor mediated inhibitory process in the "red-green"-range. Neither a blockade of glycine- nor of glycine- and GABAa-receptors has any effect what so ever on wavelength discrimination in the "blue-violet"- to "blue-green"-range. This indicates that inhibition through either glycine- or glycine- and GABAa-receptor mediated mechanisms are not involved in the retinal "color" coding for the 404-540 nm range. This also indicates, that either other retinal color coding mechanisms exist than described for the "red-green"-range or that color coding for the 404-540 nm range does not occur in the retina.

# EFFECT OF GLYCINE- AND $GABA_a$-RECEPTOR BLOCKADE ON GOLDFISH FLICKER PERCEPTION

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**Introduction:** Inhibitory mechanisms in the inner retina are essential for high frequency coding in the goldfish retina. Mainly GABAergic amacrine cells form so-called "nested feedback" loops which supposedly determine the bipolar cell to ganglion cell temporal transfer properties (Marc & Liu, 2000). Although there are only few glycinergic synapses described for these nested feedback loops, a glycinergic contribution to the temporal transfer properties can not be excluded. To examine whether $GABA_a$- and glycine- receptors interact in shaping temporal transfer properties, a behavorial two-alternative forced-choice procedure was used. **Methods:** Photopic temporal resolution of goldfish was investigated with a behavorial, two-alternative forced-choice procedure (Mora-Ferrer & Gangluff, 2002). Training stimulus was a steady white light (50-100 $cd/m^2$) and the test stimulus was a white light which flicker frequency was varied in 5 Hz steps in the 10-50 Hz range. Temporal resolution was measured pre- and post-injection of strychnine (glycine-receptor antagonist, calculated intra-ocular concentration 1 µM) and bicuculline ($GABA_a$-receptor antagonist, calculated intra-ocular concentration 100 µM), dissolved in 5 mM HEPES-buffer, into both eyes. **Results:** Relative choice frequencies for flicker frequencies 10-40 Hz were above the defined behavioral threshold, i.e. 75 % correct choices, and in the 90 % range for flicker frequencies between 10-30 Hz. For flicker frequencies 45 and 50 Hz, animals were no longer able to discriminate between the training and the test stimulus, the relative choice frequencies were below 70 %. A total of seven injection experiments with four fish were performed. The relative choice frequencies for flicker frequencies 15-30 Hz were decreased by 7, 14, 18, and 26 %, respectively. Assuming that the flicker fusion frequency can be derived from the intersection between the connecting line between data points and the threshold value, a 15 Hz reduction of the flicker fusion frequency is induced by strychnine and bicuculline. **Discussion:** The observed changes in temporal resolution are not due to a motor impairment by strychnine as animals performed equally fast pre- and post-injection and exhibited no unusual behavior. The reported impairment of temporal resolution in the high frequency range due to glycine- and $GABA_a$-receptor blockade is similar to the effect of $GABA_a$-receptor blockade alone (Mora-Ferrer & Behrend, 2004). The retinal temporal transfer properties post-injection of bicuculline alone and of bicuculline and strychnine, measured in the ERG, are very similar. Both, bicuculline alone and the combination of strychnine with bicuculline induced a nearly identical continuous decrease of the temporal resolution ability for flicker frequencies below the flicker fusion frequency. This indicates, that in the "nested-feedback"-mechanism of the inner retina (Marc & Liu, 2000), which shapes the retinal temporal transfer properties, the reported glycinergic synapses do not contribute to the temporal coding properties of the retina.

# Retinal expression and integration of the USH1G protein SANS in the USH protein interactom

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Human Usher syndrome (USH) is the most common form of combined deaf-blindness. USH is divided in three clinical distinct types USH1 to USH3. USH1 comprises 6 genetical distinct subtypes (USH1B-G) and is the most severe form. In general USH1 is characterized by profound congenital deafness, constant vestibular dysfunction and prepubertal onset of retinitis pigmentosa. The USH1G gene encodes the scaffold protein SANS (scaffold protein containing ankyrin repeats and SAM domain) which contains several functional domains capable of protein-protein interaction.

In the present study, SANS expression and subcellular localization was analyzed in the retina. For this purpose, anti-SANS antibodies were generated and the specificity of the affinity purified antibodies was evaluated. Immunocytochemistry revealed SANS expression in the outer plexiform layer and in photoreceptor cells. In photoreceptor cells, SANS was localized in the apical inner segment at the ciliary apparatus. The subcellular distribution of SANS was confirmed by Western blot analysis of tangential sections and extended by immunoelectron microscopy, that displayed SANS localization also at the periciliary region. Destabilization of microtubules by thiabendazole interfered with the cellular localization of SANS indicating an association of SANS to the microtubule cytoskeleton. Yeast-two-hybrid screens revealed the PDZ-protein whirlin as a novel binding partner of SANS. SANS-whirlin interaction was validated by co-transfection experiments, immunocytochemistry and immunoelectron microscopy.

The USH1G protein SANS is an integrative component of the USH interactome. Our results support an integrative function of the USH1G protein SANS within the USH protein network in retinal photoreceptors. This study further provides first evidence for a link of the USH interactome to the microtubule cytoskeleton. Defects of one component of the interactome may lead to dysfunction of the entire USH network and cause sensory degeneration.

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# The pharmacological profile of the contrast-dependent optomotor response in Goldfish

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**Purpose:** The retinal motion coding depends upon direction-selective ganglion cells. The pharmacological profile of the behavioural response to full-field motion (optomotor response), has been determined (Mora-Ferrer et al., 2005). Using this profile, the contrast-dependency of the retinal direction-coding mechanism (Hassenstein and Reichhardt, 1956), and the contribution of different retinal transmitter mechanisms were investigated in a behavioral- neuropharmacological experiment.

**Method:** The photopic optomotor response was elicited with pattern cylinders with sine-wave modulated contrasts (10, 20, 40, 60, and 80%) of fixed spatial frequency (4 cm/cycle), pattern velocity (10 RPM), and luminance. The experimental procedure was similar to the one reported by Mora-Ferrer and Gangluff (2000). Drugs (strychnine 1 µM, bicuculline 10 µM, curare 10 µM, AP7 30-60 µM, CGS-19755 1,5 µM, TBOA 1 and 10 µM) were injected into the vitreous of both eyes. Curare and bicuculline were used at half-maximal doses (Mora-Ferrer et al., 2005).

**Results:** Averaged and normalized pre-injection data show on a log contrast scale a near linear reduction of the optomotor response for decreasing contrasts to about 20% relative response for 10% pattern contrast. Threshold criterion was defined as half-maximal response and fullfilled pre-injection at 20% pattern contrast. GABAa- and nicotinic acetylcholine-receptor antagonists (bicuculline, curare) reduced the contrast-dependent optomotor response by approx. 30 to 50% for contrasts in the 20-80% range. Strychnine, a glycine-receptor antagonist, induced a significantly increased relative optomotor response for 20% pattern contrast and reduced the threshold contrast to approx. 13%. In contrast, blocking GABAa- and glycine-receptors simultaneously reduced the optomotor response significantly to about 35% of the pre-injection response for 80% pattern contrast, i.e. based on the threshold definition animals were no longer able to exhibit an optomotor response. TBOA, an excitatory amino acid transporter antagonist, reduced the contrast-dependent optomotor response to 0-10% relative response for 10 and 20% pattern contrast. For higher contrasts, the results were similar to the results obtained with curare and bicuculline. Blockade of NDMA-receptors with either CGS 19755 or AP7 had no effect.

**Conclusions:** Both blockade of nicotinic acetylcholine and GABAa-receptors induced a uniform reduction of the optomotor response irrespectively of the pattern contrast. The lack of effect of NMDA-receptor blockade is in accordance with electrophysiological studies. Blocking excitatory amino acid transporters impairs the contrast-dependent full-field motion detection. This indicates an important contribution of the ON-channel to the coding of full-field motion. A strychnine-induced increase of contrast sensitivity suggests an involvement of glycinergic mechanisms in either directional and/or contrast coding. Surprisingly, blocking both GABAa- and glycine-receptors reduced the full-field motion perception to a level below the defined threshold even at 80% pattern contrast. The GABAergic and glycinergic contribution to full-field motion coding does not appear to be additive, as in this case an alleviation of the bicuculline effect should be expected.

# GOLDFISH FULL-FIELD MOTION PERCEPTION IS ELIMINATED AFTER BLOCKADE OF THE ROD ON-CHANNEL UNDER SCOTOPIC ILLUMINATION CONDITIONS AND IMPAIRED UNDER MESOPIC ILLUMINATION CONDITIONS

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**Purpose**: Retinal directional coding is essential for full-field motion perception. We were interested in the contribution of the rod-driven ON-signal in directional coding under either scotopic and mesopic illumination conditions. The contribution of a retinal rod-driven ON-signal to full-field motion perception was investigated before and after the intra-ocular injection of APB, an agonist of class III mGlu-receptors. One member of this mGlu-receptor family reportedly mediates rod-ON responses in both rod and Mb-ON-bipolar cells. **Methods**: The optomotor response was elicited with a pattern cylinder with sine wave modulated contrast pattern of fixed contrast (80%) and spatial frequency (4 cm/cycle) and which was rotated at 10 RPM (Schmidt-Hoffmann & Mora-Ferrer 2006). The optomotor response was measured under scotopic or mesopic adaptation conditions (1.5 lux, white light). Light of two different wavelengths, 540 nm and 640 nm, and light intensities in the range of 0.03 - 30 % were used to elicit the optomotor response. To impair rod-driven ON-biploar cells, APB (calculated intra-ocular concentration 100 microM) was injected into both eyes and the optomotor response measured again. **Results**: Scotopic illumination conditions: For increasing test light intensities of either wavelength used the optomotor response increased. Light of 540 nm wavelength was more effective in eliciting the induced motor response both in absolute action and sensitivity. Animals sensitivity was about 0.9 log units higher for light of 540 nm. Tonic activation of mGluR6, post-injection of APB, reduced the absolute response to values smaller responses elicited with the lowest test light intensities pre-injection. Mesopic illumination conditions: Animals can be grouped into two different groups based on their pre-injection optomotor response behavior, a group of two animals which were more sensitive to light of 640 nm and a group of three animals which were nearly equally sensitive to light of either 540 nm and 640 nm. Post-injection of APB, both groups exhibited very similar optomotor responses, increasing from a relative optmotor response of approx. 0 to maximally 40 %. **Conclusions**: Under scotopic illumination conditions, tonic activation of retinal class III mGlu-receptors eliminates full-field motion perception. This is in agreement with a rod-mediated input into ON-direction-selective mechanisms. An OFF-mechanism does not seem to be involved in full-field motion coding. Under mesopic illumination condition the animals responses vary much more than under scotopic conditions. Irrespectively of this variability optomotor responses post-injection of APB were very similar. This indicates that rods do provide an input into a directional coding mechanism. The cone contribution is relatively small and increases with increasing test light intensities. It does seem that a rod-driven ON-mechanism is mainly responsible for the coding of full-field motion. Whether cone ON-direction-selective ganglion cells are involved in mesopic full-field motion coding is under investigation. More experiments are necessary to investigate the relative contribution of rods and cones to mesopic directional coding in more detail.

# APPLICATION OF ATR-FTIR MICROSPECTROSCOPY TO THE INVESTIGATION OF THE PHOTOTRANSDUCTION PROCESS

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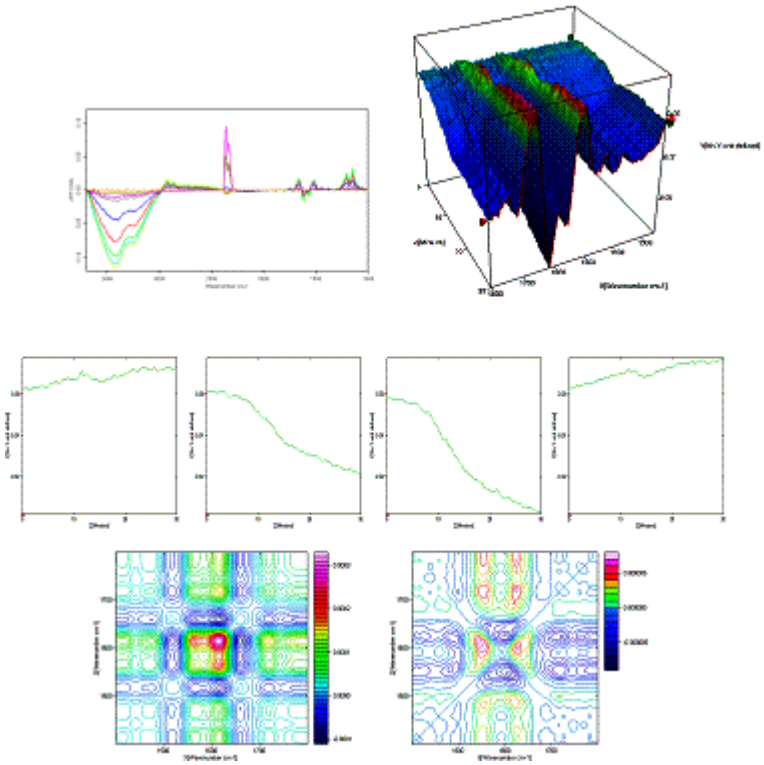
Fourier Transform Infrared (FT-IR) microspectroscopy in the Attenuated Total Reflectance (ATR) configuration has been applied to study the photoresponse of ex vivo retinas of *Bufo regularis*. This has been done to gain new insight into the molecular aspect of phototransduction cascade. We verified the possibility of acquiring accurate IR absorption spectra optimising existing instrumentation for this purpose. Then we used time-resolved FT-IR difference spectroscopy to measure changes in the visual proteins, occurring because of light exposure with technique: these changes give rise to difference spectra with a series of positive (at 1516 and 1660 cm-1) and negative peaks (1616 and 1564 cm-1)

Using 2D correlation analysis we demonstrated that some of these signals can be assigned to the variation of concentration of protein components over a time scale of tens of minutes. We can make a tentative assignment of specific proteins by considering their spectroscopic features, and they correspond to β-sheet proteins. We tentatively assign these changes to the most abundant proteins in ROS, transducin and arrestin, which are rich in β-sheets.

Previous works support this assignment: immunofluorescence studies showed that the onset of light results in a simultaneous translocation of these proteins to the opposite rod segment, proposing that these changes are due to the adaptive response of cells to intense illumination.

Overall we have shown that the use of FT-IR microspectroscopy in ATR configuration is extremely promising in providing information on changes occurring in retinal tissue as a consequence of photoexcitation. Retinas could be studied ex vivo without any particular pretreatments or modification.

*Difference ATR spectra, temporal evolution of some Amide I, II bands, synchronous and asynchronous correlation analyses*



# Expression of erg1 potassium channels in horizontal cell bodies but not axon terminals of the mouse retina

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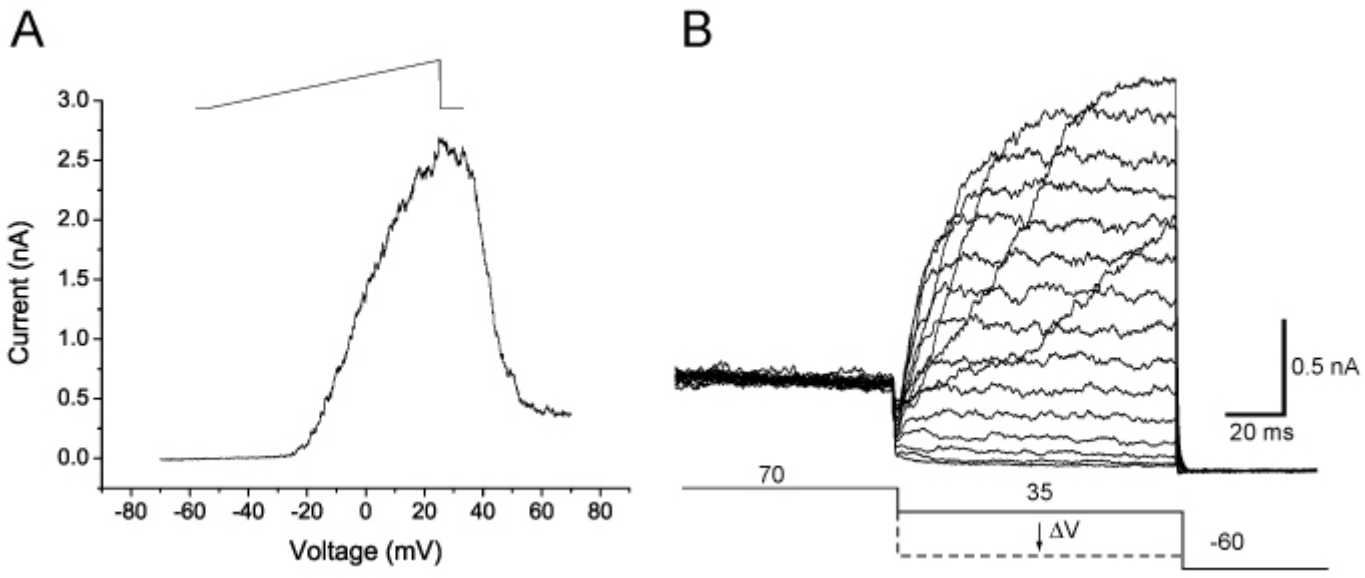
Inward rectifier $K^+$ channels of the Kir family and *ether-à-go-go*-related gene isoform 1 (erg1) $K^+$ channels were simultaneously expressed in isolated horizontal cells of the mouse retina. Whereas inward rectifier channels were present in both cell bodies and axon terminals, expression of erg1 was confined to cell bodies and primary dendrites. By patch-clamp whole-cell recordings we could identify erg1-mediated currents as voltage-dependent outward $K^+$ currents, characterized by a negative current-voltage relation at positive membrane potentials (Fig.1A). Erg channels did not contribute significantly to inward currents, which were completely blocked by extracellular application of the canonical Kir inhibitors cesium and barium, but not by selective inhibitors of erg channels. In contrast, outward currents were significantly reduced by E-4031 (45%), rBeKm-1 (25%), and haloperidol (73%). Half-maximal activation of the haloperidol-sensitive current occurred at 3.2 ± 2.1 mV, which was very similar to the value obtained with a hyperpolarizing tail current protocol (4.2 ± 1.6 mV). Inactivation was steeply voltage dependent at a half-maximal potential of 44.4 mV. Erg channels have the unique property of C-type inactivation, resulting in increasingly delayed onset kinetics of the current response (Fig. 1B). The gating properties of erg1 channels were affected by voltage- and time-dependent components. Current-clamp experiments revealed the presence of oscillatory potentials induced by injection of positive currents. As determined by fast Fourier transformations, these oscillations were centered at around 100 Hz, and they were entirely abolished by haloperidol and by large depolarizing current steps (>800 pA). Finally, horizontal cell bodies showed a bimodal voltage response, with open erg channels clamping the membrane potential change to 25.5 ± 3.0 mV. Inactivation of erg channels by large depolarizing currents led to a sharp switch in the voltage response to 141.8 ± 4.8 mV. Our results suggest a cone pathway-specific modulation by erg channels of the glutamate-induced voltage response of horizontal cells.

Supported by the Deutsche Forschungsgemeinschaft (WE 849/14-1,2).



# Localization of connexin57 in dendro-dendritic and axo-axonal gap junctions of mouse horizontal cells

Konrad Schultz[1], Ulrike Janssen-Bienhold[1], Petra Dirks[1], Jennifer Shelley[1], Gerrit Hilgen[1], Sonja Hombach[2], Klaus Willecke[2] and Reto Weiler[1]

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Horizontal cells (HCs) are second order neurons located in the outer nuclear layer of the retina. These neurons are extensively coupled by gap junctions, resulting in large receptive fields. The coupled network of HCs feeds back negatively onto the photoreceptors, contributing to the antagonistic center-surround organization of bipolar cell receptive fields. Using a Cx57-deficient mouse line, Hombach et al. (2004) demonstrated that connexin57 (Cx57) is expressed exclusively by HCs in the mouse retina. In these animals, the coding region of the Cx57 gene was replaced by a lacZ reporter gene expressed under the control of the endogenous Cx57 promoter. Deletion of Cx57 strongly reduced HC coupling (Hombach et al., 2004) and receptive field size; however, deletion did not eliminate negative feedback (Shelley et al., 2006). In order to study the distribution of Cx57 within mouse horizontal cells, we used polyclonal antibodies generated in rabbit against a 15 amino acid peptide sequence of the C-terminal end of mouse Cx57.

Specificity of the antibodies was tested in two experiments. On Western blots, the antibodies recognized a single ~57 kDa protein in total homogenate and membrane fraction samples of mouse retina. This pattern of Cx57 immunoreactivity was not detectable in control experiments in which the blot was incubated either with Cx57 antibodies preadsorbed with the immunization peptide, or with the pre-immune serum. Next, we excluded cross-reaction with other connexins by comparing the Cx57 immunostaining in vertical cryosections of retinae from wild-type and Cx57-deficient mice. Strong immunostaining was detected in the outer plexiform layer and distal inner nuclear layer of the wild-type mouse retina. This pattern of Cx57 immunoreactivity was absent in homozygous Cx57-deficient mice and significantly reduced in heterozygous animals.

Next we examined the cellular distribution of Cx57 in mouse HCs. Strong punctate Cx57 immunoreactivity was detected exclusively on the dendrites and fine axon terminal arborizations of dissociated HCs. A more detailed analysis at the electron microscopic level revealed strong labeling of HC terminals within cone pedicles and rod spherules of wild-type mice, as well as some immunoreactive processes in the outer plexiform layer. Cx57-deficient mice did not show this intense labeling pattern; a low level of immunoreactivity was detected in a small number of HC terminals in rod spherules. No immunoreactivity was detected in control retinas which lacked the primary antibody.

These data support previous studies which showed that Cx57 is the main connexin involved in the formation of both dendro-dendritic and axo-axonal gap junctions between mouse HCs (Hombach et al., 2004; Shelley et al., 2006). Whether Cx57 forms hemichannels mediating negative feedback at the HC-photoreceptor synapse needs to be clarified in a more detailed study.

Supported by the Deutsche Forschungsgemeinschaft (WE 849/14-1,2 & JA 854/1-1,2).

# Rod and cone contributions to horizontal cell responses in the mouse retina

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Interaction between the rod and cone pathways has been shown to be quite extensive. Rods and cones are electrically coupled, and rod signals are funneled into the cone pathway at several stages in visual processing. Mouse horizontal cells make contacts with both types of photoreceptors: the dendrites contact cone photoreceptors, while the axon terminals contact rods. In this study we examined the contributions of rod and cone photoreceptor inputs to horizontal cell light responses in the mouse retina. Since mouse rod and cone signals cannot be easily differentiated based on wavelength, we used an alternative approach, taking advantage of the availability of genetically modified mouse lines: rod signals were isolated by recording intracellularly from horizontal cells in the CNGA3 knock-out mouse, which lacks cone function (Biel et al., 1999), and cone signals were assessed using the rhodopsin knock-out mouse, which is a model for pure cone function (Humphries et al., 1997; Jaissle et al., 2001). These responses were compared to the responses of wild-type horizontal cells: we found that wild-type horizontal cell somata receive a mixture of rod and cone inputs.

The contributions of rod and cone signals to horizontal cell light responses could be distinguished in the wild-type mouse by altering the adaptational state of the retina: in the dark-adapted retina, rod contributions were more pronounced, whereas light adaptation facilitated cone contributions. Amplitude-intensity functions were shifted towards higher intensities under light adaptation, indicating facilitation of the cone component and suppression of the rod component. All effects seen following light adaptation could be reversed by subsequently dark adapting the retina.

Second-order neurons have been shown to form ectopic synapses in the outer nuclear layer in retinas with photoreceptor deficiencies (for example Jaissle et al., 2001). The morphology of the rhodopsin-deficient retina is normal until postnatal week 7 (Jaissle et al., 2001). We used immunohistochemistry and intracellular dye injection to examine the morphology of the horizontal cells in the CNGA3-deficient retina.

We conclude that horizontal cells receive a mixture of rod and cone signals, which they presumably feed back to both photoreceptor types in order to adjust photoreceptor sensitivity to changing levels of ambient illumination. Whether rod inputs reach the horizontal cell soma via the axon terminal or by way of rod-cone coupling will be investigated by assessing the rod contribution to horizontal cell somatic light responses in mice lacking rod-cone coupling.

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# Turtle retinal ganglion cells encode stimulus speed, direction and acceleration

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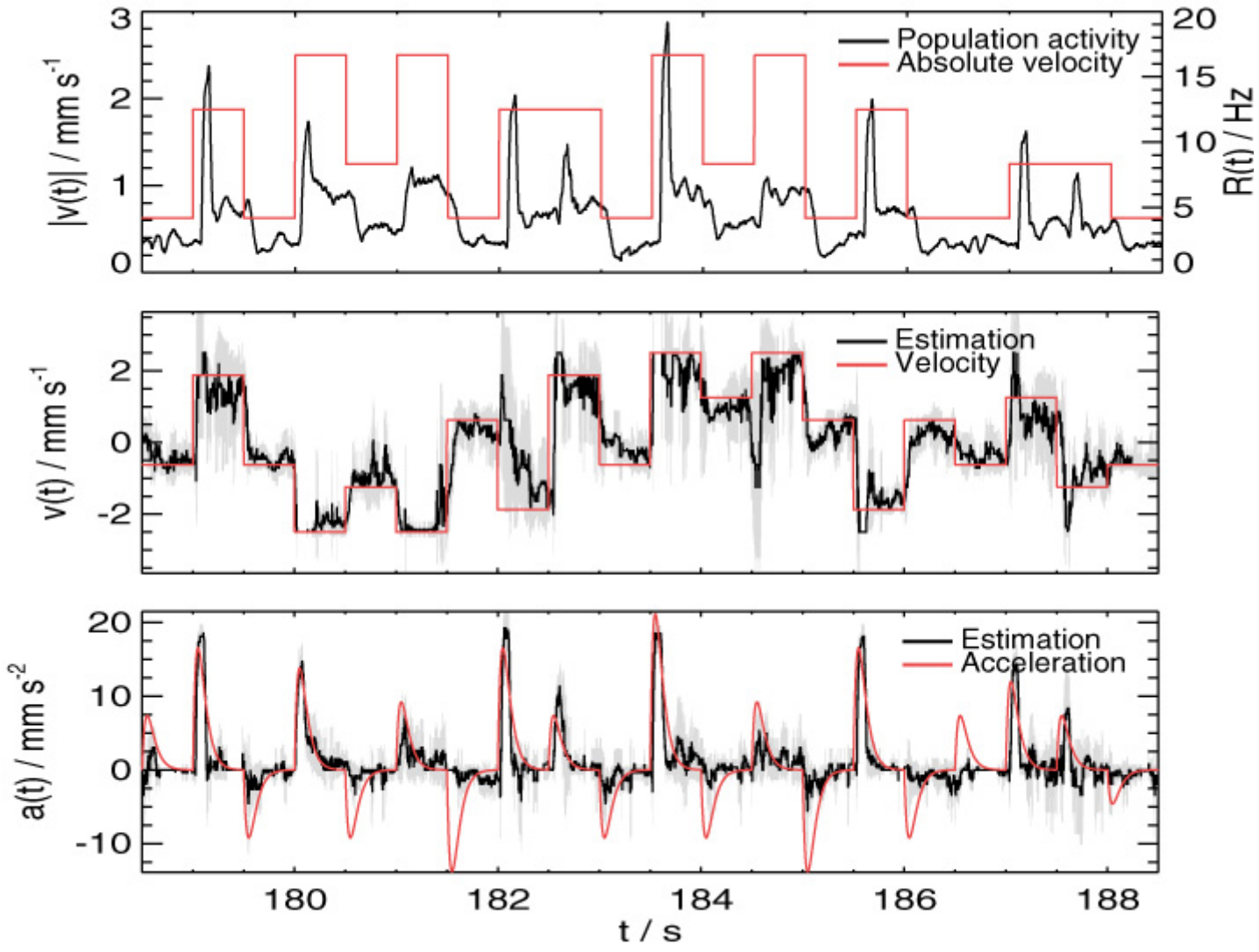
Neurons responding selectively to movement direction have been found throughout the visual systems of numerous species. For instance, in rabbit and turtle, already certain retinal ganglion cells (RGCs) encode motion direction.
We recorded from a population of turtle RGCs with a multi-electrode array, while visually stimulating the retina by a moving dot pattern performing abrupt changes in speed and direction (Fig. top). Bayesian stimulus reconstruction yields that although direction sensitive cells encode stimulus speed as well, velocity estimation is significantly improved by including RGC responses that are speed-tuned but not direction sensitive (Fig. middle).
Immediately after velocity switches, RGC activity displays transients seemingly depending on the difference between previous and new velocity (Fig. top). We confirmed that RGC responses are indeed acceleration sensitive by successfully reconstructing the acceleration time course from the cells' spike trains, again using Bayesian inference (Fig. bottom). Surprisingly, only the combination of velocity and acceleration could be estimated satisfactorily, indicating that RGCs in our ensemble signal both stimulus properties simultaneously. Intuitively, a maximum firing rate indicates fast motion but at the same time sharp speed increases. Thus, in the turtle, major features of visual motion, namely direction, speed and acceleration, are already encoded in RGC responses.



# Retinal gene expression in mice and chicks with visual experience that induces refractive errors.

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**Purpose:**
To identify retinal genes that are involved in the development of myopia (induced by covering the eye with diffusers) and hyperopia (induced by myopic defocus) in mice and chickens.

**Methods:**
Mice: Male C57/BL6 mice (aged 30 days) were treated with a diffuser over one eye and a clear neutral density filter with matched light attenuation of 0.3 log units over the other eye, for either 30 minutes or 4 hours (n = 3 for each group). Labelled cRNA was prepared from the 12 retinas and hybridised to Affymetrix GeneChip® Mouse Genome 430 2.0 arrays (> 39000 characterised genes).
Chicks: Male white leghorn chicks (aged 10 days) were bilaterally treated with +7D lenses for 24 hours, untreated chicks served as controls (n = 4 for each group). Labelled cRNA was prepared fom 8 mm retinal punches (both eyes of one chick were pooled) and hybridised to Affymetrix GeneChip® Chicken Genome array (> 28000 characterised genes).
Candidates with potential relevance are further examined by semi-quantitative real-time RT-PCR in both animal models.

**Results:**
Mice: After 30 minutes of diffuser treatment, 151 genes were down-regulated and 40 up-regulated, compared to the ND-filter treated eyes. After 4 hours, only 27 genes showed a changed regulation, with most of them being down-regulated. Among others, the down-regulation of the transcription factors Egr-1 and fos after both 30 minutes and 4 hours were confirmed with real-time RT PCR.
Chicks: Experiments are still underway.

**Conclusions:**
Degradation of the retinal image with diffusers has previously been shown to produce myopia in both animal models and humans, and treatment with positive lenses produced eye growth inhibition and hyperopia in various animal models. Genes that are regulated by these treatments are potential molecular targets for a pharmacological intervention of myopia. Furthermore, identification of these genes leads to a better understanding of the molecular mechanisms of emmetropization.

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# Visual pigment regeneration mechanisms in the *zebrafish*

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Visual transduction in vertebrates starts within photoreceptor (rods, cones) outer segments. Absorption of a photon by the visual pigment (rhodopsin, cone opsin) leads to a *cis-trans* isomerization of the chromophore 11-*cis*-retinal to all-*trans*-retinal. In order to restore vision, all-*trans*-retinal has to be recycled back to 11-*cis*-retinal in a process called "visual cycle".

Studies on the kinetics and metabolism of the rod visual cycle have shown that rods are dependent on a pool of all-*trans*-retinylesters in the RPE (retinal pigment epithelium) for regenerating their pigment. However, the existence of a second pool of retinylesters not in the RPE, but rather the retina itself, plus the finding that Müller glia cells in culture are able to isomerize retinol in chicken strongly suggest the existence of a second, cone-specific, mechanism of visual pigment recycling.

The cellular retinal binding protein Cralbp is part of the enzyme complex responsible for the *cis-trans* isomerization of the chromophore. We have cloned two orthologues, denoted as *cralbp-a* and *-b*, in the cone-dominant zebrafish. While Cralbp-a is exclusively expressed in Müller glia cells, Cralbp-b expression is restricted to the RPE (retinal pigment epithelium). In order to analyze the role of both isoforms in retinoid metabolism, we have performed a loss of function study using an antisense morpholino approach. The effect of downregulation of either protein on visual function was assessed by electrophysiology (ERG), behaviour (OKR) and biochemical (HPLC) analysis.

# Zebrafish Model for Human Usher Syndrome Shows Light Adaptation Defects and Retinal Degeneration

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**Purpose**

Mutations in *myosin VIIA* gene cause Usher syndrome 1B. Affected patients suffer from deafness, vestibular dysfunction, and blindness. In human, Myosin VIIa is expressed in inner hair cells, photoreceptors, and in the retinal pigment epithelium. Here, we characterize the retinal phenotype of 3 different myosin VIIa alleles in the mutant zebrafish *mariner*.

**Methods**

We examined light and dark adapted mutant larvae of three different alleles. The morphology of dark / light adapted retinas was assessed by standard histology and electron microscopy. Immunocytochemistry was used to identify subpopulations of retinal neurons and to localize Myosin VIIa in the zebrafish retina. Furthermore, the optokinetic response was used to analyze dark adaptation defects on behavioural level.

**Results**

The recovery of the optokinetic response is prolonged after dark adaptation in the mutants compared to siblings. Morphological and morphometrical analyses show that pigment migration during adaptation is impaired in all 3 *mariner* mutants. Immunocytochemical studies show only minor differences in the localization of synaptic proteins and receptors in wt and mutant larvae. Immunostaining for Myosin VIIa labels the photoreceptor cilia between the inner and outer segment and the part of pigment epithelial cells ensheathing the cone outer segments. Additionally a faint Myosin VIIa labeling can be observed in the cone pedicles. Preliminary electron microscopical analysis elucidates morphological alterations in the outer retina that contributes to the *mariner* phenotype. Furthermore, light damage experiments will reveal consequences of maladaptation.

**Conclusions**

These data are consistent with the hypothesis that the primary defect in the visual system of Myosin VIIa mutants is due to a defect in light adaptation. Thus, we suggest that the slow progressive blindness in human Usher disease might be caused by light damage due to impaired light adaptation.

# Glutamate transport in the zebrafish retina

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A tight control of extracellular glutamate concentration is important for accurate function of glutamatergic synapses. Crucially involved in this process of regulation are glutamate transporters that remove glutamate from the extracellular space. Since this transport is electrogenic, both by the active transport itself and possibly by a concomitant chloride conductance, glutamate transporters have been suggested as direct players in synaptic transmission. Physiological evidence for such a role stems mainly from studies of the teleost outer retina.

Five glutamate transporters are currently known; one of them is expressed primarily in the retina. Its expression pattern in the zebrafish retina makes this gene a good candidate to investigate the synaptic function of glutamate transporters in vivo. Cloning and expression studies of this gene as a prerequisite for its functional analysis are presented.

# The Zebrafish Mutant *noir* is a Model for Inner Retinal Dystrophies

Colette Michèle Maurer[1], Helia Berrit Schönthaler[2], Yuri Makhankov[1] and Stephan CF. Neuhauss[1]

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The zebrafish mutant *noir (nir)* was identified in a large-scale mutagenesis screen due to its dark external appearance. This phenotype results from an absence of background adaptation, an indication of lack of light sensing. Electroretinography of 5 day old *nir* larvae indicates a block in signal transduction between photoreceptor cells and second order neurons in the retina. Histological analysis of the retina of 5-7 day old *nir* larvae reveals a cell loss in the inner nuclear layer starting at day 6 and degeneration of the whole retina at day 7. As shown by Electron microscopy the synapse does not seem to be affected by the mutation. Immunohistochemical staining of dopaminergic amacrine cells and müller glia cells does not show differences between mutant and wildtype retinas. The cell type which is affected remains to be determined. To locate the mutation on the zebrafish genome a linkage analysis with simple sequence length polymorphisms (SSLP) was performed. Fine mapping and chromosomal walking narrowed the genomic interval to a region of 1.3 cM on chromosome 22. Single nucleotide polymorphisms (SNPs) in recombinant larvae confirmed the presence of the mutation in this interval. Currently, candidate genes of this region are cloned and their expression pattern is analyzed by *in situ* hybridization. Genes like Glutaminase (converts Glutamine into Glutamate) and TBC1 family domain member 20, encoding a GTPase activating protein are very good candidates.

# Symposium

## S5: Cannabinoids and the Nervous System: Different Views on Multiple Actions

***Dirk Czesnik, Goettingen***

### Slide

**S5-1** Opening remarks for Symposium 5
*Ivan Manzini and Dirk Czesnik, Goettingen*

**S5-2** Endocannabinoid retrograde modulation of retinal photoreceptors
*Stephen Yazulla and Shih-fang Fan, Stony Brook (USA)*

**S5-3** Endocannabinoid-mediated short- and long-term plasticity of the spinal circuitry
*Abdel El Manira, Stockholm (S)*

**S5-4** Cannabinoid action in the olfactory system of *Xenopus laevis* tadpoles
*Dirk Czesnik, Ivan Manzini and Detlev Schild, Goettingen*

**S5-5** Endocannabinoid control of energy balance: food intake and beyond
*Vincenzo Di Marzo, Pozzuoli (NA) (I)*

**S5-6** The Yin and Yang of endocannabinoid action in fear and anxiety
*Carsten T. Wotjak, München*

**S5-7** Cannabinoids in neuropathic and inflammatory pain
*Maulik Durgeshbhai Jhaveri, Nottingham (UK)*

### Poster

**TS5-1A** The abnormal-Cannabidiol-sensitive receptor and excitotoxic neuronal damage in organotypic hippocampal slice cultures
*S. Kreutz, M. Koch, HW. Korf and F. Dehghani, Frankfurt*

Introductory Remarks to Symposium 5

# Cannabinoids and the Nervous System: Different Views on Multiple Actions

Dirk Czesnik, Göttingen

The derivates of Cannabis sativa have been used medicinally and recreationally for thousands of years. Over the last two decades our knowledge of the chemistry and physiology of endocannabinoids increased enormously. As the endocannabinergic system is one of the most abundant neuromodulation system its effects on the functionality of the nervous system are inspiring manifold. Endocannabinoids are able to influence cognition, memory, perception and motor activity, to mention only a few of its effects. Though there are several molecular mechanisms described in many brain regions the connection to the systemphysiological effects are at large not clear. The goal of this symposium is to summarize important recent findings concerning both molecular effects and their systemic consequences in physiological, pathophysiological and pharmaceutical aspects. It will be most interesting to compare and discuss results obtained using different functional subsystem of the nervous system in different animal models.

# Endocannabinoid retrograde modulation of retinal photoreceptors

Stephen Yazulla and Shih-fang Fan

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Evidence in mammalian and non-mammalian preparations indicate that visual effects induced by marijuana may be due in part to retinal endocannabinoids. Previous work from our lab on goldfish retina demonstrated an extensive cannabinoid presence in cone photoreceptors. Cones contain presynaptic CB1 receptors and the hydrolyzing enzyme FAAH; they accumulate and hydrolyze [3H]-anandamide; voltage-gated Ca and K currents of cones are suppressed by CB1 activation of Gi/o. Despite these data, a functional role for retinal endocannabinoids has not been determined. Our purpose was to demonstrate an effect of endocannabinoids by measuring retrograde suppression of the cone K current (IK(V)). Whole-cell recordings were obtained from cone inner segments, in a retinal slice preparation, under voltage clamp. IK(V) was elicited by a +124 mV pulse from a holding potential of -70 mV. A single 50 msec puff of saline with 70 mM KCl or Group I mGluR agonist DHPG added was applied through a pipette directly at a mixed rod/cone (Mb) bipolar cell body/ascending dendrite. The rationale was that appropriate stimulation of postsynaptic dendrites could lead to the release of a retrograde transmitter, the effect of which could be measured by modulation of presynaptic membrane currents in cones. This turned out to be the case as the amplitude of cone IK(V) decreased 25% compared to the pre-puff control. All effects were unaffected by combined application of GABAA, GABAC, AMPA and kainate antagonists but were blocked completely by the CB1 receptor antagonist SR 141716A. The FAAH inhibitor URB597 had no effect on the suppression of IK(V), whereas nimesulide, a COX-2 inhibitor, prolonged the effects of the K+ puff 10-fold. Orlistat, a blocker of 2-AG synthesis, blocked the effect of the K+ puff. In context of data obtained from rat brain, we concluded that the retrograde transmitter was 2-AG rather than anandamide. Group I mGluR activation of Gq/11 was demonstrated in that a puff with DHPG decreased IK(V) of cones by 32%, an effect blocked by SR141716A. The effect of DHPG was not blocked by the mGluR5 antagonist MPEP, indicating involvement of mGluR1. The large Mb bipolar cells are the only cells in the fish retina that contain mGluR1, suggesting that they are the source of the released 2-AG. The suppressive effect of the K+ puff vanished in a Ca2+-free, 2mM Co2+ saline. TMB-8 or ryanodine, blocked the effect of DHPG, but not that of the K+ puff, showing that calcium influx or release from intracellular stores could mediate retrograde 2-AG release. We suggest that retrograde suppression of cone IK(V) is mediated by Ca2+-dependent release of 2-AG from Mb bipolar cell dendrites by separate mechanisms: 1. voltage-dependent, mimicked by the K+ puff, that may be activated by the depolarizing ON response to light ; 2. voltage-independent, occurring under ambient illumination, mediated by tonic mGluR1/ Gq/11 activation. The negative feedback of this latter mechanism could regulate tonic glutamate release from cones within narrow limits, regardless of ambient illumination, thus maintaining sensitivity to detect contrast. These data also show that the presence of anandamide and FAAH in cones has a function other than retrograde suppression.

Grant support: NIH grant R01 EY001682

# Endocannabinoid-mediated short- and long-term plasticity of the spinal circuitry

Abdel El Manira

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Endocannabinoids released from dendrites can act as retrograde signals to modulate synaptic transmission. We have examined the physiological effects of endocannabinoids in the lamprey spinal cord. The spinal circuitry offers the possibility to determine the role of endocannabinoid retrograde signaling in the generation and regulation of integrated activity underlying locomotion. Our results show that endocannabinoids are released during locomotor activity and participate in setting the baseline burst rate. In addition, we showed that the release of endocannabinoids is triggered by activation of mGluR1. This on demand release of endocannabinoids produces short- and long-term depression and potentiation of inhibitory and excitatory synaptic transmission, respectively. These changes results in short- and long-term plasticity of the activity of the locomotor circuitry. Thus our results show that mGluR1-induced release of endocannabinoids transforms motoneurons and interneurons into modulatory neurons by enabling them to regulate synaptic strength and thereby the activity of the spinal circuitry as a whole.

# Cannabinoid action in the olfactory system of *Xenopus laevis* tadpoles

Dirk Czesnik, Ivan Manzini and Detlev Schild

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Olfactory information processing is influenced by a variety of neuromodulators at different stages of the olfactory system. Here, we demonstrate for the first time that odor processing is influenced by the cannabinergic system and discuss its possible function. We report on cannabinergic actions in the olfactory system of *Xenopus laevis* tadpoles. By means of calcium imaging and patch clamp we show that cannabinergic substances alter odor induced neuronal activity patterns and spike associated currents. Furthermore we describe the cellular distribution of the CB1 receptors using CB1 immunoreactivity.
[Supported by the "Research program, Faculty of Medicine, Georg-August-University, Goettingen"]

# Endocannabinoid control of energy balance: food intake and beyond

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Endocannabinoids (ECBs) are endogenous lipids, mostly derived from arachidonic acid, capable of binding to, and subsequently activating, the two cannabinoid CB1 and CB2 receptors. The two most studied ECBs are anandamide and 2-arachidonoylglycerol. Cannabinoid (CB) receptors are G-protein-coupled receptors discovered in the early 1990's as molecular targets of Cannabis psychoactive principle, delta-9-tetrahydrocannabinol, to which they bind with high affinity. Of the two CB receptor subtypes cloned so far, CB1 is the one most widespread in mammalian tissues, with the highest concentrations in some brain areas, but also present in many other peripheral organs, including the gastrointestinal system, the liver, the airways, the reproductive organs and the cardiovascular system.

ECBs are biosynthesized from neurons following the Ca2+-dependent remodelling of membrane phospholipids, followed by the enzymatic hydrolysis of specific lipid precursors. This means that ECBs are not stored in neurons prior to their release, but are instead released "on demand" immediately after their de novo biosynthesis. In other words, the basal levels of ECBs are barely detectable as they are produced only "when and where needed", to be then rapidly inactivated by hydrolytic enzymes. ECBs are produced, and CB1 receptors stimulated, in response to stressful stimuli to help re-establishing the steady-state homeostasis of other neurotransmitters, mediators, hormones and cytokines. Therefore, CB1 receptor stimulation is short-lasting, is limited to those cells or tissues that have been subjected to stress or damage, and it normally ends once that the organism has recovered from a transient "unbalanced" condition. However, some chronic pathological states lead to a long-lasting up-stimulation of ECB synthesis (or down-regulation of their degradation), resulting in permanent over-activation of CB1 receptors, which may then contribute to the symptoms of these disorders.

The ECB system is present in all those brain and peripheral regions involved in the control of energy balance and body weight, as well as in neurons of the mesolimbic system that participate in the "liking" and "wanting" of foods. It has been shown that CB1 receptors are necessary to induce food intake after a short period of food deprivation, to accumulate fat into adipocytes, and to consume palatable food and substances of abuse. Their stimulation, in fact, leads to the modulation of the release and/or expression of some hypothalamic anorexigenic and orexigenic mediators, as well as of dopamine in the nucleus accumbens shell, whereas hypothalamic ECB levels are depressed by leptin. However, we now know that these physiological responses can be transformed into pathological ones upon repeated consumption of fatty foods and substances of abuse, when a permanent stimulation of CB1 receptors can occur, leading to further food consumption, fat accumulation, dyslipidemia and insulin resistance. These conditions, their pathological consequences and how to correct them will be discussed in this lecture.

# The Yin and Yang of endocannabinoid action in fear and anxiety

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Fear comes in different shades of gray, reaching from rapidly waning to excessive and sustained responses. Recent data suggest that endocannabinoids are essential for guaranteeing adequate fear adaptation. It turned out that endocannabinoids exert anxiolytic actions in exploration-based anxiety tests. In addition, they are specifically involved in extinction of aversive memories, most likely via habituation-like processes. However, the anxiolytic consequences of endocannabinoids are controversially discussed, since pharmacological blockade or genetic ablation of the cannabinoid receptor type 1 (CB1) either reduced, failed to affect or even increased behavioral measures of fear and anxiety in rats and mice. Here we provide a possible solution of this conundrum: By using mutant mouse strains with cell type-specific ablation of CB1 we demonstrate that the nature of a neuron dictates as to whether CB1 exerts anxiogenic or anxiolytic effects, and that the behavioral relevance of CB1 depends on the averseness of the test situation. This Yin and Yang of endocannabinoid action largely extends our knowledge about the involvement of CB1 in the control of fear and anxiety. In addition, it might be of considerable significance for a future exploitation of the of endocannabinoid system as a therapeutic target for the treatment of anxiety disorders.

# Cannabinoids in neuropathic and inflammatory pain

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Recent studies suggest that cannabis based medicines have therapeutic potential for the treatment of inflammatory and neuropathic pain states. Previously we have demonstrated that activation of both the cannabinoid CB1 and CB2 receptors reduces nociceptive processing in animal models of inflammatory and neuropathic pain. A novel experimental approach to treat pain is the augmentation of levels of endocannabinoids. Fatty acid amide hydrolase (FAAH) is the main enzyme responsible for the metabolism of several endogenous fatty acid amides including anandamide (AEA), palmitoylethanolamide (PEA) and oleamide (OEA). Augmentation of levels of endocannabinoids, with inhibitors of fatty acid amide hydrolase (FAAH), is analgesic in models of acute pain states. The aim of our study was to investigate the effects of local administration of the FAAH inhibitor URB597 in the carrageenan model of inflammatory and the selective spinal nerve ligation model of neuropathic pain. The effects of URB597 on levels of endocannabinoids (AEA and 2AG) and related fatty acid amides (PEA and OEA) have also been measured in the hindpaws of neuropathic and carrageenan inflamed rats.

Here we report that intraplantar injection of URB597 (25 μg in 50 μl) reduces nociceptive responses in sham-operated rats, but not in neuropathic rats. Furthermore, levels of AEA and 2AG were elevated by URB597 (25 μg in 50 μl) in sham-operated rats, but not in neuropathic rats. A higher dose of URB597 (100 μg in 50 μl) inhibited nociceptive processing in neuropathic rats. Effects of URB597 were blocked by pre-administration of a selective CB1 receptor antagonist. In carrageenan model of inflammation, intraplantar injection of URB597 (25 μg in 50 μl) was antinociceptive in incapacitance test, a measure of weight bearing on inflamed paw, while a higher dose was pro-nociceptive (100 μg in 50 μl) suggesting dose-related contrasting role of endocannabinoids in inflammatory conditions.

These data demonstrate that peripheral FAAH metabolism of endocannabinoids is altered in neuropathic pain states, these differences may arise as a result of up-regulation of FAAH in neuropathic pain states or a greater contribution of alternative metabolic pathways. In inflammatory conditions, inhibiting metabolism of endocannabinoids could lead to activation of anti- or pro-nociceptive pathways, depending on the intensity of FAAH inhibition.

# The abnormal-Cannabidiol-sensitive receptor and excitotoxic neuronal damage in organotypic hippocampal slice cultures

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In the aftermath of acute CNS pathologies a wide variety of metabolic and immunological mechanisms contribute to the delayed loss of neurons, which were initially unaffected by the lesion. Cannabinoids have been shown to be involved in neuroprotection. It is known that activated microglial cells mainly express the CB2 and the abn-CBD receptor. Abn-CBD receptors are activated by abnormal Cannabidiol (abn-CBD) and 2-Arachidonoylglycerol (2-AG) and are antagonized by the phytocannabinoid Cannabidiol (CBD) and the synthetic cannabinoid O-1918, respectively. In the present study we investigated the effects of agonists/antagonists of the abnormal-Cannabidiol-sensitive receptor (abn-CBD receptor) on microglial cells and neurons after excitotoxically lesioning of organotypic hippocampal slice cultures (OHSC). In glial single cell cultures we further investigated the effects of abn-CBD receptor agonists/antagonists on glial secretion of proinflammatory cytokines and nitric oxide (NO) and on glial proliferation. OHSC derived from 8-day-old (p8) Wistar rats were lesioned by the application of N-methyl-D-aspartate (NMDA) after 6 days in vitro (div) and treated with abn-CBD (0.1-10µM) or 2-AG (0.001-1µM) up to 9 div. Propidium iodide (PI) and IB4 were used to visualize the extent of neuronal damage and to determine the number of microglial cells, respectively. Abn-CBD and 2-AG had no effect on microglial cells and neuronal cell death in non-lesioned OHSC. NMDA-treated cultures displayed a massive increase in PI+ neurons and large numbers of amoeboid microglial cells accumulating at the sites of neuronal injury. Treatment of lesioned OHSC with abn-CBD resulted in a concentration-dependent decrease in the number of microglial cells, compared to OHSC lesioned with NMDA only. Maximal reduction occurred at an abn-CBD concentration of 10µM. This effect was blocked by the abn-CBD receptor antagonists CBD and O-1918. Abn-CBD protected granule cells from excitotoxic injury in a concentration dependent manner with maximal reduction of degenerating neurons at 10µM abn-CBD, which was only reversed by the CBD but not by O-1918. In lesioned OHSC 2-AG also reduced the number of microglial cells with maximal reduction at 0.001µM, and as compared to abn-CBD this effect was antagonized by CBD and O-1918. Similar to abn-CBD, 2-AG significantly reduced the amount of degenerating neurons and surprisingly this was only antagonized by O-1918, but not by CBD.

Our findings show that the number of microglial cells and the amount of neuronal cell death induced by excitotoxicity is reduced by both abn-CBD and 2-AG. Different binding sites may be responsible for the different effects of natural and synthetic antagonists of the abn-CBD receptor.

# Symposium

## S6: The Cortical Nerve Impulse

***Fred Wolf and Maxim Volgushev, Göttingen and Bochum***

### Slide

**S6-1** Reverse Physiology of Action Potentials in the Vibrissal System
*Michael Brecht, Berlin*

**S6-2** An analytically tractable model of a spatially extended spiking neuron.
*Carl van Vreeswijk, Paris (F)*

**S6-3** A dendritic switch for synaptic plasticity in neocortical pyramidal cells
*Michael Hausser and Jesper Sjöström, London (UK)*

**S6-4** Adaptation of coding to input statistics in V1
*Kenneth D Miller, Michael P Stryker and Tatyana Sharpee, New York (USA) and San Francisco (USA)*

**S6-5** Axonal sodium channels control excitability of cortical pyramidal neurons
*Ilya A. Fleidervish, Rehovot (Israel)*

**S6-6** Unique features of action potential initiation in neocortical neurons
*Maxim Volgushev, Pavel Balaban, Marina Chistiakova, Aleksey Malyshev, Stanislav Volgushev and Fred Wolf, Bochum, Moscow (RUS) and Göttingen*

### Poster

**TS6-1A** Membrane resonance shapes the pattern of spontaneous firing in the entorhinal cortex
*TA. Engel, S. Schreiber, L. Schimansky-Geier, AVM. Herz and I. Erchova, Berlin*

Introductory Remarks to Symposium 6

# The Cortical Nerve Impulse

Fred Wolf and Maxim Volgushev, Göttingen and Bochum

Recent progress in cortical physiology and theoretical neuroscience calls for a reevaluation of our view of cortical action potentials (APs). This symposium will bring together scientists addressing the functional role of APs using combinations of advanced theoretical and experimental approaches to present recent research achievements on the biophysics, cellular physiology and computational role of AP generation in cortical cells and networks.

Action potentials are electrical pulses that are actively generated by neurons and can propagate unattenuated along axons for distances up to the meter range. Therefore it is traditionally assumed that the primary purpose of APs is to enable long distance communication among neurons. AP generation, however, also provides a highly nonlinear operation that fundamentally expands the information processing capabilities of neuronal networks. Because APs are relatively expensive in terms of metabolic cost, one expects substantial evolutionary pressure to use APs sparsely and in a computationally optimal fashion. Recently, it was discovered that apparently minor modifications of the AP generating mechanism of a neuron can qualitatively change the nature of neuronal encoding. In particular, computational analyses showed, that conventional Hodgkin-Huxley type AP initiation mechanisms impose severe constraints on the encoding capabilities of neural networks, especially when rapidly changing stimuli need to be processed. These theoretical results are complemented by novel neurophysiological data on biophysical mechanisms of AP initiation, precise spatial localisation of the AP initiation zone, the interplay of different neuronal compartments in AP encoding, adaptation of neuronal encoding, and on the impact of individual APs on behavioral reactions of the animal.

# Reverse Physiology of Action Potentials in the Vibrissal System

Michael Brecht

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The relationship between the activity of individual neurons and behavior is a core interest of neurobiology. Extracellular recording and stimulation techniques and have demonstrated that single neuron activity of neurons is closely correlated with behavior in mammals, but both techniques are not suited to pinpoint the impact of single neuron activity on behavior. We confront this problem by stimulating single neurons in the rat vibrissal system and testing the effect on vibrissal movement and sensation. This approach reverses conventional physiological research, where APs are mainly studied as correlates of sensorimotor processing. Specifically we addressed this issue by assessing effects of single cell stimulation in (i) the vibrissal motor cortex (ii) the vibrissal motor neurons in the facial nucleus, (iii) in the somatosensory cortex of awake rats. In vibrissa motor cortex we find that AP initiation in individual cells causes long sequences of small and slow multi whisker movements. AP number had only little effect on whisker movement amplitude but it strongly affected movement latency. AP frequency in contrast did not affect movement latency but determined movement amplitude and direction. In the facial nucleus we find that AP initiation in individual cells causes mainly but not exclusively single whisker movements. Movements are brief and usually fast and each spike causes a very similar fixed latency movement. Thus, motor cortical neurons and cells in the facial nucleus code movements in very different ways: Cortical APs affect movements on long time scales and APs are read as sequences or "words", such that the effect (movement latency and direction) of an AP depends on the AP context. In contrast, facial nucleus APs are translated spike by spike to movement twitches. We also investigated sensory effects of single cell stimulation in the somatosensory cortex. To this end animals were first trained to report trains of cortical microstimulation pulses by a tongue lick. Once the animal reported small microstimulation currents, microstimulation trials were mixed with trials in which we evoked ~ 14 APs in single cortical neurons. Rats responded significantly more often after single cell stimulation than in catch trials without stimulation. The bias introduced by single cell stimulation was weak on average (responses were induced in ~ 6% of trials), but could be strong for individual cells. We conclude that the activity of single sensory cortical neurons can lead to a behaviorally reportable effect. Thus, single neuron stimulation in general and the parametric variation of initiated AP patterns in particular, allow us to decode (i.e. measure effects of APs and AP train parameters) single neuron activity in an unprecedented fashion.

# An analytically tractable model of a spatially extended spiking neuron.

Carl van Vreeswijk

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I present a model of a spatially extended spiking neuron that is analytically tractable. The model consists of a soma, modeled as an integrate and fire unit, coupled to a passive dendritic cable. I will show how this model can be treated analytically. I will present the results of a study of the effects of shunting inhibition. I show that depending on the distribution of excitatory and inhibitory inputs the effect of shunting inhibition can either be divisive or subtractive. I will also discuss the effect of dendro-dendritic gap junction on the synchronization of a pair of neurons and show that, depending on the distance from the soma at which the gap junctions occur, the effect of the gap junction can be either to synchronize or desynchronize the neurons. Finally, I will show that adding shunting inhibition can change the effect of the gap junction on neuronal synchronization.

# A dendritic switch for synaptic plasticity in neocortical pyramidal cells

Michael Hausser and Jesper Sjöström

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Pyramidal neurons in the cerebral cortex span multiple cortical layers. How the excitable properties of pyramidal neuron dendrites allow these neurons to both integrate activity and store associations between different layers is not well understood, but is thought to rely in part on dendritic backpropagation of action potentials. Using a combination of quadruple patch-clamp recordings from connected neurons, dendritic recordings, and two-photon calcium imaging, we demonstrate that the sign of synaptic plasticity in neocortical L5 pyramidal neurons is regulated by the spread of the backpropagating action potential to the synapse. This creates a progressive gradient between LTP and LTD as the distance of the synaptic contacts from the soma increases. At distal synapses, cooperative synaptic input or dendritic depolarization can switch plasticity between LTD and LTP by boosting backpropagation of action potentials. This activity-dependent switch provides a mechanism for associative learning across different neocortical layers that process distinct types of information.

# Adaptation of coding to input statistics in V1

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How does neural encoding adapt to the statistics of the inputs received? We have applied a new information-theoretic method that allows unbiased estimation of a neuron's receptive field from its responses to natural stimuli or other non-Gaussian stimulus ensembles. We have used this to compare a neuron's receptive field when responding to natural stimuli to its receptive field when responding to noise stimuli. While the two are similar in major features such as preferred orientation or preferred spatial frequency, they differ systematically in their details. These differences are of a form that tends to maintain the neuron's output statistics unchanged despite changes in input statistics. The times over which the neuron's encoding strategies change as it adapts to a new stimulus ensemble are extraordinarily long, on the order of 1 minute to tens of minutes.

# Axonal sodium channels control excitability of cortical pyramidal neurons

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It has long been assumed that the site of action potential (AP) initiation in layer 5 pyramidal neurons is axonal, and recent studies indicate that it is localized in the very proximal part of the axon, about 35 μm from the hillock. In order to comprehend the complex, non-linear mechanisms that underlie AP initiation, it is necessary to understand precisely the behavior of transient and persistent Na+ channels in this region. We have used a combination of patch clamp recording and high-speed fluorescence imaging of the Na+-sensitive indicator SBFI to explore this behavior. The TTX-sensitive Na+ transients elicited by a single AP are prominent in axons and are not detectable in soma, apical and basal dendrites. Axonal [Na+]i grows rapidly and decays monoexponentially with a time constant between 200 and 600 ms. The decay of [Na+]i is not affected by Ouabain, indicating that it reflects diffusion of axonal Na+ into the large volume of the soma rather than extrusion by a pump. Na+ signals elicited by trains of more than two spikes can be detected in all compartments, but they are larger and faster in the proximal axons. Subthreshold depolarizing current steps lasting 0.3 to 3 s elicit [Na+]i elevations that are only seen in the proximal axon. [Na+]i grows throughout the current step and falls at its termination, indicating that the underlying Na+ current is truly non-inactivating. Direct comparison of the axonal Na+ transients generated by a subthreshold depolarization with those elicited by a single AP (persistent vs. transient Na+ conductance) reveals that the amplitude of the former is significantly larger, despite the fact that it must be greatly affected by Na+ diffusion. These findings indicate that in layer 5 pyramidal neurons, the density of Na+ channels in proximal axons is significantly higher than in the rest of the cell membrane. At functionally critical subthreshold voltages, persistent Na+ current in these neurons is predominately axonal, and it reflects activation of a remarkably high percentage of the local Na+ channels. The high density and distinctive biophysical properties of the Na+ channels in the proximal axon confer on this region a major role in controlling the excitability and integrative properties of the cortical pyramidal neuron.

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# Unique features of action potential initiation in neocortical neurons

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Generation of action potentials (AP) and encoding of the incoming information into sequences of discharges is an ubiquitous step in operation of neurons and neural networks. Recent evidence from theoretical and experimental studies has questioned the direct applicability of the reigning theory of cellular electrogenesis - the Hodgkin-Huxley theory - to the AP initiation in central mammalian neurons.

We have shown, that two salient features of AP initiation in neocortical neurons in vivo: their sharp, step-like initiation dynamics and large variability of the onset potential are virtually impossible to describe by Hodgkin-Huxley type models with realistic parameter settings. Our quantitative analysis of AP waveforms and initiation dynamics in a large population of mammalian neocortical neurons and invertebrate (snail) neurons showed, that the Hodgkin-Huxley formalism could explain AP initiation in most of snail neurons, but not in vertebrate neocortical neurons. To describe the AP initiation dynamics, we used the ratio of errors of exponential over the piecewise linear fits of the initial portion of AP in the phase-plot representation. This quantitative measure segregates the AP initiation dynamics in two fundamentally different classes: a gradual Hodgkin-Huxley-type AP initiation usual in the snail neurons, and the fast AP initiation typical for the neocortical neurons. Segregation of neurons by the ratio of fit errors corresponded well to the segregation by other AP parameters. Under the conditions which diminish functioning of voltage-gated sodium channels (TTX or reduced extracellular Na+), not only the amplitude if APs in neocortical neurons was decreased, as the canonical Hodgkin-Huxley theory predicts, but also their initiation dynamics was altered, becoming slow and gradual. These results support the hypothesis that sharp, step-like onset dynamics of neocortical APs is due to cooperative activation of voltage-gated sodium channels.

Furthermore, we show that the mechanisms, which make AP initiation in neocortical neurons fundamentally different from predictions of the classical Hodgkin-Huxley theory, are not present at birth, but appear during the development. In young animals, AP initiation dynamics in most neurons does follow the Hodgkin-Huxley description. The proportion of such cells decreased during the 1-3 postnatal weeks, in parallel with the maturation of the other electrophysiological characteristics. After postnatal day 17 all cells encountered exhibited non-Hodgkin-Huxley type AP initiation.

We conclude, that Hodgkin-Huxley description of AP generation is not as general as is widely assumed, and basic principle(s) of AP generation in adult neocortex neurons remain to be elucidated.

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# Membrane resonance shapes the pattern of spontaneous firing in the entorhinal cortex

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Subthreshold frequency preference of neuronal membranes is assumed to exert influence beyond the level of the individual neuron to rhythmic activity and frequency preference in large neuronal networks. Because fast signaling between neurons is largely dependent on action potentials, it is vital to understand how subthreshold resonance properties influence neuronal firing. In this study, we investigate two cell types with different subthreshold frequency preference - one resonant and one nonresonant - in the entorhinal cortex and systematically analyze their spontaneous firing statistics. We find that resonant cells show characteristic clustering of action potentials, reflected in interspike interval (ISI) distributions of spontaneous activity, which is not typical of nonresonant cells. Analysis with a simple resonate-and-fire model reveals that while subthreshold frequency preference is sufficient to qualitatively account for spike clustering, it cannot fully explain the observed interspike interval distributions. We show that correlations on a time scale of 200-300 ms, which are equally present in the recorded resonant and nonresonant cells, prove necessary to capture interspike interval statistics. We propose a modification to the resonate-and-fire model which allows to better represent ISI statistics and correlations. Action potential generation in this model is nonrenewal and thus depends on spike history. The model captures frequency preference and ISI correlations and is still efficient to use in network models.

# Symposium

## S7: Molecular Aspects of Synapse Function and Dysfunction in the Mammalian Brain

***Matthias Kneussel, Hans-Jürgen Kreienkamp and Stefan Kindler, Hamburg***

### Slide

**S7-1** Opening remarks for Symposium 7
*Matthias Kneussel, Hans-Jürgen Kreienkamp and Stefan Kindler, Hamburg*

**S7-2** Molecular assembly of the active zone
*Eckart D. Gundelfinger, Magdeburg*

**S7-3** Role for the spine apparatus organelle in synaptic plasticity
*Michael Frotscher, Shanting Zhao, Thomas Deller and Alexander Drakew, Freiburg and Frankfurt*

**S7-4** Activity and translational dependent regulation of spines morphology
*Carlo Sala, Milano (I)*

**S7-5** Dendritic mRNA transport: *cis*-elements, *trans*-factors and motor proteins
*Stefan Kindler, Hamburg*

**S7-6** Neuronal cotransport of glycine receptor and the scaffold protein gephyrin
*Matthias Kneussel, Hamburg*

**S7-7** KCl cotransport, cytoplasmic chloride and GABA response: insight from KO animals
*Thomas J. Jentsch and Christian A Hübner, Berlin and Hamburg*

### Poster

**TS7-1B** Attenuated effects of carbamazepine and phenytoin on CA1 neurons in chronic experimental epilepsy
*C. Schaub, M. Uebachs and H. Beck, Bonn*

**TS7-2B** Glutamine synthetase becomes partially inhibited by the nitration of tyrosine residues after repeated PTZ-induced seizures.
*HJ. Bidmon, B. Görg, N. Palomero-Gallgher, A. Schleicher, EJ. Speckmann and K. Zilles, Düsseldorf and Münster*

**TS7-3B** Characterization of a novel synaptic vesicle protein identified by a proteomic approach
*W. Volknandt, J. Burré, T. Beckhaus, H. Schägger, M. Karas and H. Zimmermann, Frankfurt/Main*

**TS7-4B** mAbp1 deficiency suggests a role for actin in vesicle fission
*N. Glyvuk, Y. Tsytsyura, C. Thiel, J. Wienands and J. Klingauf, Göttingen*

**TS7-5B** Hierarchical ability of SNAP-25 homologues to support neuronal function
*ID. Martinez and JB. Sorensen, Göttingen*

**TS7-6B** Adenoviral expression of multiple proteins of interest to study exocytosis in chromaffin cells
*R. Mohrmann and J. Soerensen, Göttingen*

**TS7-7B** Disruption of clathrin-mediated endocytosis in hippocampal synapses causes a shift to a bulk endocytotic mode of membrane retrieval, but not to kiss and run
*Y. Tsytsyura, N. Glyvuk, M. Krikunova, N. Jung, V. Haucke and J. Klingauf, Göttingen and Berlin*

**TS7-8B** Dendritic mRNA transport: *cis*-elements, *trans*-factors and motor proteins
*S. Kindler, M. Rehbein and KH. Zivraj, Hamburg*

**TS7-9B** The Voltage-Gated Calcium Channel is the Calcium Sensor Protein of Secretion
*D. Atlas and I. Lerner, Jerusalem (Israel)*

**TS7-10B** TRPC channel-mediated signaling in cerebellar Purkinje cells
*J. Hartmann, E. Dragicevic, R. Blum, M. Freichel, A. Dietrich, L. Birnbaumer, V. Flockerzi and A. Konnerth, München, Homburg, Marburg and Research Triangle Park (USA)*

**TS7-11B** Overexpression of individual gamma-Protocadherins in Transgenic Mouse Models
*M. Frank, M. Ebert and R. Kemler, Marburg and Freiburg*

**TS7-12B** ubMunc13-2 enhances vesicles recruitment in a calmodulin dependent manner
*A. Mezer, D. Zikich, M. Gutman, N. Esther, R. Melamed, H. Junge, N. Brose and U. Ashery, Tel Aviv (Israel) and Göttingen*

Introductory Remarks to Symposium 7

# Molecular Aspects of Synapse Function and Dysfunction in the Mammalian Brain

Matthias Kneussel, Hans-Jürgen Kreienkamp and Stefan Kindler, Hamburg

The aim of the symposium is to discuss various aspects of synaptic function and dysfunction in the mammalian central nervous system. Mutation in genes encoding synaptic proteins are associated with a wide range of human desease ranging from epilepsy to mental retardation and autism. The trafficking of molecular components toward the pre- and postsynaptic compartment, as well as the function of protein complexes at the axo-dendritic contact site are in focus of the proposed topics.

Eckart D. Gundelfinger (Leibniz Institute of Neurobiology, Magdeburg) will discuss aspects of presynaptic active zone formation and function, including mouse models addressing the role of the cytomatrix proteins bassoon and piccolo. Michael Frotscher (University of Freiburg, Medical School) will continue with new data on synaptopodin, an actin-binding protein thought to participate in morphological adaptations of dynamic spine structures underlying the regulation of synaptic strength.

A particular challenge for neuronal cells is to provide fast protein turnover at synaptic sites. Carlo Sala (CNR Institute for Neuroscience, Milano) will describe how modifications in spine number and morphology are regulated by synaptic activity through specific control of the translation of dendritically localized mRNAs. Stefan Kindler (University of Hamburg, Medical School) will discuss the aspect of dendritic mRNA transport, which is required for local protein synthesis at distal dendrites. This will be complemented by Matthias Kneussel (Center for Molecular Neurobiology Hamburg) who will present data about motor protein-driven transport complexes underlying anterograde and retrograde transport of neurotransmitter receptors toward and from postsynaptic sites.

The symposium will be concluded by consequences of neuronal dysfunction analysed with mouse mutants of K-Cl cotransporters, which regulate different aspects of chloride concentrations in neurons. Thomas J. Jentsch (Leibniz Institut für Molekulare Pharmakologie und Max Delbrück Zentrum Berlin) will illustrate how K-Cl proteins underlie the regulation of synaptic inhibition.

# Molecular assembly of the active zone

Eckart D. Gundelfinger

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Neurotransmitter release is restricted to specific sites of the pre-synaptic cell membrane, called active zones, which are positioned exactly opposite to the postsynaptic neurotransmitter reception apparatus. At active zones, a cycle of membrane trafficking events - the synaptic vesicle cycle - is organized by a complex meshwork of proteins - the cytomatrix assembled at the active zone or CAZ. With Bassoon and Piccolo we have identified two related proteins that are exquisitely localized at neurotransmitter release sites and serve as important scaffolding elements of the CAZ. Both molecules turned out to be useful tools to monitor assembly mechanisms of the active zone during synaptogenesis. This work has given rise to the active zone transport vesicle hypothesis, which predicts that major parts of the active zone are pre-assembled inside the neuron and transported to sites of synaptogenesis as a precursor vesicle - the Piccolo-Bassoon transport vesicle (PTV).
Recent work has focused on assembly and transport mechanisms of PTVs. In young cultured neurons, the two proteins co-localize with markers of the trans-Golgi network. Disruption of the Golgi apparatus causes aggregation of Bassoon and Piccolo in the soma and impairing vesicle exit from the Golgi complex prevents transport of Bassoon out of the soma. Our results indicate that transport via vesicles is essential for delivery of cytomatrix proteins to the synapse at least during the major period of synapse formation. Moreover, they establish Golgi transit as an obligatory step in subcellular trafficking of these scaffolding proteins [1]. Thus, active zone formation at CNS synapses may indeed involve three spatially and mechanistically distinct events, including cytomatrix formation at the Golgi-complex, vesicular precursor transport, and en-bloc deposition at pre-synapses.
In hippocampal primary cultures, primordial synapses accumulating synaptic marker proteins and cycling vesicles are formed in the absence of functional Bassoon and Piccolo. This indicates that the two proteins are not essential for synatogenesis. Our data rather suggest that they play a role in synaptic maturation and plasticity.

This work has been supported by the Deutsche Forschungsgemeinschaft, the German-Israeli Foundation, the State Saxony-Anhalt and the European Commission (SynScaff).

[1] Dresbach T, Torres V, Wittenmayer N, Altrock WA, Zamorano P, Zuschratter W, Nawrotzki R, Ziv NE, Garner CC, Gundelfinger ED (2006) J Biol Chem 281:6038-47

# Role for the spine apparatus organelle in synaptic plasticity

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Current concepts of synaptic plasticity mainly involve mechanisms of transmitter release, transmitter receptor recruitment, and the formation of new synaptic sites, in particular the de novo formation of dendritic spines. Cytoplasmic organelles such as the spine apparatus present in many forebrain dendritic spines have not been studied in great detail, and their roles in synaptic transmission and synaptic plasticity remain to be determined. We have recently shown that mutant mice lacking synaptopodin fail to form a spine apparatus and show deficits in synaptic plasticity and learning and memory (Deller et al., 2003). However, these studies did not elucidate synaptopodin's role in the formation of the spine apparatus nor did they determine the mechanism(s) by which the spine apparatus contributes to synaptic transmission. Here, we will summarize our recent attempts aimed at characterizing the functional significance of the spine apparatus. Evidence will be provided that the spine apparatus is a calcium store involved in the regulation of hippocampal synaptic plasticity such as long-term potentiation.

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Reference:

T. Deller et. al. (2003) Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity. Proc. Natl. Acad. Sci. USA 100:10494-10499.

# Activity and translational dependent regulation of spines morphology

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Synaptic activity regulates and promotes dendritic spines formation and maturation but the molecular mechanisms involved in these processes are still not well characterized.
In this study we have developed a new proteomic approach, based on a conservative sub-cellular fractionation, to reveal changes in protein synthesis, degradation and post-translational modifications in hippocampal neuron cultures where a change in spines number and morphology has been induced by long-term stimulation/depression of synaptic activity. Experiments were performed on neurons fractionated by sequential exposition to different detergent-containing buffers after long-term treatment with Bicuculline or TTX. Four proteins fractions for each condition have been isolated and differentially analyzed by 2D-SDS-PAGE maps and MALDI-TOF. This analysis revealed how long-term stimulation/depression of synaptic activity regulates cell metabolic status, cytoskeletal dynamic, local protein synthesis, mitochondrial distribution, actin network organization and protein metabolism. Interestingly we have also fond that phosphorylation of eEF2 by eEF2K/CamKIII can link glutamate receptors activation to an increase of local dendritic proteins synthesis required to induce spines maturation.

# Dendritic mRNA transport: *cis*-elements, *trans*-factors and motor proteins

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In neurons, the diverse protein composition of distinct cellular regions demands elaborate sorting mechanisms. In addition to intrinsic protein targeting signals, cytoplasmic localization and local translation of mRNAs contributes to differential protein sorting. In contrast to most neuronal mRNAs, which are restricted to somata, transcripts encoding the microtubule-associated proteins 2 (MAP2) are found in dendrites. Along dendritic shafts, MAP2-mRNAs form granules that appear to serve as transport units. A cis-acting dendritic targeting element (DTE) in the 3'-untranslated region of MAP2-transcripts mediates dendritic localization in neurons. Two 90 and 65 kDa MAP2-RNA trans-acting proteins, MARTA1 and MARTA2, specifically interact with the MAP2-DTE. In neurons, MARTA1 preferentially resides in the nucleus, whereas MARTA2 is a nucleocytoplasmic protein that is mainly present in the somatodendritic cytoplasm where resides in granular structures. In primary neurons, overexpression of a truncated MARTA2 isoform that contains only the RNA-binding domains exerts a strong dominant-negative effect as it completely disrupts dendritic targeting of endogenous MAP2 mRNAs. In contrast, extrasomatic trafficking of MAP2 transcripts is not affected by overexpression of two other RNA-binding proteins. Furthermore, truncated MARTA2 does not noticeably alter the concentration and subcellular distribution of the entire pool of polyadenylated mRNAs. Finally, dominant-negative kinesin I, but not dynamitin, disrupts extrasomatic trafficking of MAP2 mRNA granules. Thus, in neurons kinesin I and MARTA2 appear to act in concert to specifically target MAP2 mRNAs into dendrites. Supported by the Deutsche Forschungsgemeinschaft (Ki 488/2-6)

# Neuronal cotransport of glycine receptor and the scaffold protein gephyrin

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The dynamics of postsynaptic receptor-scaffold formation and remodelling at inhibitory synapses remains largely unknown. Gephyrin, a multimeric scaffold protein, interacts with cytoskeletal elements and stabilizes glycine receptors (GlyRs) and individual subtypes of GABA-A receptors (GABA-ARs) at inhibitory postsynaptic sites.
Our data identify gephyrin as a component of intracellular transport complexes in the recruitment of motor protein-dependent trafficking along microtubules.
We report intracellular mobility of gephyrin transport packets over time. Gephyrin units enter and leave active synapses in the range of several minutes. High potassium and receptor blockade alter the transport parameters of gephyrin units.
In addition to previous reports of GlyR-gephyrin interactions at plasma membranes, we show cosedimentation and coimmunoprecipitation of both proteins from vesicular fractions. Moreover, GlyR and gephyrin are cotransported within neuronal dendrites and further coimmunoprecipitate and colocalize with and functionally depend on motor complexes. Our data support the concept that gephyrin-motor interactions contribute to a dynamic and activity-dependent rearrangement of postsynaptic GlyRs, a process thought to underlie the regulation of synaptic strength. Supported by grants of the Deutsche Forschungsgemeinschaft (SFB444/B7).

# KCl cotransport, cytoplasmic chloride and GABA response: insight from KO animals

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The intraneuronal chloride concentration determines the response of neurons to the neurotransmitters GABA and glycine. During brain development, this concentration decreases, resulting in a shift from an excitatory to an inhibitory response to these neurotransmitters in most neurons. The major transport process lowering cytoplasmic chloride is the electroneutral K-Cl cotransporter KCC2, with a minor role also played by KCC3. We have generated KO mouse models for these transporters to gain insights into their physiogical roles. The constitutive KO of KCC2 led to perinatal death due to an inability to breathe, as well as to a spastic phenotype. Perforated patch clamp measurements on spinal cord motoneurons revealed a large shift in the reversal potential in the presence of GABA, indicating that KCC2 is the major player in setting neuronal Cl concentration. We are currently generating various conditional KOs to avoid lethality and to investigate the role of intraneuronal Cl in older animals. The KO of KCC3 led to a severe degeneration of the CNS and PNS, resembling the human Anderman syndrome in which KCC3 is mutated, as well as to deafness and hypertension.

# Attenuated effects of carbamazepine and phenytoin on CA1 neurons in chronic experimental epilepsy

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Epilepsy is a common and devastating neurological disorder. A substantial (about 30%) proportion of patients continue to have seizures despite carefully optimized treatment with currently available antiepileptic drugs (AEDs). One key concept to explain the development of pharmacoresistance is that epilepsy-related changes in the properties of drug targets in the brain result in AED-insensitivity of these targets, and that this changes contribute to the drug-resistance on a clinical level. This notion is supported by a loss or reduction of carbamazepine-induced use-dependent block of voltage-dependent $Na^+$ currents ($I_{Na}$) in hippocampal granule neurons, but it has remained unclear whether these findings are generalizable to other neuron types. Our results suggest that epilepsy-associated changes in AED sensitivity of ion channels are both AED-specific, as well as cell-type specific.

We thoroughly examined the effects of carbamazepine (CBZ) and phenytoin (PHT) on $I_{Na}$ in hippocampal CA1 neurons of sham-control and chronically epileptic rats. We find that there were no epilepsy-associated changes in efficacy of these AEDs on voltage-dependent activation of $I_{Na}$. In contrast, there were significant changes in the effects of PHT, but not CBZ on the voltage-dependence of inactivation, resulting in a significant reduction in voltage-dependent blocking effects in chronically epileptic animals. Conversely, CBZ effects on the time course of recovery from inactivation of $I_{Na}$ were significantly less pronounced in epileptic compared to sham-control animals, whereas PHT effects remained unaltered. It is noteworthy that the epilepsy-associated reduction in CBZ effects was much less pronounced compared to the findings previously reported for dentate granule cells.

Thus, our findings indicate that AED sensitivity of $Na^+$ currents may be differentially modified in different hippocampal subregions. Furthermore, changes in AED sensitivity cannot be generalized across different AEDs acting on $Na^+$ channels. Despite this emerging complexity, our data suggest that target mechanisms contribute to the development of drug-resistant epilepsy, in concert with other mechanisms such as altered function of multidrug transporters.

# Glutamine synthetase becomes partially inhibited by the nitration of tyrosine residues after repeated PTZ-induced seizures.

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The astrocyte-specific glutamine synthetase (GS) holds a key role in glutamate recycling, providing also precursors for GABA and glutathione metabolism. Therefore, changes in the expression or activity of GS are proposed to contribute to the pathology of epilepsy. However, clear evidence for the mechanisms involved is still lacking, except for a reduced expression in sclerotic hippocampal tissue (Eid et al. 2004), whereas in other conditions no alterations have been found (Steffen et al. 2005). Here we used the rat model of repeated, PTZ-induced epileptic seizures (Bidmon et al. 2005, 2004) to investigate how these seizures may affect astrocytic GS. Saline treated rats and rats treated with the specific inhibitor of GS, L-methionine sulfoximine (L-MSO) served as controls. Similar to our previous studies PTZ-induced seizures resulted in a focal heat shock response in affected astrocytes as seen by increased HSP-27 induction, whereas L-MSO induced GS-inhibition caused a widespread much more homogeneous astrocytic HSP-27 induction throughout the whole brain. There occurred no changes in GS-immunoreactivity (GSIR) during repeated treatment with PTZ compared to the highly significant reduction of GSIR after treatment with L-MSO in situ. Employing quantitative, standard western blotting (WB), no changes in the GSIR were found for all treatment conditions, whereas the dot-blot technique, using native protein extracts reproduced the loss of GSIR seen in situ. These data clearly show that neither PTZ-induced seizures nor GS-inhibition by L-MSO results in a loss of GS but that L-MSO interferes with antibody binding resulting in a loss of GSIR in situ. L-MSO treatment (100mg/kg) reduced GS-activity to 20-30%, whereas PTZ-treatment resulted in a slight non-significant reduction of GS-activity in whole cerebral cortex and hippocampus. However, when we specifically resected the piriform-entorhinal cortices from PTZ-treated rats in which the packing density for HSP-27 positive astrocytes is highest, we found a highly significant reduction of GS-activity (57% of control) indicating that PTZ-induced seizures do affect GS-activity selectively within affected astrocytes without interfering with GSIR. As revealed by immunoprecipitation this inhibition has been caused by the nitration of tyrosine-residues within the GS-protein providing direct evidence that PTZ-induced seizures affect astrocytes focally within the epileptic circuitries by oxidative and nitrosative stress and that GS is among the nitrated proteins. This nitration-induced inhibition of GS may result in a reduction of a timely glutamate clearance from the synaptic cleft by perisynaptic astrocytes which may contribute to the progression of further pathological consequences. These data further indicate that various cerebral pathological processes which result in nitrosative stress and which occur in regions belonging to the so called epileptic circuitry could set the starting point for seizure activity.

Eid et al., (2004) Lancet; 363: 28-37; Steffens et al., (2005) Neurochem Int.;47: 379-84; Bidmon et al., (2005) J. Chem. Neuroanat., 30: 1-16; (2004) Epilepsia 45:1549-59.

# Characterization of a novel synaptic vesicle protein identified by a proteomic approach

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Synaptic transmission relies upon synaptic vesicles, abundant organelles of the presynaptic nerve terminal of neurons. Therefore, the analysis of the synaptic vesicle proteome is essential for identifying components involved in vesicle mobilization, migration to the presynaptic plasma membrane, docking and fusion as well as recycling of synaptic vesicles via endocytosis. To date, a considerable variety of synaptic vesicle proteins has been identified that govern these processes.

To identify novel synaptic vesicle proteins, we isolated synaptic vesicles from rat brain synaptosomes to high purity. Vesicles were separated according to density (sucrose density gradient centrifugation) and specific protein contents (immunopurification). For protein analysis, we subjected synaptic vesicle proteins to different gel-based approaches in combination with mass spectrometry. Novel proteins were characterized by immunoblotting, immunocytochemistry and immunohistochemistry. In addition, the tissue expression was investigated by RT-PCR.

Out of a total of 185 proteins we identified eight hitherto unknown proteins. One of these proteins (SV35) was subjected to a detailed characterization. RT-PCR data suggests a restricted tissue expression. mRNA can be detected at high levels in all brain regions. Analysis of the subcellular distribution of recombinant SV35 in PC12 cells revealed an association with punctuate and reticular structures and suggests a vesicular distribution. The protein is enriched in growth cones and colocalizes with endogenously expressed synaptic vesicle proteins. Immunohistochemical analyses of rat brain slections revealed a restricted distribution within a subpopulation of nerve terminals.

Taken together, these data suggest that SV35 is a synaptic vesicle protein that is localized to subpopulations of neurons. Further analyses focus on functional aspects of the newly identified protein.

# mAbp1 deficiency suggests a role for actin in vesicle fission

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Clathrin-mediated endocytosis plays a fundamental role in presynaptic vesicle cycling. A large variety of proteins and protein complexes participates in regulation of this process. To clarify the possible role of actin in clathrin-mediated endocytosis, we focussed on proteins, which could functionally link the actin network to the endocytic protein machinery. Mammalian Abp1 (mAbp1) specifically binds F-actin with a stoichiometry of 1:5 by two different N-terminal domains as well as dynamin via its SH3 domain. Knock-out of mAbp1 lead to a moderate reduction of synaptic vesicle endocytosis and a severe delay of vesicle recycling in cultured hippocampal neurons (Connert, S., et al.(2006) The EMBO journal 25, 1611-1622).

Ultrastructural analysis of mAbp1 KO hippocampal synaptic boutons did not show any significant changes in the total number of synaptic vesicles per 1 µm of synapse area compared to control. However we observed a significant increase in synaptic vesicle size as well as an accumulation of endosome-like structures (>60 nm), presumably endocytic intermediates, in synaptic boutons even under resting conditions. These cisternae may either represent true endosomal intermediates or, more likely, may arise from bulk endocytosis of large infoldings from the plasma membrane. Upon stimulation with 900 APs three-fold more such intermediates are found in mAbp1 KO hippocampal synaptic boutons compared to wild type. Notably the number of visibly docked or vesicles closer than half of the vesicle diameter to the plasma membrane per active zone length was not changed in knockout synapses compared to wild-type under resting conditions, whereas 900 APs stimulation lead to a complete depletion of vesicle pools at the active zone in knockout synapses.

When overall endocytic activity was measured using synaptopHluorin, a fusion construct of the vesicle protein synaptobrevin and a pH-sensitive form (pHluorin) of GFP, a significant slowing of endocytosis in mAbp1 knockout neurons was observed.

Thus, the physiological and ultrastructural data demonstrate an important role of mAbp1 and hence actin in clathrin-mediated endocytosis of synaptic vesicles.

# Hierarchical ability of SNAP-25 homologues to support neuronal function

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The neuronal SNARE complex, consisting of SNAP-25, synaptobrevin and syntaxin, is required for presynaptic exocytosis of neurotransmitter-filled vesicles during synaptic transmission. However the SNARE complex undertakes neuronal developmental roles as well. SNAP-25 has been involved in vesicular fusion during axonal outgrowth, synaptic formation and trafficking of glutamate receptors. Its expression is developmentally regulated by alternative splicing from SNAP-25a to SNAP-25b. In GABAergic neurons, SNAP-25 seems to be replaced by SNAP-23, a ubiquitous SNAP-25 homologue, after synaptogenesis. The development of a Snap25 knock-out mouse line has provided a genetically clean background in which SNAP-25 can be studied (Washbourne et al., 2002). However, deletion of SNAP-25 in cultured neurons compromised arborisation, leading to a dramatic reduction in survival after 5-6 days. Here, this loss was recovered by early reintroduction of SNAP-25a, SNAP-25b or SNAP-23 using the long-term expression lentiviral system. Surviving knock-out neurons presented complete arrest of evoked release, decreased frequency of spontaneous release and 50% reduction in the size of single vesicle events. Overexpression of SNAP-25 isoforms restored synaptic transmission in both glutamatergic and GABAergic neurons. However, SNAP-23 overexpression was unable to recover synchronous release. Evoked responses were slow and attenuated (0.198±0.046 nA, n=52), resembling those observed in synaptotagmin-I null neurons (Geppert et al., 1994). Evoked responses driven by SNAP-25a were smaller than by SNAP-25b (1.94±0.22 nA, n=43; 2.88±0.30 nA, n=53, respectively; $p<0.02$ 2-way ANOVA). This reduction was accompanied by unchanged synaptic density and release probability and an overall reduction in the readily-releasable pool measured either by functional staining with styryl dyes or depletion by a hyperosmolar solution or by electrical stimulation. This finding would indicate that developmental splicing of SNAP-25 produces larger releasable pools after synaptic maturation.

The results revealed SNAP-25 as key component for neuronal survival and outgrowth, regulation of the synchronous and asynchronous release and spontaneous activity and demonstrate a hierarchical ability of the SNAP-25 homologues to support neuronal function.

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# Adenoviral expression of multiple proteins of interest to study exocytosis in chromaffin cells

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Catecholamine release from large dense-core vesicles in chromaffine cells is often employed as a model system to study exocytosis. Recent progress in identifying the molecular components of the release machinery indicates that vesicle fusion requires the orchestrated interaction of multiple proteins. So far, however, insights into the molecular mechanism of vesicle fusion have been mostly gained by manipulating only a single protein at a time, either by changing its expression level or by introduction of a protein variant bearing site-directed mutations. Naturally, it would be extremely useful to target multiple components of the release machinery in parallel, e.g. to manipulate expression levels of two different proteins independently of each other. Here, we present a strategy to use adenoviral gene transfer to simultaneously express two proteins in chromaffine cells under the control of CMV promotors. It is well established that the formation of the SNARE complex is at the heart of the fusion machinery. SNAP25, whose two SNARE domains participate in the formation of this complex, is required for dense-core vesicle exocytosis, and was therefore used to test the feasibility of adenoviral gene transfer to chromaffin cells. In a first experiment we established that adenoviral expression of SNAP25 can restore secretion in cultured chromaffine cells derived from SNAP25 deficient mice. To monitor expression we tagged SNAP25 via N-terminal fusion to eGFP or mCherry. Mouse chromaffin cells were infected after one day in vitro or earlier, and first fluorescent signals could be detected 12-24h later depending on the used virus titer. A solid rescue of dense-core vesicle exocytosis could be demonstrated by capacitance measurements and amperometric recordings around 36-48h post infection. As expected eGFP SNAP25 was localized to the plasmalemma of chromaffine cells. We noticed that infected cells exhibited a broad distribution of fluorescence intensities even when exposed to a higher virus titer (>3*109 particles/2ml Medium). Moreover, we observed very limited morphological changes in infected cells, which sets the adenoviral system off against Semliki-Forrest virus infections. Using two adenoviruses expressing eGFP- or mCherry-tagged SNAP25 versions we examined whether a double infection of chromaffine cells is effective. Indeed, corresponding fluorescence signals indicated the presence of both tagged SNAP25 variants in the vast majority of infected cells. Using equal amounts of viruses for infection, we observed that both proteins were expressed at considerably varying ratios in different chromaffin cells. This spectrum of expression ratios could be further manipulated by infection with different amounts of both types of virus particles.

In summary, our results suggest that the adenoviral infection of chromaffine cells is a valid alternative to the established viral expression systems for chromaffin cells. Especially, multiple infections with adenoviruses allow for new exciting possibilities to study exocytosis in chromaffine cells.

# Disruption of clathrin-mediated endocytosis in hippocampal synapses causes a shift to a bulk endocytotic mode of membrane retrieval, but not to kiss and run

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Clathrin-mediated endocytosis (CME) is a major form of synaptic vesicle retrieval pathway. Alternatively, a fast mechanism, where the vesicles connect to the plasma membrane only transiently, without full collapse ('kiss-and-run'), might restore vesicular components more efficiently. To determine the possible contribution of a clathrin-independent mechanism like kiss-and-run, we sought to interfere with CME by perturbing the molecular interactions between the accessory protein amphiphysin 1 and proteins of the clathrin coat. Mice hippocampal synapses were transfected with either the amphiphysin SH3 domain (that binds a PxRPxP sequence of dynamin) or the B/C fragment (containing clathrin/AP2-binding sites). Endocytosis was assayed by fluorescence imaging using FM 1-43 and the exo-endocytic fluorescent probe synaptopHluorin as well as by electron microscopy. Overexpression of the SH3 domain of amph1 impaired membrane retrieval after stimulation. Overexpression of the B/C domain of amph1 containing clathrin/AP2 binding sites dramatically altered synaptic vesicle turnover. The total number of stained, i.e. recycled vesicles reached already a plateau for short stimuli ≥ 40 action potentials. Overexpression of either interfering peptide slowed down endocytosis modestly, when assayed by FM 1-43, but dramatically, when measured with synaptopHluorin. Moreover during prolonged stimulation the regeneration of fusion-competent synaptic vesicles was severely impaired and delayed four-fold in terminals over-expressing the B/C domain of amph1. The striking difference between the results obtained with FM 1-43 and synaptopHluorin after perturbing CME could be explained, if excess membrane gave rise large infoldings, later retrieved by bulk endocytosis. In fact ultrastructural analysis of synaptic boutons revealed more large (≥ 60 nm) endosome-like profiles after stimulation, while the numbers of synaptic vesicle profiles per synapse area was only modestly decreased prior to stimulation, and their distribution was normal. These findings are in conflict with a kiss-and-run mechanism of vesicle retrieval and support the notion that CME is the dominant form of synaptic vesicle recycling under physiological conditions, while bulk endocytosis may play a role when CME is impaired or saturated, like under strong stimulation.

# Dendritic mRNA transport: *cis*-elements, *trans*-factors and motor proteins

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In neurons, the diverse protein composition of distinct cellular regions demands elaborate sorting mechanisms. In addition to intrinsic protein targeting signals, cytoplasmic localization and local translation of mRNAs contributes to differential protein sorting. In contrast to most neuronal mRNAs, which are restricted to somata, transcripts encoding the microtubule-associated proteins 2 (MAP2) are found in dendrites. Along dendritic shafts, MAP2-mRNAs form granules that appear to serve as transport units. A cis-acting dendritic targeting element (DTE) in the 3'-untranslated region of MAP2-transcripts mediates dendritic localization in neurons. Two 90 and 65 kDa MAP2-RNA trans-acting proteins, MARTA1 and MARTA2, specifically interact with the MAP2-DTE. In neurons, MARTA1 preferentially resides in the nucleus, whereas MARTA2 is a nucleocytoplasmic protein that is mainly present in the somatodendritic cytoplasm where resides in granular structures. In primary neurons, overexpression of a truncated MARTA2 isoform that contains only the RNA-binding domains exerts a strong dominant-negative effect as it completely disrupts dendritic targeting of endogenous MAP2 mRNAs. In contrast, extrasomatic trafficking of MAP2 transcripts is not affected by overexpression of two other RNA-binding proteins. Furthermore, truncated MARTA2 does not noticeably alter the concentration and subcellular distribution of the entire pool of polyadenylated mRNAs. Finally, dominant-negative kinesin I, but not dynamitin, disrupts extrasomatic trafficking of MAP2 mRNA granules. Thus, in neurons kinesin I and MARTA2 appear to act in concert to specifically target MAP2 mRNAs into dendrites. Supported by the Deutsche Forschungsgemeinschaft (Ki 488/2-6)

# The Voltage-Gated Calcium Channel is the Calcium Sensor Protein of Secretion

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The coupling of voltage-gated $Ca^{2+}$ channel (VGCC) to exocytotic proteins suggests a regulatory function for the channel in depolarization-evoked exocytosis. To explore this possibility we have examined catecholamine secretion in PC12 and chromaffin cells. We found that replacing $Ca^{2+}$ with $La^{3+}$ or other lanthanide ions, supported exocytosis in divalent ion-free solution. $Cd^{2+}$, nifedipine, or verapamil inhibited depolarization-evoked secretion in $La^{3+}$, indicating specific binding of $La^{3+}$ at the pore of L-type VGCC, most likely at the poly-glutamate (EEEE) locus. Lanthanide efficacy was stringently dependent on ionic radius with $La^{3+} > Ce^{3+} > Pr^{3+}$, consistent with a size selective binding interface of trivalent cations at the channel pore. $La^{3+}$ inward currents were not detected and the highly sensitive $La^{3+}$/fura-2 imaging assay (~1 pM) detected no $La^{3+}$ entry, cytosolic $La^{3+}$-buildup, or alterations in cytosolic $Ca^{2+}$. These results provide strong evidence that occupancy of the pore of the channel by an impermeable cation leads to a conformational change that is transmitted to the exocytotic machinery upstream to intracellular cation buildup ($[Ca^{2+}]_i$). Our model allows for a tight temporal and spatial coupling between the excitatory stimulation event and vesicle fusion. It challenges the conventional view that intracellular $Ca^{2+}$ ion-buildup via VGCC permeation is required to trigger secretion and establishes the VGCC a plausible $Ca^{2+}$ -sensor protein in the process of neuroendocrine secretion. Acting simultaneusly as the voltage-sensor and the $Ca^{2+}$-sensor protein of secretion, the channel controls both the initiation and termination of the release process.

Lerner I, Trus M, Cohen R, Yizhar O, Nussinovitch I, Atlas D. Ion interaction at the pore of Lc-type Ca2+ channel is sufficient to mediate depolarization-induced exocytosis. J Neurochem. 97:116-27 (2006)

# TRPC channel-mediated signaling in cerebellar Purkinje cells

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Cation channels of the TRPC subfamily are widely expressed in the brain. However, their functional roles are largely unknown. It has been suggested that the mGluR-dependent slow excitatory postsynaptic currents (sEPSCs) require the activation of the TRPC1 subunit (Kim et al., *Nature* 2003, 426: 285). In order to determine the role of TRPC channels *in vivo*, we studied mGluR-dependent synaptic transmission in mice deficient for different TRPC-subunits (combinations of TRPC1-/-, TRPC4-/- and TRPC6-/-) by using whole-cell recordings and $Ca^{2+}$ imaging in acute cerebellar slices. Surprisingly, we found that repetitive parallel fiber stimulation-evoked sEPSCs persist in these mutant mice, indicating that non of these TRPC channel subunits is needed for sEPSC function. Furthermore, the mGluR1-specific agonist DHPG, when pressure-ejected to dendrites of Purkinje cells, was still able to activate robust sEPSC-like inward currents in the absence of TRPC1, TRPC4 and TRPC6.

Because $Ca^{2+}$-permeable receptor-operated channels have been implicated in the maintenance of $Ca^{2+}$ homeostasis in intracellular $Ca^{2+}$ stores in other cell types, we tested the role of TRPC1, TRPC4 and TRPC6 in store refilling in Purkinje cells. We found that somatic store replenishment following depletion with caffeine is functional in the absence of all three TRPC subunits. However, in Purkinje cell dendrites in the absence of TRPC1, TRPC4 and/or TRPC6 subunits the rundown of mGluR-mediated $Ca^{2+}$-release signals when repeatedly evoked with DHPG is faster than in wild type mice. This suggests that TRPC subunits contribute to dendritic store refilling.

We next determined the expression pattern of all TRPC subunits in Purkinje cells. For this purpose we used a recently published approach for quantitative single cell RT-PCR analysis that involves the harvesting of individual neurons from living brain slices (Durand et al., *Pflügers Arch.* 2006, 451: 716). The single cell RT-PCR analysis demonstrated that, surprisingly, TRPC3 is the by far dominating TRPC subunit expressed in Purkinje cells. TRPC1, TRPC4 and TRPC6 are also present, but much less abundantly found in this cell type. TRPC5 was not detected. Taken together, our results fail to support the earlier suggestion of an important role of TRPC1 channels for sEPSC activation, but, instead, reveal a new role of various TRPC subunits in $Ca^{2+}$ store signaling in cerebellar Purkinje cells. Importantly, we identified TRPC3 as the dominating subunit expressed in these cells. Work concerning the role of TRPC3 is in progress.

# Overexpression of individual gamma-Protocadherins in Transgenic Mouse Models

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Three sequential clusters of protocadherins (Pcdhα, Pcdhβ, Pcdhγ) comprise more than 50 genes in man and mouse in a striking genomic organization. The Pcdhγ cluster contains 22 arrayed Pcdh genes (termed "variable" exons), which are each spliced to three separate exons (termed "constant" exons) that code for a common cytoplasmic domain. Pcdhγ proteins are strongly expressed in neurons of the developing and adult nervous system and are localized to subsets of synapses, consistent with functions in neuronal differentiation and synaptic adhesion. Loss of Pcdhγ function in genetically altered mice leads to early postnatal lethality and severe degeneration of the spinal cord.

For our overexpression approach, two genes of the Pcdhγ cluster, PcdhγC3 and PcdhγC5, were selected. The cDNA of PcdhγC5, tagged with the yellow fluorescent protein (YFP), was inserted into the ROSA26 locus via homologous recombination in ES cells (knock-in). The ROSA26 promoter is ubiquitously expressed during embryonic and postnatal development of the mouse. Whereas PcdhγC5 has a late postnatal onset in the wildtype, its precocious expression in the knock-in mice does not yield an overt phenotype. Analysis of the transgenic PcdhγC5-YFP proteins in different tissues suggests that Pcdhγ expression is regulated by a post-transcriptional, tissue-specific mechanism. For example, moderate transcript expression but highest expression of PcdhγC5-YFP is found in the brain, the preferred site of wildtype Pcdhγ expression. In contrast, PcdhγC5-YFP is undetectable in the liver, as are wildtype Pcdhγ proteins, although high levels of the recombinant PcdhγC5-YFP transcript are made in this tissue.

Similarly, we have established a conventional transgenic mouse line expressing PcdhγC3 tagged with the hemagglutinin sequence (HA) under the control of a chicken β-actin promoter. Again, these mice were viable and showed no overt phenotype, despite high levels of recombinant PcdhγC3 expressed in the brain.

When we crossed our Pcdhγ-overexpressing mice to a gene-trap mouse line with loss of Pcdhγ function we found that the expression of PcdhγC5-YFP or of PcdhγC3-HA was not able to rescue the lethal phenotype in homozygous gene-trap mice. However, the expression of such single Pcdhγ genes in the null background of the gene-trap mice will be a valuable tool to further analyze functions and interaction partners of individual Pcdhγ.

Supported by the Max-Planck Society.

# ubMunc13-2 enhances vesicles recruitment in a calmodulin dependent manner

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Catecholamine release from chromaffin cells is coordinated by a large number of proteins and involves the fusion of large dense core vesicles with the cell plasma membrane. We study the roles of Munc13 in exocytosis and the effects of calcium on these processes. Munc13 protein family contains a conserved calmodulin-binding domain and calcium-binding motifs. As calcium is known to accelerate vesicle priming in chromaffin cells, we investigated the physiological relevance of calmodulin binding to Munc13 activity. The exocytotic response of cells overexpressing ubMunc13-2W387R, a mutant that is calmodulin-binding deficient, was considerably lower compared to the responses of cells overexpressing the WT protein, yet still larger as compared to control cells. To evaluate if Munc13's priming activity depends on [Ca2+], exocytosis was evoked under conditions of low basal calcium concentration (<100nM), conditions that do not support vesicle priming. Under these conditions, both proteins showed proportionally reduced priming activity. However, when calcium was kept high for several seconds, secretion accelerated only in cells overexpressing the wild type ubMunc13-2 (that bind calmodulin), giving rise to S-shaped release kinetics. These results demonstrate that the interaction of calmodulin with ubMunc13-2 enhances vesicle recruitment at high [Ca2+]. Next, we kinetically analysis the result using our new kinetic model that is comprehensibly reproduces the dynamics of exocytosis with high accuracy. Using the model we conclude that calmodulin's interaction with ubMunc13-2 has a regulatory nature and that this interaction is important for boosting the activity of ubmunc13-2 when intracellular [Ca2+] rises. Furthermore, it seem that the ubMunc13-2 have a unique effect on the release which is different from the WT chromaffin cells.

# Symposium

## S8: Olfactory Development: Common Principles and Differences across Phyla

***Joachim Schachtner and Wolfgang Rössler, Marburg and Würzburg***

### Slide

**S8-1** Opening remarks for Symposium 8
*Joachim Schachtner and Wolfgang Rössler, Marburg and Würzburg*

**S8-2** Molecular basis of bidirectional neuron-glia signaling in the developing olfactory system of the moth *Manduca sexta*
*Leslie P. Tolbert, Lynne A. Oland, Nicholas J. Gibson, Mark R. Higgins and Alan Nighorn, Tucson (USA)*

**S8-3** How the fly's brain knows what the fly's nose knows
*Gregory S. X. E. Jefferis, Cambridge (UK)*

**S8-4** Brain targeting and glomerulus formation of olfactory neuron populations in mouse
*Jörg Strotmann, Olga Levai, Sidonie Conzelmann, Karin Schwarzenbacher, Jörg Fleischer and Heinz Breer, Stuttgart*

**S8-5** Developmental control of zebrafish olfactory receptor gene repertoires and odor responses
*Sigrun I. Korsching, Köln*

**S8-6** Concluding remarks to Symposium 8
*Joachim Schachtner and Wolfgang Rössler, Marburg and Würzburg*

### Poster

**TS8-1B** A novel olfactory receptor gene family in teleost fish
*LR. Saraiva and SI. Korsching, Cologne*

**TS8-2B** Odor tuning curves for zebrafish olfactory receptor neurons in vivo
*C. Potting, L. Saraiva and SI. Korsching, Köln*

**TS8-3B** Functional characterisation of an universal
olfactory receptor class C promoter
*YY. Kwan, Koeln*

**TS8-4B** Semaphorin-1a and Dscam control synaptic specificity in Drosophila olfactory system development
*T. Hummel, Münster*

**TS8-5B** Pupal staging and metamorphic development of the antennal lobes of the red flour beetle *Tribolium castaneum*
*S. Dippel, D. Dreyer, W. Huetteroth and J. Schachtner, Marburg*

**TS8-6B** The *Tribolium* brain: 3D reconstruction and immunocytochemical mapping during metamorphosis and in the adult
*D. Dreyer, S. Dippel, W. Huetteroth and J. Schachtner, Marburg*

**TS8-7B** 3D reconstruction of *Manduca sexta* adult brain and of brains during metamorphic development
*B. el Jundi, W. Huetteroth and J. Schachtner, Marburg*

**TS8-8B** Time course of NO dependent cGMP regulation and its influence on antennal lobe neuropil development in the sphinx moth *Manduca sexta*
*W. Huetteroth, S. Utz and J. Schachtner, Marburg*

**TS8-9B** Promotor-motifs governing the spatial expression pattern of olfactory receptors
*YQ. Zhang, H. Breer and J. Strotmann, Stuttgart*

**TS8-10B** Psychophysical evidences of the cross-modal interaction of human faces and of smelling gender specific sex hormon-like substances
*Z. Zsadányi-Nagy and G. Kovács, Szeged (H) and Budapest (H)*

**TS8-11B** Short range detection and neural representation of nestmate recognition cues in the ant *Camponotus floridanus*
*AS. Brandstätter and CJ. Kleineidam, Würzburg*

**TS8-12B** Caste-specific differences in synaptic development within olfactory neuropils of the female honeybee brain
*C. Groh and W. Rössler, Würzburg*

**TS8-13B** Plasticity of synaptic microcircuits in the mushroom-body calyx of the honeybee brain
*T. Münz, C. Groh and W. Rössler, Würzburg*

**TS8-14B** Neuromodulation and synaptic plasticity within olfactory centers in the brain of the carpenter ant, *Camponotus floridanus*
*C. Ziegler, NK. Starke, C. Zube, S. Kirschner and W. Rössler, Würzburg*

**TS8-15B** Organization of glomeruli and olfactory processing of trail pheromone in the ant, *Camponotus floridanus*
*C. Zube, S. Kirschner, J. Neef, C. Kleineidam and W. Rössler, Würzburg*

**TS8-16B** Neuroanatomical sub-castes in leaf-cutting ants:
Differences in antennal lobe design correlate with olfactory guided behavior
*LS. Kuebler, C. Kelber, W. Roessler and CJ. Kleineidam, Wuerzburg*

Introductory Remarks to Symposium 8

# Olfactory Development: Common Principles and Differences across Phyla

Joachim Schachtner and Wolfgang Rössler, Marburg and Würzburg

The primary integration centers for olfactory information in the brain of vertebrates (olfactory bulb) and insects (antennal lobe) not only share their principal morphological organization into so called olfactory glomeruli, but also a number of basic physiological properties with respect to information processing. Glomeruli represent functional units for odor processing containing thousands of synapses between olfactory receptor neurons (ORNs) from the olfactory epithelium / antenna and neurons of the olfactory bulb / antennal lobe. Each glomerulus receives input from ORNs expressing particular odorant receptors. Odors are finally encoded by activation patterns of defined sets of glomeruli, resulting in a spatial odor map and a chemotopic representation of odor information in the brain.
Despite great efforts of many groups working with various animal models over the years, it is still not clear how these odor maps form during development, how the neuronal network in and between the glomeruli is established. Comparative approaches on the formation of chemotopic maps in olfactory systems are not only important for understanding general principles in the wiring of brains, but also become more and more of public interest as the importance of olfaction for ecosystems and human health and wellness emerges. In this symposium we compare knowledge on the ontogeny of the olfactory bulb and the antennal lobe from four important model systems for olfactory development - mouse, zebrafish, fruitfly and sphinx moth. Four representative speakers, well known in their fields will give state of the art insights into olfactory system development in the four animal models. The organizers will add an introduction and a conclusion to underline similarities and obvious differences obtained from the different systems.

Leslie Tolbert will summarize recent work in the sphinx moth on problems of receptor-axon sorting and glomerular targeting. Gregory Jefferis will discuss the logic of wiring specificity in the Drosophila antennal lobe and compares this with other insects and vertebrates. Jörg Strotmann will give latest insights on developmental expression patterns of odorant receptors and projection patterns of ORN axons in the mouse olfactory bulb. Sigrun Korsching will highlight recent work on the expression of odorant receptors during development of the zebrafish olfactory pathway.

# Molecular basis of bidirectional neuron-glia signaling in the developing olfactory system of the moth *Manduca sexta*

Leslie P. Tolbert, Lynne A. Oland, Nicholas J. Gibson, Mark R. Higgins and Alan Nighorn

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As they extend toward their CNS targets, the axons of olfactory receptor neurons (ORNs) in many species sort by odor specificity, grow to particular sites in the target neuropil, and induce the formation of synaptic glomeruli. In the experimentally advantageous moth *Manduca sexta*, developing olfactory neurons influence glial-cell behaviors, which in turn influence neuronal growth behavior. We are exploring the molecular bases for these cellular events.

Previous work (reviewed by Oland & Tolbert, Annu. Rev. Entomol. 48:89-110, 2003; Tolbert et al., Prog. Neurobiol. 73:73-105, 2004) has shown that axonal sorting occurs in a discrete, glia-rich region where the nerve enters the antennal lobe of the brain. ORN axons entering this "sorting zone" (SZ) dramatically change their trajectories and sort into fasciclin II-positive and fasciclin II-negative bundles after encountering the glial cells. Glia-reduction experiments have shown that the glial cells are essential for axonal sorting. Recent experiments show that fibroblast growth factor receptors (FGFRs) on the SZ glia and epidermal growth factor receptors (EGFRs) on ORN axons are phosphorylated, a sign of activation, in the SZ, precisely where the axons express a Triton-resistant form of the IgCAM neuroglian. Pharmacological disruption of EGFR signaling leads to axon stalling and abnormal fasciculation in the SZ and to small glomeruli in the antennal lobe (Gibson & Tolbert, 2006, J. Comp. Neurol. 495:554-572). Using parallel *in vitro* and *in vivo* experiments, we have adduced evidence that SZ glial cells alter axonal behavior in part by regulating interactions between the IgCAMs, which in turn affect activation of growth factor receptors. Control of this signaling pathway appears to be exerted by inclusion of these membrane-associated molecules in lipid rafts, since, when raft assembly is pharmacologically disrupted, axonal growth into the lobe and the glomerular array are abnormal.

Our previous work also revealed that, as they grow into the antennal lobe, ORN axons navigate through another set of glial cells, the neuropil-associated glia, and then form the protoglomeruli that are the templates for mature glomeruli. Here, too, reciprocal interactions are critical: the presence of the axons causes neuropil-associated glial cells to migrate to surround the protoglomeruli, and the glial envelope then is essential to constrain the growth of the dendrites of target central neurons. Nitric oxide appears to be a key factor in the signal telling glial cells to migrate. Recent studies show that neuroglian is expressed transiently in the axon terminals as the protoglomeruli develop and appears in neuropil-associated glial cells as they migrate, and that activated EGFRs are present during dendritic growth and synapse formation in the maturing glomeruli. Our results thus suggest that molecular interactions among IgCAMs and growth factor receptors may also underlie the neuron-glia interactions affecting glomerulus formation and maturation.

Supported by NIH DC004598 and P01-NS28495.

# How the fly's brain knows what the fly's nose knows

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The fruitfly brain receives information about the olfactory world through the activity of about 50 distinct channels of incoming information. The olfactory receptor neurons that define each channel have their own distinctive odour response profile governed by a specific seven-transmembrane receptor protein. The axons of these receptor neurons form highly specific connections in the first olfactory relay of the fly brain, the antennal lobe, each synapsing with specific second order partner neurons.

I will use our own results from the *Drosophila* system to discuss the logic of wiring specificity in the brain and to review the cellular and molecular mechanisms that allow such precise wiring to develop, before making comparisons with other insects and vertebrates. A key theme will be how developmental and organisational principles relate to the ability of the central brain to read and interpret incoming sensory information.

# Brain targeting and glomerulus formation of olfactory neuron populations in mouse

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Olfactory information is conveyed from the nasal neuroepithelium to the main olfactory bulb, the first relay station in the brain, via axons of the olfactory sensory neurons. Each neuron extends a single axon to the bulb where it synapses onto second order neurons in a spherical region of neuropil, called glomerulus. A glomerulus represents the site of convergence for neurons expressing the same olfactory receptor. The glomeruli from neurons expressing the highly homologous receptors of the mOR37 subfamily are located in immediate vicinity, nevertheless each glomerulus receives input with very high precision from only one, receptor-subtype specific population of neurons. To monitor the unfolding of this precise olfactory map during development, the onset of receptor expression, outgrowth of axons as well as glomerulus formation for two neuron populations expressing different mOR37 subtypes was investigated on transgenic mouse lines. The data indicate a synchronous onset of receptor expression at about embryonic day 10 (E10). From E15, axons of both populations terminate in a common, small area of the presumptive olfactory bulb. During a short postnatal phase, the two axon populations segregate into distinct, protoglomerular structures.

Between E11 and 16, populations of cells expressing distinct olfactory receptor types are located in the cribriform mesenchyme, between the prospective olfactory epithelium and the developing telencephalon. Molecular phenotyping demonstrated that these 'extraepithelial' cells co-express key elements characteristic for neurons in the nasal epithelium. Studies on transgenic mice showed that they are positioned along the axon tracts, and each population expressing a given receptor gene is specifically associated with the axons of those olfactory sensory neurons with the same receptor type. The data suggest that they either might be guide posts for the outgrowing axons or migrate along the axons into the brain.

This work was supported by the Deutsche Forschungsgemeinschaft, SFB 495.

# Developmental control of zebrafish olfactory receptor gene repertoires and odor responses

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Zebrafish, Danio rerio, is a simple vertebrate which has become an increasingly important model system to study olfactory coding. Fish smell water-borne odors which comprise several well-defined substance groups, including amino acids, nucleotides and bile acids. Olfactory receptor gene repertoires have been identified both by data mining and classical cloning techniques. However, with one exception no ligands are known for these receptor genes. Expression of olfactory receptor genes is regulated both spatially and temporally, but little is known about the mechanisms involved in this regulation. At the level of the olfactory bulb (first synapse) detailed studies have demonstrated the neuronal representation of different odors and odor groups. We have used data mining to identify a novel olfactory receptor gene repertoire. We have systematically examined ligand spectra of olfactory bulb response units (glomeruli) to infer characteristics of the underlying receptor gene repertoires. We have performed detailed quantitative developmental expression studies for several olfactory receptors to extract information about their regulation of expression. We are currently investigating developmental control of odor responses in an in vivo system.

Saraiva, L.R. and Korsching, S.I. (2006) A novel olfactory receptor gene family in teleost fish encompasses the whole mammalian V1R pheromone receptor superfamily, submitted.

Argo, S., Weth, F. and Korsching, S.I. (2003) Analysis of penetrance and expressivity during ontogenesis supports a stochastic choice of zebrafish odorant receptors from pre-determined groups of receptor genes, Eur J Neurosci.17, 833-843.

Fuss, S.H. and Korsching, S.I. (2001) Odorant feature detection: activity mapping of structure response relationships in the zebrafish olfactory bulb, J Neurosci. 21, 8396-8407.

# A novel olfactory receptor gene family in teleost fish

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Pheromones mediate sexual and social communication between individuals. Reproduction in fish is governed by a handful of identified pheromones, which actually are sexual hormones that double as ligands for as yet unidentified olfactory receptors. In mammals two large families of up to 200 vomeronasal receptor genes (V1Rs and V2Rs) may serve as pheromone receptors. V2R receptors have been recognized as an evolutionary old family - with about 50 members present already in several fish species, but an extensive search of the fish genome so far has unearthed only a single V1R-like receptor gene. Thus the V1R family appeared to be evolutionary rather recent, originating as a family with tetrapods. We now identified five ancestral V1R-related genes, with orthologues detected for each gene in every teleost species analysed (zebrafish, two pufferfish, medaka). These genes form a coherent family in the phylogenetic analysis, containing in a single branch the whole mammalian V1R superfamily.
Expression pattern, phylogenetic position and family size support a role as olfactory pheromone receptors.

# Odor tuning curves for zebrafish olfactory receptor neurons in vivo

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In recent years zebrafish, Danio rerio, has become an increasingly important model system to study information processing in the olfactory nervous system. Fish smell water-borne odors which comprise several well-defined substance groups, including amino acids, nucleotides and bile acids. Olfactory receptor gene repertoires have been identified both by data mining and classical cloning techniques. At the level of the olfactory bulb (first synapse) detailed studies have demonstrated the neuronal representation of different odors and odor groups. However, nearly nothing is known about the intervening steps between these two levels. Very few studies have analysed the odor tuning characteristics of single olfactory receptor neurons in any fish species and none in zebrafish. Ligands have been characterized only for a single olfactory receptor of class C. Here we report odor responses of single olfactory receptor neurons in vivo and compare their specificity to previously reported odor responses in the olfactory bulb.

Friedrich, R.W. and Korsching, S. (1997) Combinatorial and chemotopic odorant coding in the zebrafish olfactory bulb visualized by optical imaging, Neuron 18, 737-752.

Fuss, S.H. and Korsching, S.I. (2001) Odorant feature detection: activity mapping of structure response relationships in the zebrafish olfactory bulb, J Neurosci. 21, 8396-8407.

# Functional characterisation of an universal olfactory receptor class C promoter

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Teleost fish, in particular zebrafish, Danio rerio, have emerged as a valid model system to understand the mechanisms of odor processing in the brain. Odors are recognized by olfactory receptor neurons, which are divided into at least three different subtypes that differ with respect to morphology as well as molecular characteristics. These subtypes are composing neurons with long cilia, neurons of microvili and crypt cells which resemble both the cilia and microvili. Ciliated receptor neurons express class A olfactory receptor genes, whereas microvillous receptor neurons express class C olfactory receptor genes. Hence, it would be desirable to address these three subtypes separately in order to understand olfactory coding. This segregation requires the characterization of promoters that are specific for a particular subtype, and in addition, to be as ubiquitous as possible within the subtype. For example, OMP promoter serves such a role for ciliated receptor neurons and has been used successfully in many studies. The promoter region of a signal transduction component (TRPC2) has been used to drive expression in microvillous receptor neurons, while no suitable gene has so far been described for crypt cells, the third subtype of olfactory receptor neurons.

A small group of three genes with properties rather divergent from the main group was observed in a search for class C olfactory receptor genes. One of the genes displayed a broad but specific expression in microvillous receptor neurons. As a result, this gene is a suitable candidate to develop a microvillous receptor neuron-specific genetic marker. Consequently, BAC screening was performed and we observed that an upstream region consisting of several kb bases is sufficient for strong and specific expression.
Further characterisation is ongoing.

# Semaphorin-1a and Dscam control synaptic specificity in Drosophila olfactory system development

Thomas Hummel

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In the adult fly, olfactory receptor neurons (ORNs) project from the peripheral sense organs in a highly organized fashion into the first synaptic CNS region, the antennal lobe (AL). Here, all axons of functional similar ORNs sort out from the other 50 ORN classes and converge onto only one of the roughly 50 possible glomerular units, establishing synapses with AL relay neurons. Although strikingly similar to the mouse olfactory system organization, the development of the Drosophila odortopic map does not involve the function of the odorant receptors itself, suggesting the existence of a different regulatory mechanism to couple sensory and synaptic specificity during ORN differentiation.

To uncover the molecular basis of wiring and functional specificity in the Drosophila olfactory system, we have performed a large scale genetic mosaic screens and identified mutations that lead to ORN class specific changes in axon targeting or odorant gene expression. Further phenotypic and molecular characterization of the mis-targeting mutants uncovered members of 3 main families of neuronal receptors, the Cadherins, Plexin/Semaphorins and Ig-domain molecules. The combinations of differential loss-of-function and gain-of-function studies allowed us to distinguish between intra- and inter-glomerulus as well as axon-axon and axon-dendrite interactions mediated by these signaling systems. Based on these data a model of how synaptic specificity is achieved during fly olfactory system development will be presented.

# Pupal staging and metamorphic development of the antennal lobes of the red flour beetle *Tribolium castaneum*

Stefan Dippel, David Dreyer, Wolf Huetteroth and Joachim Schachtner

Biology - Animal Physiology, Philipps-Universität Marburg, Karl-von-Frisch Str., Marburg, Germany

*Tribolium castaneum* is emerging as a further standard insect model beside *Drosophila*. Its genome is fully sequenced and it is susceptible for genetic manipulations. *Tribolium* provides an ideally suited organism to study postembryonic brain development. As a beetle, *Tribolium* occupies a basal pyhlogenetical position among the holometabola and thus contrasts the highly derived Diptera. Developmental features of *Tribolium* resemble that observed in basal insects e.g. short-germ development, larval heads.

To study brain metamorphosis we developed a suitable staging system for *Tribolium* pupae based on easily recognizable external markers including eye pigmentation and sequential sclerotization of mouthparts, legs and wings (see figure).

During metamorphosis, the transition from the larva to the adult, the insect brain undergoes considerable remodeling: new neurons are integrated while larval neurons are remodeled or eliminated. The most obvious morphological changes in brain architecture concern brain neuropils which are newly formed during metamorphosis, including antennal lobes and optic lobes, centers responsible for olfactory and visual information processing.

As we are particularly interested in the development of the olfactory system we study the time course of metamorphic development of the antennal lobes of *Tribolium* and compare it with the antennal lobe development in holometabolous model insects including fruit fly, sphinx moth, and honey bee.

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# The *Tribolium* brain: 3D reconstruction and immunocytochemical mapping during metamorphosis and in the adult

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*Tribolium castaneum* is emerging as a further standard insect model beside *Drosophila*. Its genome is fully sequenced and it is susceptible for genetic manipulations. *Tribolium* provides an ideally suited organism to study postembryonic brain development. As a beetle, *Tribolium* occupies a basal phylogenetical position among the holometabola and thus contrasts the highly advanced Diptera.

The goal of this study was to provide suitable morphological references of defined brain areas at defined postembryonic stages including freshly emerged first instar larva, pupa of different stages, and adults of both sexes. To obtain the desired datasets we labeled whole brains with an antiserum against the synaptic vesicle protein synapsin to visualize neuropil areas, analyzed the staining using a confocal laser scanning microscope (Leica TCS SP2), and 3D reconstructed the major brain neuropils (including antennal lobes (ALs), mushroom bodies etc.; see figure) using AMIRA (Mercury).

As we are particularly interested in the development of the olfactory system we started to further characterize the olfactory glomeruli of the ALs with respect to their numbers, individual localization, shape, and volume. Additionally, we analyze the neurochemistry of the ALs using antisera against several neuromediators including biogenic amines, neuropeptides and the nitric oxide producing enzyme nitric oxide-synthase.

To the best of our knowledge this study provides for the first time data on brain morphology and neurochemistry of *Tribolium* and is thought as a basis for future genetic manipulation experiments.

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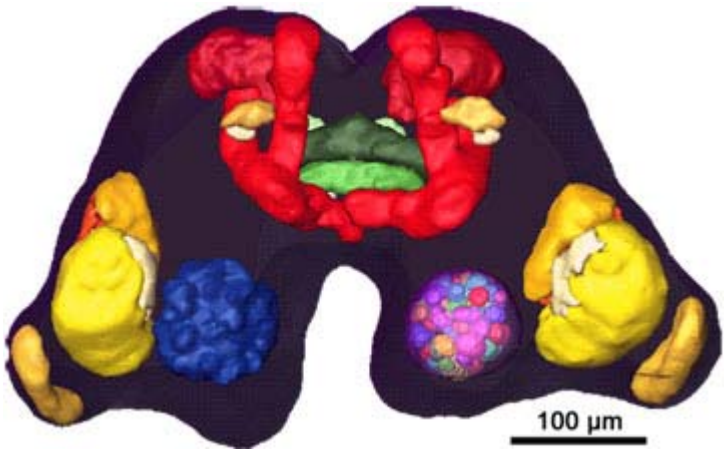

# 3D reconstruction of *Manduca sexta* adult brain and of brains during metamorphic development

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During metamorphosis, the transition from the larva to the adult, the insect brain undergoes considerable remodeling: new neurons are integrated while larval neurons are remodeled or eliminated. The most obvious morphological changes in brain architecture concern brain neuropils which are newly formed during metamorphosis, including antennal lobes and optic lobes, centers responsible for olfactory and visual information processing. One well acknowledged model to study metamorphic brain development is the sphinx moth *Manduca sexta*.

To further understand mechanisms involved in the metamorphic transition of the brain we 3D reconstructed selected brain areas of adults (see figure) and of defined stages during pupal development. Selected brain areas include for example mushroom bodies, central complex, antennal- and optic lobes. In addition to the 3D reconstructions of brain areas, we are mapping defined neuronal populations into these brain reconstructions.

With this approach we eventually want to quantify developmental changes in neuropilar architecture, but also quantify changes in the neuronal complement and in the projection pattern of selected neuronal populations or single identified neurons.

To obtain datasets of the *M. sexta* brain areas, we stained whole brains with an antiserum against the synaptic vesicle protein synapsin. To obtain datasets of defined neuron populations or single neurons we use antisera against various neuromediators, including antisera against biogenic amines, neuropeptides etc., or intracellular filled identified neurons. Such labeled brains were then scanned with a confocal laser scanning microscope and selected neuropils and neurons/ populations of neurons were reconstructed with the 3D software AMIRA 4.1.

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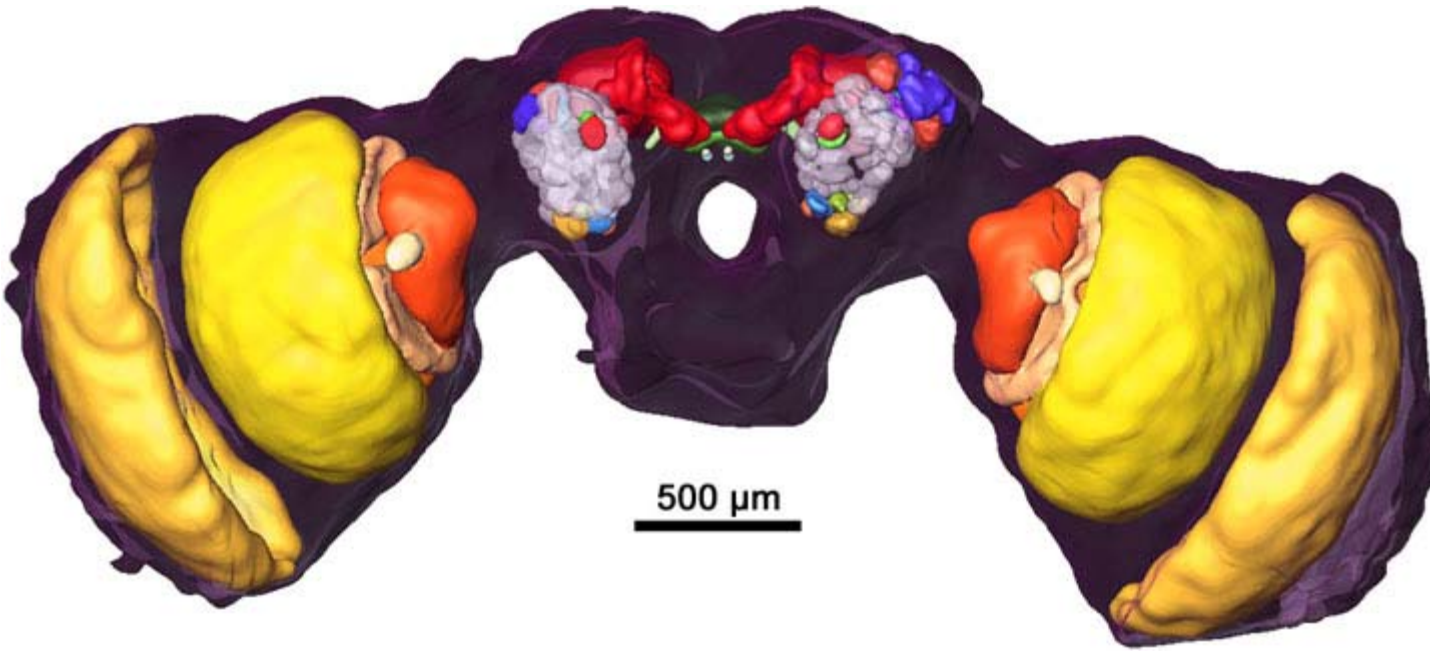

# Time course of NO dependent cGMP regulation and its influence on antennal lobe neuropil development in the sphinx moth *Manduca sexta*

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The antennal lobe (AL) is the first integration center for olfactory signals in insects. The neuropil of the AL of *M. sexta* is organized into about 63 glomeruli which are arranged around a core of coarse neuropil. Olfactory glomeruli are sphere-like structures, commonly found in most animal groups including the olfactory bulb of vertebrates and thus seem to be a universal feature for odor recognition. In *M. sexta* the AL is formed during 21 days of pupal development (P0 - P20). AL development can be subdivided in three main phases: in phase I (P0 - P7/8) all neurons comprising the AL are born and glomerular templates are laid out, during phase II (P7/8 - P12) the main wave of synaptogenesis occurs in the AL and glomeruli are formed, and finally in phase III (P12 - adult eclosion) the established synaptic network matures and undergoes further refinement.
Glomerulus formation in phase II is accompanied by transient cGMP elevation in a subset of local AL interneurons.
We defined time course and number of somata transiently upregulating cGMP during phase II. Of this neuronal population we determined a subset which upregulates cGMP dependent on antennal nerve activity. We showed that antennal nerve activity-dependent cGMP elevation is completely blocked with the soluble guanylyl cyclase inhibitor 1H-[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (ODQ). A possible synaptogenic effect of the nitric oxide (NO)/cGMP pathway in the AL was quantified using antibodies against the ubiquitous synapse proteins synaptotagmin and synapsin. Three different approaches were applied: ELISA of AL homogenates and optical density measurements in slices and defined 3D glomeruli as described previously. All methods indicate a reduction of synaptic marker antigenes in the AL after ODQ application.
[DFG Scha 678/3-3]

# Promotor-motifs governing the spatial expression pattern of olfactory receptors

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Odorant receptors (ORs) of the OR37 subfamily are only expressed in olfactory sensory neurons (OSNs) which are segregated within a small area in the center of olfactory epithelium. The encoding genes comprise highly conserved DNA motifs immediately upstream of the transcription start site which might be candidate elements for governing the spatial expression pattern. To scrutinize this hypothesis, transgenic mouse lines were generated which carry random integrated DNA constructs with the coding region of OR37C and the 5'-region including the conserved DNA motifs. In 6 out of 7 independent mouse lines, the transgene was found to be expressed in cells segregated in the characteristic clustered pattern. The number of transgene expressing OSNs varied considerably between the different lines. The transgene was expressed in a mutually exclusive manner and only one allele per neuron. The axons of transgene expressing OSNs in all mouse lines projected to the ventral domain of the olfactory bulb; those axons of OSNs located within the OR37 area generally co-converged with the axons of cells expressing the endogenous OR37C gene in the same glomerulus. Ectopically positioned transgene expressing cells formed novel glomeruli. These results demonstrate that the major features of the special OR37 topography are recapitulated by the short transgene; thus, indicating that the conserved DNA elements are indeed involved in controling the distinct expression pattern of the OR37 receptor types.

# Psychophysical evidences of the cross-modal interaction of human faces and of smelling gender specific sex hormon-like substances

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Recently more and more studies test the cross-modal interactions of the human visual and olfactory systems. In our present paper we studied the effect of an odorous male gender specific sex hormon-like steroid (5-α-androgenst-16-en-3-one, TESTO) on the face perception of healthy adult males. We tested how facial gender discrimination varies in the presence or absence of TESTO in a two-alternative forced-choice task. According to our results subjects judge a particular face significantly more masculine when inhaling TESTO, than in the control condition. In a second experiment we used a facial adaptation paradigm where subjects first were exposed for 5 sec to a female or male face. As a result, the subsequent test faces were judged more masculine or feminine, respectively. This facial adaptation effect, that involves higher ventral visual cortical areas was also strongly biased by olfactory presentation of TESTO, when compared to control conditions. Our experiments illustrate that the olfactory presentation of sex hormon-like steroids can alter the gender discrimination of faces, suggesting a close interaction between olfactory and visual modalities, and this relationship manifest on socially important odorous substances and human faces.

# Short range detection and neural representation of nestmate recognition cues in the ant *Camponotus floridanus*

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Ants are able to discriminate nestmates (colony members) from non-nestmates (members of a different colony) by means of long chain hydrocarbons on their cuticle. The hydrocarbon profile of non-nestmates elicits aggressive behavior, even when presented on a dummy, while dummies with nestmate hydrocarbon profile are attacked less. The post-pharyngeal gland (PPG) contains relatively large amounts of hydrocarbons in ratios similar to what was found on the cuticle. The colony-specific profile is thought to be spread throughout the colony via tactile interaction and trophalaxis between colony members (Boulay, Katzav-Gozansky et al. 2004). Due to environmental factors (e.g. feeding), the colony specific profile is not constant, but changes over time in the course of months or years. Therefore, experience dependent plasticity of the recognition system is expected, in order to account for the changes in the cuticular hydrocarbon profile. Experience dependent plasticity was described e.g. in the antennal lobe of honey bee (Brown, Napper et al. 2004). It is not known how long chain hydrocarbons are represented in the antennal lobe of ants (or bees) and whether the representation of hydrocarbons involved in nestmate recognition changes with experience. To investigate this, our first step was to examine how long chain hydrocarbons are represented in the antennal lobe, using a functional imaging technique.

As a stimulus we used PPG extracts of single glands applied on dummies. The ants were not allowed to touch the dummies which were presented close to the antennae at a distance of 0.5 to 1 cm. First, we showed in a behavioral assay that ants are able to discriminate nestmate from non-nestmate cues presented on the dummies, even without physical contact. PPG extracts of nestmates elicited aggressive behavior less often than PPG extracts of non-nestmates or PPG extracts of nestmates with addition of cis-9-tricosene. Thus, nestmate recognition cues can be received with olfactory sensilla, and contact chemosensilla are not crucial for detecting nestmate recognition cues.

The same types of dummies treated with the three different PPG extracts were used in Calcium imaging experiments. Projection neurons were stained retrograde with the calcium sensitive dye Fura2-dextran. Moving dummies with different PPG extracts close to the antenna resulted in a persistent activation of projection neurons innervating different glomeruli as long as the stimulus was presented.

Boulay, R., T. Katzav-Gozansky, et al. (2004). "Odour convergence and tolerance between nestmates through trophallaxis and grooming in the ant Camponotus fellah (Dalla Torre)." Insectes Sociaux 51(1): 55-61.

Brown, S. M., R. M. Napper, et al. (2004). "Foraging experience, glomerulus volume, and synapse number: A stereological study of the honey bee antennal lobe." Journal of Neurobiology 60(1): 40-50.

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# Caste-specific differences in synaptic development within olfactory neuropils of the female honeybee brain

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Research on neuronal mechanisms of behavioral plasticity represents a fascinating field in neurobiology. The main goal of this study was to investigate cellular components of neuronal plasticity during metamorphic adult development of olfactory neuropils in the brain of the honeybee *Apis mellifera*. We focused on the two female castes, queens and workers and investigated caste-specific differences in the formation of olfactory glomeruli in the primary olfactory centers (antennal lobe, AL) and synaptic complexes (microglomeruli, MG) in higher olfactory centers in the mushroom-body (MB) calyx.

The differences between workers and queens are not based on genetic differences, but rather reflect phenotypic plasticity caused by differential gene expression initiated via larval feeding and endocrine mechanisms. During postembryonic pupal metamorphosis, queens develop much faster than workers, are larger, live much longer, and are devoted to reproductive behavior. The developmental trajectory for female larvae is determined by the third day of larval development. From there on the developmental pathway is fixed for the queen or the worker phenotype.

To study ontogenetic plasticity at the level of the brain and to begin to unravel possible neuronal correlates for the obvious differences in behavior, we raised synchronized worker and queen brood under controlled conditions in incubators at temperatures within the natural, thermoregulated range (~35°C). Brains of worker and queen pupae were dissected at various stages for both immunofluorescent staining (anti synapsin-immunocytochemistry and f-actin labeling with phalloidin) and prepared for electron microscopy.

The results of immunolabeling experiments revealed substantial differences in the timetable of synaptic development at the level of AL glomeruli as well as MB-calyx MG within olfactory and visual input regions showing that ontogenetic plasticity among the female castes is reflected in the development of the brain. These findings were substantiated by analyses at the ultrastructural level. The results show that differences found in the synaptic organization of adult workers and queens (Groh et al. 2006, Brain Behavior & Evolution 68:1-14) are mainly caused by developmental plasticity during postembryonic pupal metamorphosis. Our findings on MB-calyx MG further indicate that structural plasticity affects both pre- and postsynaptic components. The underlying mechanisms and regulation of this remarkable developmental synaptic plasticity will be the focus future studies.

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# Plasticity of synaptic microcircuits in the mushroom-body calyx of the honeybee brain

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Workers of the honeybee (*Apis mellifera*) perform a variety of different tasks. The behavioural repertoire depends on age, and workers go through two major phases during their lifetime of around six weeks. After about three weeks of various indoor duties as nurses and as guardians at the hive entrance, bees become foragers and start to collect nectar and pollen depending on the needs of the colony. This life phase as a forager requires completely new behavioural profiles for the bees. Visual and olfactory cues, for example, are totally different and become very important during foraging. Previous work has shown that the transition from in-hive duties to foraging is correlated with a volume increase in the olfactory and visual input regions of the mushroom bodies (MBs), which are prominent higher sensory integration centers in the hymenopteran brain. The underlying cellular and subcellular causes of these volume changes are still unclear.

In the present study we investigated structural plasticity of synaptic complexes (microglomeruli, MG) during maturation of olfactory and visual subregions in the MB calyx. To visualize pre- and postsynaptic compartments of these synaptic microcircuits we used markers for synaptic proteins and cytoskeletal elements, which were analysed by laser-scanning confocal microscopy and 3D image processing. Presynaptic boutons of olfactory and visual projection neurons were labeled with an antibody to synapsin, while postsynaptic structures of Kenyon cells (KCs) were labeled with fluophore-conjugated phalloidin and anti-tubulin antibody. We examined the density and number of MG, the size of presynaptic boutons as well as changes in KC dendrites. Additionally, electron microscopy studies were performed to investigate synaptic changes at the ultrastructural level. The results revealed substantial changes in the structural organisation of MG during the transition from nurse bees to foragers. The changes in MB-calyx microcircuits are very likely to underlie previously observed volume changes and provide an ideal substrate to further investigate mechanisms and regulation of synaptic plasticity in the insect brain.

Supported by DFG: SFB 554 (A8)

# Neuromodulation and synaptic plasticity within olfactory centers in the brain of the carpenter ant, *Camponotus floridanus*

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Neuromodulatory systems reversibly adjust neuronal information processing to various conditions and may be regarded as mediators between environmental changes and neuronal plasticity. Despite the wealth of information about olfactory guided behavior and chemical communication in ants, the structural and functional aspects of neuromodulatory systems and neuronal plasticity are largely unknown. In the present study we investigated a putative correlation between two neuromodulatory systems, serotonin (5HT) and nitric oxide (NO), and synaptic plasticity within primary and secondary olfactory centers in the brain of the carpenter ant, *Camponotus floridanus*. 5HT-immunohistochemistry was used to visualize serotonergic neurons. The NO/cGMP system was investigated using cGMP-immunoreactivity (IR) following NO stimulation via SNP and pre-treatment of the brains with IBMX to detect NO-induced production of cGMP. Synapsin-immunohistochemistry was used to detect areas of synaptic plasticity. The results show that a subpopulation of antennal lobe (AL) glomeruli associated with the lateral antenno-cerebral tract (lACT) was preferentially innervated by serotonergic processes. Correspondingly, only the central lACT projection area in the olfactory input region in the mushroom-body (MB) calyx lip contained serotonergic processes. In contrast to the serotonergic innervation pattern, most AL glomeruli were innervated by NO-induced cGMP positive neuronal processes of mainly projection neurons (PNs), with a distinct pattern of cGMP- IR in a cluster of glomeruli innervated by the medial (m) ACT. In the MB-calyx lip, the outer region showed the strongest cGMP-IR - the region that lacked 5HT-immunoreactivity and exclusively contains terminals of mACT PNs. Interestingly, this outer region of the MB-calyx lip showed a substantial increase in the number and density of synapsin-IR synaptic complexes in old workers compared to young workers. The results suggest that the two prominent neuromodulatory systems differentially influence distinct subsystems of the olfactory pathway, which may undergo, at the level of the olfactory MB-calyx lip, a different degree of synaptic plasticity during a period of maturation. Supported by DFG: SFB 554 (TP A8)

# Organization of glomeruli and olfactory processing of trail pheromone in the ant, *Camponotus floridanus*

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Pheromones play an important role in ant communication, especially in organizing their complex social behavior. Therefore, proper recognition and processing of such odors is essential for survival and reproductive success of ant colonies and requires advanced olfactory capabilities. In this study we used neuroanatomical and -physiological techniques to investigate the organization and function of the olfactory pathway in the carpenter ant, *Camponotus floridanus*. We address the question whether this olfactory system exhibits anatomical and/or physiological adaptations to pheromone processing within the primary olfactory centers, the antennal lobes (ALs).

3D-analyses of whole brains revealed a total of ~ 460 olfactory glomeruli in the AL. Antennal-nerve (AN) backfills with fluorophore-conjugated dextrans showed a division of the AN in seven tracts innervating distinct clusters of glomeruli. Olfactory information leaves the AL via the axons of projection neurons (PNs) along two prominent antennocerebral output tracts (medial, m- and lateral, l-ACT). Selective staining of the output tracts by double-labeling with different fluo-dextrans revealed that PNs leaving the AL via the m- and l-ACT innervate two separate subsets of glomeruli in the AL. l-ACT PNs innervate a cluster of glomeruli in the anterior part, and m-ACT PNs innervate a cluster of glomeruli in the posterior part of the AL. Despite a higher number of glomeruli, this organisation is similar to the conditions found in the honeybee (Kirschner et al. 2006, J. Comp. Neurol., in press) indicating that in hymenoptera olfactory information from topographically separated clusters of glomeruli is transferred via different pathways. We did not find glomeruli of particularly large volume or specific shape that might indicate a special function in pheromone processing as, for example, reported in moths or recently in leaf-cutting ants (Kleineidam et al. 2005, Chem. Senses 30:383-392). We used calcium imaging techniques to visualize and analyse the spatial and temporal representations of odors in AL glomeruli of *C.floridanus*, to test whether responses to pheromonal odors differ from responses to general odors regarding sensitivity and/or spatio-temporal representation. Axons of AL PNs were retrogradely filled by injection of fura-2 dextran into the m- and l-ACT. Changes in calcium levels were measured during odor stimulation with a releaser component of the trail pheromone (nerolic acid), an alarm pheromone (n-undecane) and two general odors (heptanal, octanol), all applied at dilutions ranging from $10^{-1}$ to $10^{-12}$. Each odor elicited an odor specific glomerular activation pattern. No obvious spatial separation of pheromone responsive glomeruli was observed compared to glomeruli activated by general odors. Response thresholds for both pheromonal and general odors ranged between dilutions of $10^{-11}$and $10^{-10}$ indicating a high sensitivity to both pheromonal and general odors. Interestingly, the glomerular activation patterns in response to the releaser component of the trail pheromone (nerolic acid) were remarkably invariant over a broad range of concentrations. Supported by DFG:SFB 554 (TP A6, A8)

# Neuroanatomical sub-castes in leaf-cutting ants: Differences in antennal lobe design correlate with olfactory guided behavior

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Ants have a well-developed olfactory system, and pheromone communication is essential for regulating social life within their colonies. We investigated the primary olfactory neuropils (antennal lobes, ALs) in the brain of the leaf-cutting ant *Atta vollenweideri*. Leaf-cutting ants express a striking size polymorphism, and size is related to the behavior of workers (alloethism). We discovered that workers of different size express a disproportional allometry of glomerular volumes. The ALs of large workers contain a substantially enlarged glomerulus (MG, macroglomerulus) located at the entrance of the antennal nerve. Correlation of body size and relative volume of the largest glomerulus at the entrance of the AL revealed two distinct groups of workers. Thus, according to the AL design, two neuroanatomical sub-castes of workers (with and without MG) can be distinguished.

In the late pupal stage, large workers already have an MG indicating that this is based on developmental rather than experience dependent plasticity. The size of the MG most probably is related to a higher number of olfactory receptor neurons (ORNs), and mass staining of ORNs of the antenna revealed a strong sensory innervation of the MG.

We asked the question about the odors represented in the MG and therefore tested trail pheromone components and other odors in electroantennogram (EAG) experiments. Stimulation with the releaser component of the trail pheromone (sufficient to trigger trail following behavior) elicited strong EAG signals. In a comparative study with *Atta vollenweideri* and *Atta sexdens* we found that the EAG response to the species-specific releaser component was stronger compared to the response to a second main component of the trail pheromone. The differences in EAG signals and the species specific MG location in large workers provide correlative evidence that the MG may be involved in the detection of the releaser component of the trail pheromone.

In behavioral experiments we asked how differently sized workers assess trail pheromones. Trail-following behaviour (responsiveness and preference) of small and large leaf-cutting ant workers was investigated on artificially laid trails. Preference was measured by testing the discrimination between two competing trails, one conspecific and the other interspecific. At a balanced concentration of conspecific and interspecific trail, large workers showed no preference for any of the two trails, whereas small workers preferred the conspecific trail over the interspecific trail. We suggest that large workers primarily respond to the releaser component present in both, conspecific and interspecific trails, whereas small workers focus more on the conspecific traits provided by the blend of components contained in the trail pheromone.

The phenotypic plasticity found in the antennal lobe of *Atta vollenweideri* correlates with differences in olfactory guided behavior and possibly promotes division of labor in leaf-cutting ant colonies.

Supported by DFG SFB 554/A6

# Symposium

## S9: Recent Advances in the Use of Cell Penetrating Peptides

***Gunnar P.H. Dietz, Goettingen***

### Slide

**S9-1** The import mechanism of cationic cell-penetrating peptides and its implications for the cellular delivery of inhibitors of signal transduction
*Roland Brock, Nijmegen (NL)*

**S9-2** Biophysical studies of cell penetrating peptides
*Astrid Gräslund, Stockholm (S)*

**S9-3** PTD-DRBD Mediated Delivery of Macromolecular siRNA Therapeutics
*Steven Dowdy, La Jolla (USA)*

**S9-4** A new signaling mechanism based on the presence of natural transduction domains
*Alain Louis Prochiantz, Paris (F)*

### Poster

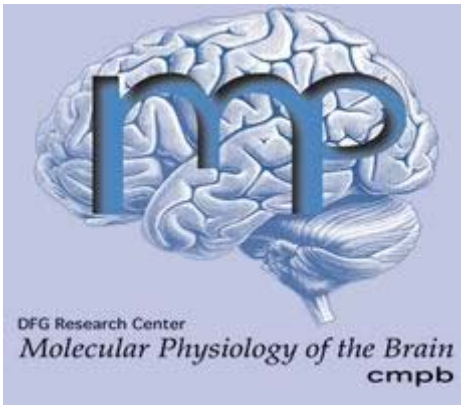
**TS9-1B** Application of Tat-Hsp70 in the MPTP-mouse model for Parkinson's disease
*F. Nagel, B. Falkenburger, S. Kowsky, C. Pöppelmeyer, JB. Schulz, M. Bähr and GPH. Dietz, Göttingen*

Introductory Remarks to Symposium 9

# Recent Advances in the Use of Cell Penetrating Peptides

Gunnar P.H. Dietz, Goettingen

Over the last 15 years, many publications described the use of peptide sequences that have been dubbed cell penetrating peptides (CPP), Trojan horse peptides, protein transduction domains, or membrane-translocating sequences. These mostly positively charged domains bring attached cargo across biological membranes. One of the reasons for the interest in CPP is their potential as delivery tools to enhance the pharmacodynamics of drugs otherwise poorly bioavailable. In particular, the neuroscientist aiming to deliver a protein or other compounds into the brain for analytical or therapeutic reasons is faced with the challenge that few drugs cross the blood-brain barrier. CPP are valuable tools to overcome the plasma membrane or the blood-brain barrier in basic research, and in relevant models of neural disease, and will hopefully help to increase the precious few treatments or even cures for people with diseases of the brain and nervous system. In this symposium, the most acclaimed pioneers and world leaders of the field will give an update on recent developments using cell penetrating peptides.




With generous support from the DFG Research Center for Molecular Physiology of the Brain (CMPB)

Astrid Gräslund examines the structure, dynamics, interaction and function of cell penetrating peptides and their cargo, by high resolution NMR, EPR and optical spectroscopy including CD and fluorescence. Steven F. Dowdy heads the innovations on CPP derived from the HIV-Tat protein in cell cycle, cancer, and the mechanism of cell penetration. Alain Prochiantz leads the development of homeodomain-derived peptides as delivery tools and studies the physiological significance of homeoprotein transduction. Roland Brock will address the mechanism of uptake of cationic CPPs, cytotoxic effects, the interference with cellular signalling and the dependence of uptake on fluorescent reporter groups and on the peptide cargoes.

# The import mechanism of cationic cell-penetrating peptides and its implications for the cellular delivery of inhibitors of signal transduction

Roland Brock

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Cell-penetrating peptides (CPPs) possess the highly attractive ability to accomplish a non-invasive cellular delivery of membrane impermeable cargo molecules. The CPP-based approach provides the possibility of a selective interference with cellular signal transduction by import of peptides interfering with protein-protein interaction. However, sequestration of CPP conjugates in the endolysosomal compartment and proteolytic break-down represent major challenges for the efficient delivery of peptides into the cytoplasm.

The bioactivity of a proapoptotic cargo-peptide, delivered into the cells either via electroporation or via conjugation to the cationic CPPs nonaarginine, was quantified following activation of cells with TNF using a caspase-3 activity assay and cellular assays. The uptake of this conjugate was mediated by endocytosis and led to a significant reduction of cargo bioactivity compared to its direct transfer via transient membrane permeation. This finding was related to the sequestration of peptides within endocytic vesicles and proteolytic degradation, as demonstrated for cell-penetrating peptides terminally labeled with different fluorophores for the detection of break-down by fluorescence resonance energy transfer.

Moreover, in the case of the TNF response the CPPs induced the internalization of receptors during cell entry thereby compromising the ability of the cell to respond to external stimuli. These latter results demonstrate that cationic CPPs may themselves act as bioactive molecules interfering with cellular signalling.

For the successful application of CPPs in biological and biomedical research a careful consideration of the cell biology of the vectors themselves is therefore required.

# Biophysical studies of cell penetrating peptides

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Cell-penetrating peptides, CPPs, are water-soluble but have the ability to transport themselves and an attached cargo, such as polypeptides, oligonucleotides, or particles many times their own molecular mass, into cells with high efficiency and low lytic activity. These peptides are therefore of high biotechnological and pharmacological interest. A common denominator for the CPPs is that they are more or less positively charged at pH 7, and have a considerable fraction of hydrophobic residues. The mechanisms for the translocation of the CPPs are under debate: recent evidence suggests that endocytotic mechanisms including macropinocytosis dominate for most peptides, and particularly when they carry a large cargo. In this case, escape from the engulfed endosomes has to take place while the cargo is still intact. Direct interactions between peptides and the cell membrane may also participate. In either case the molecular details of the peptide-bilayer interactions are of fundamental importance for the translocation process.

High resolution NMR, CD and fluorescence spectroscopies have been used to investigate peptide interactions with various membrane mimetic solvent systems, such as detergent micelles, small unilamellar phospholipid vesicles, or phospholipid bicelles (small disc shaped assemblies composed of phospholipids with two different fatty acid chain lengths). Solvent induced secondary structures and positioning within the membrane model systems have been determined for various translocating peptides.

Leakage induction and pore formation in unilamellar vesicle bilayers have been studied in parallel with peptide translocation. The effects of pH and electrochemical gradients on these processes have been studied. A pH gradient across the membrane was found to be crucial for direct membrane translocation of vesicle entrapped peptides. This observation is relevant for the possible mechanisms of endosomal escape for peptides which have entered the cell via endocytosis.

Besides the biotechnical relevance of the CPPs, interesting questions arise about the potential roles of such sequences as parts of native proteins or peptides. Certain dynorphin neuropeptides, dynorphin A and big dynorphin, have CPP activity and induce membrane leakage. These properties of the dynorphins may be related to their non-receptor mediated activities in neurons. The N-terminus of the prion protein (residues 1-28 in the mouse prion protein, which include the signal sequence and a basic NLS-like segment) is another example of a CPP sequence which may play a role in the pathology of prion diseases.

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# PTD-DRBD Mediated Delivery of Macromolecular siRNA Therapeutics

Steven Dowdy

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Our laboratory is focused on treating malignancies by in vivo delivery of macromolecular anti-cancer therapeutics to specifically kill tumor cells, while leaving surrounding normal cells unharmed. We have focused our attention on utilization of small cationic peptide transduction domains (PTDs), typified by the TAT peptide, to deliver macromolecular therapeutics. Protein transduction occurs by a rapid, lipid raft-mediated macropinocytosis mechanism that is independent of receptors and transporters that all cells perform. Utilizing this information, we have developed fusogenic peptides that dramatically enhance escape of cargo from macropinosome vesicles and into the cytoplasm of cells. Previously, we have shown the ability to deliver p53-activiating peptides to treat mouse models of terminal invasive peritoneal carcinomatosis resulting in disease free animals. These observations serve as a proof-of-concept that delivery of macromolecular therapeutics can modulate tumor biology in vivo. However, not all tumors sufficiently respond to the p53-activiating peptide. Therefore, we have focused considerable attention over the last three years on delivery of short interfering RNAs (siRNAs) to induce RNA-interference (RNAi) mediated targeted mRNA degradation. Recent efforts from our lab have resulted in the ability to deliver siRNAs and induce a RNAi response in ~100% of all cells tested, including: glioma, lung adenocarcinoma, leukemic T cells, lymphoma B cells, melanoma, macrophage, primary human (and murine) fibroblasts, keratinocytes and human embryonic stem cells (hESCs). Thus, protein transduction delivery has great potential to deliver exquisitively selective anti-cancer siRNA therapeutics.

# A new signaling mechanism based on the presence of natural transduction domains

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In the early 90s, it was discovered that the DNA-binding domain (homeodomain) of Antennapedia, a fly homeoprotein transcription factor, was internalized by live cells gaining access to their cytoplasm and nuclei. The study of this phenomenon revealed that internalization is due to the third helix of the homeodomain, composed of sixteen amino acids highly conserved among most homeoproteins. This short peptide later baptized Penetratin was the first of a large series of transduction peptides or Cell Permeable Peptides (CPPs) widely used for the internalization of all sorts of cargoes in vitro and in vivo. Although all CPPs are being developed with the latter practical goal, it has rapidly appeared that different CPPs use different entry routes and mechanisms. Before addressing more physiological issues, I want to underscore that Penetratin internalization, in contrast with that of most CPPs, is independent of endocytosis.

However, the most intriguing outcome of our initial observation is that full-length homeoproteins are transferred between cells and have non-cell autonomous transcriptional and translational activities. This new signaling mechanism requires that homeoproteins be internalized and secreted. Secretion is Golgi independent and requires a small sequence also present in the homeodomain but distinct from the Penetratin sequence. Three models have been developed that illustrate how homeoprotein transfer is used at three distinct times during the development of the visual system. Data will be presented that illustrate how Pax6, Engrailed and Otx2 transduction play a role in the development of the eye anlagen (Pax6), in the establishment of retino-tectal maps (Engrailed) and in the opening of the critical period for binocular vision (Otx2).

# Application of Tat-Hsp70 in the MPTP-mouse model for Parkinson's disease

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The molecular chaperone Hsp70 plays an important role in protein folding and degradation and protects cells against oxidative stress and apoptotic stimuli. Protein misfolding and aggregation are associated with many neurodegenerative diseases, including Parkinson's disease. The pathological hallmarks of Parkinsons's disease are the progressive loss of dopaminergic neurons and the presence of intraneuronal proteinaceous cytoplasmic inclusions ("Lewy bodies") in the substantia nigra. Hsp70 reduces protein misfolding and aggregation and inhibits apoptosis in various neurodegenerative models. However, to be used as neuroprotective agent, Hsp70 is too large to freely pass biological membranes or the blood-brain barrier. Therefore we linked it to an 11-amino acid-domain derived from the HIV Tat protein, which facilitates such delivery. As a model for Parkinson's disease, we used the subacute toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 30mg/kg on 5 consecutive days) to test the therapeutic potential of Tat-Hsp70. Survival of dopaminergic neurons was assayed by counting the number of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the density of dopaminergic fibers in the striatum. A potential effect of Tat-Hsp70 on MPTP metabolism was ruled out by measuring levels of its active metabolite MPP+ in the brain. Our preliminary results suggest that Tat-Hsp70 reached dopaminergic neurons in the SNpc and protected them from cell death as revealed by counting the number of tyrosine-hydroxylase-immunoreactive neurons and Nissl-positive cells. Furthermore, Tat-Hsp70 diminished the MPTP-induced decrease in striatal fiber density.The use of Tat-fusion proteins might therefore be a valuable tool to deliver proteins of interest into the brain and molecular chaperons like Hsp70 may be the basis to develop neuroprotective strategies for neurodegenerative disorders.

# Symposium

## S10: Generating Rhythmic Movement: From Microcircuits to Complex Motor Programs

*Ansgar Büschges and Hans-Joachim Pflüger, Köln and Berlin*

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**TS10-3B** Changes in aimed limb movements during growth and development in an insect
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**TS10-4B** Acetylcholine rather than octopamine activates the locust flight CPG
*E. Buhl, K. Schildberger and PA. Stevenson, Leipzig*

Introductory Remarks to Symposium 10

# Generating Rhythmic Movement: From Microcircuits to Complex Motor Programs

Ansgar Büschges and Hans-Joachim Pflüger, Köln and Berlin

A primary function of the central nervous system is the generation of motor programs, many of which are rhythmic, like breathing, chewing or locomotion. Recent results have unravelled new insights into the organization and operation of the microcircuits underlying the generation of rhythmic motor programs. Firstly, recent studies emphasize that a functional partitioning of neural networks generating rhythmic motor outputs exists. There is one level generating rhythmicity and another level generating the actual output pattern of the circuits. Further subdivisions are observed with respect to the functional organisation of the output stage. For example, the segmentation of a locomotor organ can form independent subunits that are coordinated in a flexible fashion to form complex motor outputs. The symposium will dwell on recent advances in the construction of central pattern generators and to what extent "microcircuits within the CNS" can be used as "common building blocks" of network operation and network formation. The symposium will start with basic new findings in the lamprey spinal cord (Cangiano). It will then place these findings in the light of new results on development (Simmers), coordination (Mentel) and neuromodulation (Pflüger, Stein) and the generation of targetted limb movements (Matheson) within neural networks generating rhythmic movements

# Organization of Central Pattern Generating Networks in the Lamprey Spinal Cord

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Animals propel themselves by rhythmic oscillatory movements of their body and limbs, mainly generated by specialized neuronal circuits called central pattern generators (CPGs) for locomotion. In vertebrates these lie in the spinal cord and integrate sensory feedback with supra-spinal commands, to produce precise and goal-directed locomotion. The lamprey, an aquatic vertebrate that swims by rhythmic left-right bending of its body, serves as a model system for the study of CPGs. Its isolated spinal cord, when activated pharmacologically, produces at the ventral roots (VR) the motor pattern of swimming thus allowing the experimenter to investigate in vitro, the neuronal mechanisms responsible for locomotion.

This system has been recently exploited to address the fundamental question of whether the rhythmic contraction of each group of muscle synergists during locomotion is generated by a distinct subnetwork within the CPG, endowed with intrinsic rhythmic capability. In many vertebrates including the lamprey it has been claimed instead that rhythmicity depends on reciprocal inhibition between antagonists.

Answering this question in the lamprey required testing if, at any given spinal segment, the rhythmic motor output, normally alternating between left and right VRs, could be still produced when the left and right hemisegments were surgically separated. We completely sectioned pieces of spinal cord sagittally along the midline, and activated the hemisegmental network with D-glutamate, NMDA, or by brief electrical stimulation. Fast rhythmic bursting (2-15 Hz) coordinated across VRs was observed with all three methods. Being these frequencies in the mid-higher range of swimming, we developed intermediate preparations having only a partial separation of the two sides, through fine intermittent lesion of the midline. These expressed intermediate frequencies thus linking the swimming rhythm in the intact cord to that of the hemicord. Swimming is thus produced by pairs of intrinsically rhythmic unilateral networks, with reciprocal connections (mainly inhibitory) that not only ensure left/right alternation but also downregulate frequency.

The mechanisms underlying rhythm generation in the hemicord were explored by recording the unperturbed firing pattern of individual neurons along their axons and their synaptic input at the soma. Locomotor output is accompanied by a sustained depolarization of motor and premotor interneurons, onto which synaptically-driven membrane potential oscillations and spikes occur, in either case in phase with the VR bursts. Motoneurons and interneurons fire at most one spike per locomotor cycle. Thus, a further effect of the reciprocal inhibition between opposite hemisegments in the intact cord, is to promote a shift from single to multiple action potentials per cycle in network neurons. We finally showed that ipsilateral glycinergic inhibition is not required for rhythm generation in the hemicord.

In conclusion the simplest interpretation of our data is that the core module in the swim CPG consists of a network of excitatory interneurons that partially synchronize action potentials through fast synaptic interactions, thereby relaying phasic excitation to ipsilateral motoneurons.

# From tail- to limb-based swimming: development of amphibian locomotory networks

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Metamorphosis of the frog, *Xenopus laevis*, involves a complete restructuring of the organism's locomotory system from axial-based swimming using alternating contractions of left and right trunk muscles, to bilaterally-synchronous kicking of the hindlimbs in the young adult. At critical stages during this behavioural switch, functional larval and adult locomotor systems co-exist in the same animal, implying a progressive and dynamic reconfiguration of spinal circuitry and neuronal properties as limbs are added and the tail regresses. Using *in vitro* preparations of the spinal cord and brainstem from animals at different metamorphic stages, in combination with *in vivo* EMG recordings and neuroanatomical approaches, we are investigating the neurobiological processes that govern the emergence of secondary limb motor circuitry as it supersedes the primary larval network responsible for tail-based axial swimming. This transformation spans a developmental period when limb circuitry is initially absent, present but not functional, functional but co-opted into the axial network, functionally separable from the axial network, and ultimately alone after axial circuitry disappears with tail resorption. Furthermore, experiments on spontaneously active brainstem/spinal cord preparations from intermediate metamorphic stage animals have indicated that neuroamines present in supraspinal projections can modulate the dynamic interaction between the axial and hindlimb spinal networks: bath application of exogenous serotonin or noradrenaline causes, respectively, an immediate and sustained re-coupling or a complete dissociation of the two fictive locomotor rhythms. Thus during *Xenopus* metamorphosis, the circuitry responsible for limb movements is progressively segregated from an axial precursor and throughout this developmental transition, the functional coordination between the two networks remains susceptible to short-term neuromodulatory control.

# Coordination of Different Motoneuron Pools in the Lamprey Spinal Cord

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In locomotion the accurate activation of different sets of motoneurons, whether it be the sets of antagonistic motoneurons driving the joints in a leg or the trunk-muscles of swimming fish depends on the precise timing of in a mostly rather complex activation-pattern. The underlying networks and mechanisms that establish different phasing of different sets of motoneurons during locomotion are mostly not known yet. We choose the lamprey spinal cord to investigate this issue, because it contains only two different subsets of motoneurons: the trunk-muscle innervating myotomal motoneurons (mMN) and the fin muscle innervating fin motoneurons (fMN). As shown previously both sets of motoneurons are active out-of-phase to each other in one hemi-segment during free swimming as well as during fictive locomotion evoked by NMDA (Mentel et al. 2006, Europ. J. Neurosci.). As demonstrated by sagittal lesions of the midline the drive of the own contralateral hemi-segment is not necessary to push fMN into a different phase compared to the activtiy of the ipsilateral mMNs. It has recently been shown by Cangiano & Grillner (2005, J. Neurosci.) that basic rhythmicity underlying the rhyhtmic activity in mMNs is produced by networks of excitory interneurons located in each half of the cord. In the present investigation we applied progressive lesions to the isolated spinal cord by perforating the midline with a scalpel at 30%-50% and 50%-70%. In all experimental conditions (intact, perforated, complete hemi-section) rhythmic activity was evoked by electrical stimulation as well as by perfusion with NMDA. All mMNs recorded showed an excitatory drive when rhythmic activity was evoked by electrical stimulation. In the intact cord and in 30%-50% perforated spinal cord the same was true for fMN. When NMDA was added to the bath the same fMNs showed a phase-shift from in phase to an out-of-phase activity. This phase shift was no longer present by perforating the midline 50%-70%. These results suggest that all sets of motoneurons in the spinal hemi-cord of the lamprey receive a common excitatory drive contributing to their activation. The actual patterning for the phasing of motoneuron activity is produced on a different level in the segmental locomotor networks by the contribution of contralateral intersegmental inputs.

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# Motor Pattern Selection in the Stomatogastric Nervous System by Nitric Oxide

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Neuronal circuits that control behavior are modulated by a host of neuroactive substances (Nusbaum & Beenhakker, 2002, Nature 417). We aim to understand how the nervous system uses these substances to select and generate distinct motor patterns from multifunctional neural networks. For this, we are using two motor patterns which are generated in the stomatogastric nervous system of the crab, *Cancer pagurus*. The rapid pyloric rhythm and the slow gastric mill rhythm are produced by central pattern generators in the stomatogastric ganglion (STG), and control the processing of food by different regions of the crab foregut. Distinct modulatory projection neurons from upstream ganglia and the brain provide neuromodulatory input to the STG circuits and alter the pyloric and gastric mill motor patterns. One of these modulatory transmitters is nitric oxide (NO; Scholz, de Vente, Truman & Graubard, J Neurosci 21, 2001), a highly mobile and membranepermeant gas that has numerous modulatory effects on muscle and nervous system function in both vertebrates (Garthwaite & Boulton, 1995, Annu Rev Physiol 57; Denninger & Marletta, 1999, Biochim Biophys Acta. 1411) and invertebrates (e.g. Mahadevan, Lappe, Rhyne, Cruz-Bermudez, Marder & Goy, 2004, J Neurosci 24; Jacklet, 1999, Invert Neurosci 3).

We focused on the effects of NO on the gastric mill rhythm and on the resulting network actions on two identified upstream projection neurons, modulatory commissural neuron 1 (MCN1; Nusbaum, Weimann, Golowasch, Marder, 1992, J Neurosci 12) and commissural projection neuron 2 (CPN2; Norris, Coleman, Nusbaum, 1994, J Neurophysiol 72). Both neurons have been shown previously to have a strong impact on the motor patterns in the STG.

Bath application of different *NO donors* exclusively to the STG elicited gastric mill rhythms which were similar to those elicited by the combined activity of the projection neurons MCN1 and CPN2. Indeed, we found that the activity of MCN1 and CPN2 had increased. These neurons, however, are located in the commissural ganglia and thus had not been perfused with the donors.

In contrast, *NO blockers* applied exclusively to the STG terminated spontaneously active gastric mill rhythms. Furthermore, a reduction in the activity levels of MCN1 and CPN2 was observed, suggesting that changes in the NO concentration in the STG influence feedback mechanisms that affect upstream projection neuron activity. When either neuronal feedback to these projection neurons was eliminated or projection neurons were tonically activated, an NO blockade did not terminate the gastric mill rhythm, indicating an indirect ascending control of the projection neurons.

We conclude that NO affects the central pattern generator of the gastric mill rhythm mainly indirectly via feedback to the projection neurons in the commissural ganglia. Together, our results show that state-dependent ascending neuronal feedback from a motor network controls the activity of upstream projection neurons which, in turn, are vital for selecting motor patterns from the motor circuits.

Currently, we are investigating the nature of the ascending neuronal pathway.

# Coupling between neuromodulatory pathways and microcircuits for locomotor pattern generation in the locust central nervous system.

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Insect thoracic ganglia contain octopaminergic unpaired median neurones (UM neurones) with their somata located on the dorsal or ventral midline and with their axons projecting bilaterally. They innervate skeletal and visceral muscles, glands and mechanosensory organs, and they modulate various aspects of motor behaviour such as neuromuscular transmission, muscle contraction kinetics, and metabolism but also sensory sensitivity. In locusts, these neurones are located dorsally (DUM neurones) and are divided into functionally different sub-populations which are activated during different motor behaviours. They are phasically activated or inhibited in parallel to specific motor patterns, and their action has been suggested to poise the whole muscular system to dynamic rather than static, postural, performance. This hypothesis is well supported by their metabolic role for boosting glycolysis. For example, during locust flight the DUM neurones innervating wing muscles are inhibited during flight which correlates well with the predominant lipid oxidation of wing muscles. In contrast, DUM neurones innervating leg muscles are activated during flight behaviour, which correlates to the predominant carbonhydrate oxidation of these muscles.
Different types of DUM neurones were previously characterized according to the peripheral nerves which their axons exit, and this corresponds to particular sets of target muscles that they innervate. The different types of DUM neurones also differ in their responsiveness to sensory stimuli. In general, the DUM neurones innervating wing muscles did no respond to mechanosensory, visual or acoustic stimuli except for antennal stimulation in some of these neurones. In all other DUM neurones two classes of sense organs, cuticular exteroreceptor mechanosensilla, such as tactile hairs and campaniform sensilla, and compound eyes elicited excitatory responses. Chordotonal organ afferences caused no responses. The tympanal organ (Müller's organ) elicited weak excitatory responses most likely via generally increased network activity due to increased arousal. Vibratory stimuli to the hind leg subgenual organ never elicited responses. Interestingly, after cutting both cervical connectives all mechanosensory activation is lost, even for sensory inputs from the abdomen. This suggests that in contrast to motor neurones the sensory inputs to octopaminergic neuromodulatory cells are pre-processed in the suboesophageal ganglion, or suboesophageal inputs gate the sensory pathways to neuromodulatory neurones.

# Generation of aimed limb movements in a locust

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We seek to understand the neuronal basis of aimed limb movements in an insect, the locust *Schistocerca gregaria*. Touching different locations on a wing elicits scratching movements of a hind leg that are aimed towards the stimulus (target) site. The movements are graded within a large workspace, are sensitive to proprioceptive information signalling leg position, and are robust against changes in load or start posture. To begin to address how the target is encoded in the CNS we have analysed the distribution patterns, response properties and central projections of wing hair sensory neurones that encode the stimulus. To address how the aimed movements are generated we have quantified the activity of the two identified excitatory motor neurones innervating the hind leg tibial extensor muscle, and many of the 9 excitatory motor neurones innervating the tibial flexor muscle. We simultaneously recorded limb kinematics using a motion-tracking system so that we can correlate motor activity to movement parameters. In unloaded animals, changes in femoro-tibial joint angle and angular velocity were controlled by variations in the number of tibial motor neurone spikes and the length of bursts, rather than the instantaneous firing frequency within bursts. There was little co-activation of tibial extensor and flexor motor neurones, but the long time-constant of muscle activation indicates that there can be considerable co-contraction of the antagonistic tibial muscles, and thus high joint stiffness. Tibial fast extensor and fast flexor motor neurones were recruited during both unloaded and loaded scratching movements. The fast extensor motor neurone was generally recruited at relatively flexed joint angles, when it fired 1 or 2 spikes. This pattern of activation differs considerably from the pattern seen in kicking, when it produces sustained bursts of high frequency, and contrasts to the situation in walking, when it is not active. Supported by the BBSRC (TM, DK, KP, JVS), FiF (VD) and Studienstiftung des Deutschen Volkes (JZ).

# Sevoflurane but not nitrous oxide enhances presynaptic Ia-inhibition in humans.

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Introd.:The suppression of movement responses to noxious stimuli during anesthesia involves the reduction of spinal motoneuron excitability. In vitro studies have given evidence that anesthetics inhibit spinal motoneuron excitability by both pre- and postsynaptic actions. Here we used heteronymous Ia facilitation of the soleus H-reflex from the femoral nerve (1) as a specific pathway involving GABAergic presynaptic inhibition to demonstrate presynaptic and probably GABAergic effects of the sevoflurane in humans. We compared the effects of sevoflurane (sevo) that interacts with glutamate-, GABAA-, and glycine receptors with the effects of NO2 that interacts more specifically by blocking NMDA- and AMPA-receptors with no or only little affinity to GABA-receptors.

Method: The study was carried out on 10 volunteers for each drug. The subjects inhaled either 0.8 Vol% sevo or 45% NO2. The H-reflex (test reflex) was elicited by stimulating the tibial nerve and was recorded over the soleus muscle. The H-reflex was conditioned by an additional stimulation of Ia-afferents from the quadriceps muscle. Since these Ia-afferents project to soleus motoneurons, the H-reflex is increased when both the test reflex and the conditioning stimulus reach the soleus motoneuron simultaneously. The increase of the H-reflex caused by the conditioning stimulus reflects the ongoing presynaptic inhibition on the quadriceps Ia-afferent. The smaller the increase the stronger are presynaptic inhibitory effects.

Conditioned and uncond. reflexes were tested in random order. Measurements of the amplitude of the reflex response were performed under 3 conditions: a) prior to drug administration (1st control) b) during drug administration c) 35min after drug administration was stopped (2nd control). A series of 100 uncond. and 100 conditioned H-reflexes was recorded in each condition. We compared the size of the conditioned H-reflex during drug administration with both control conditions. To quantify the suppressive effect of the drugs on the H-reflex itself the maximum size of the H-reflex (Hmax) was verified under each condition. The size of the reflex is expressed as fraction of the maximal muscle response to electrical stimulation of the tibial nerve (±SEM). Statistics: repeated measure ANOVA of the individual mean values ($p<0.05$).

Results: Under sevoflurane the average values of the conditioned H-reflex from all subjects were significantly reduced in comparison to both control values (1st contr. 0.211±0.009; drug: 0.195±0.005; 2nd contr. 0.213±0.009). During inhalation of NO2 the cond. H-reflex did not change significantly (1st contr. 0.207±0.008; drug: 0.199±0.006; 2nd contr. 0.202±0.007). Hmax was equally suppressed during NO2 ( 1st contr. 0.696±0.063; drug: 0.443±0.055; 2nd contr. 0.642±0.061) and sevo( 1st contr. 0.532±0.079 drug: 0.391±0.061; 2nd contr. 0.532±0.072).

Conclusion: The results show that sevo but not NO2 reduces heteronymous Ia facilitation of the soleus H-reflex from the femoral nerve. Our data are the first confirmation in humans that sevoflurane also acts via a presynaptic effect and that this effect is probably involves GABAA receptors.

(1) Hultborn H et. al. J.Physiol 1987; 389: 729-56

# Gamma-range cortico-muscular coherence during dynamic force output

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**Introduction**

The beta-range synchronization between cortical motor and muscular activity as revealed by EEG/MEG-EMG coherence has been extensively investigated for maintained voluntary contractions. However, there is a lack of information on the modulation of EEG-EMG coherence during dynamic force output. Our studies addressed this issue by comparing EEG-EMG coherence in healthy subjects and in the deafferented patient GL.

**Methods**

We recorded EEG (48 scalp positions), surface EMG from the right extrinsic finger flexors, and the motor performance in 22 healthy subjects as well as in the deafferented subject GL. The visuomotor task consisted in isometric compensation for forces generated by a manipulandum and applied vertically to the index finger. Target and exerted forces were projected on a screen. In healthy subjects, three different conditions with the same mean generated force (4 % MVC) were investigated: (1) static force: steady force at 4% MVC, (2) small dynamic force: sinusoidal modulation of the 4 % MVC force at 0.7 Hz with a peak-to-peak amplitude of 1.6 % MVC, (3) large dynamic force: sinusoidal modulation of the force at 0.7 Hz with a peak-to-peak amplitude of 4% MVC. In the deafferented subject, only conditions (1) and (2) were applied. The cortico-muscular coherence (CMC), spectral power (SP), and the error in motor performance (calculated as the difference between exerted and target force) were analyzed.

**Results**

During static force output we found significant beta-range CMC over the contralateral sensorimotor cortex. Gamma-band CMC occurred for both small and large dynamic forces. The difference between both dynamic conditions was not statistically significant. The deafferented subject exhibited beta-band coherence both in the static and in the dynamic force conditions and had conspicuous difficulty in performing the dynamic task.

**Discussion**

These findings suggest that the cortico-muscular network oscillates at gamma frequencies during predictable dynamic force output to yield the rapid integration of visual and somatosensory information (Omlor et al., 2006). The absence of gamma-range coherence in the deafferented subject indicates that the contribution of proprioception in this integration is of particular importance for the generation of corticospinal gamma oscillations (Patino et al., in preparation). Finally, the unchanged relative errors between both dynamic conditions suggest that the gamma CMC is mainly associated with the present internal state of the sensorimotor system.

Omlor W., Patino L., Hepp-Reymond M.C., Kristeva R. Gamma-range corticomuscular coherence during dynamic force output, in reviewing.
Patino L., Omlor W., Hepp-Reymond M.C., Kristeva R. A deafferented subject does not have gamma-range corticomuscular coherence during dynamic force output, in preparation.

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# Changes in aimed limb movements during growth and development in an insect

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Touching an exposed wing of the locust, *Schistocerca gregaria*, elicits a scratching movement of a hindleg that is aimed toward the site of stimulation. We are interested in how locusts adapt these movements to changes in the size and position of the wings during growth, and in particular how the central representation of the real-world position of sensory receptors changes.

The wings of a locust expand from less than 1 cm in length to around 6cm as the animal undergoes its imaginal moult. This represents a change from 30% of body length to 100%. Furthermore, the exposed wing surface in juveniles (the ventral side of the hindwing bud) becomes covered by the forewing, so that in adults it is the dorsal side of the forewing that is exposed. To remain functional, movements must be recalibrated to the new size and position of the wings. We have tracked the position of the hindleg throughout movements aimed at different sites on the wings, and related the resultant movement trajectories to the distribution of the sensory hairs that detect the tactile stimulation, determined by scanning electron microscopy. Aiming does adapt to changes in the exposed wing surface. When the tip of the juvenile wing bud is stimulated, juveniles aim toward this point, which lies relatively anteriorly, near the thorax. In adults, touching the tip of the wing, which now lies posterior to the end of the abdomen, elicits movements that are also correctly aimed at the point of stimulation. The position of the target in space, and hence the movement trajectory, therefore differ between juveniles and adults. Movements elicited by stimulation of the ventral hindwing also adapt to the change in wing length between juvenile and adult. Stimulation of the dorsal forewing only elicits aimed movements in adults, and this coincides with the appearance of tactile hairs on the dorsal forewing at this stage of development. The new hairs have a dramatically different distribution in the adult than in the juvenile. Long and medium length hairs are dispersed across the entire juvenile wing surface, but become localised along single veins in the adult.

These additions and modifications in both behaviour and the sensory hairs that elicit it, suggest that there may be alterations to the central projections of the associated neurones. We have used Neurobiotin to stain the neurones of individual sensory hairs in the 5th instar to determine if their position on the wing is represented somatotopically within the ganglia. Some hind wing hair neurones project to both the metathoracic and mesothoracic ganglia, suggesting they may contribute to a range of behaviours in addition to hind leg scratching.

# Acetylcholine rather than octopamine activates the locust flight CPG

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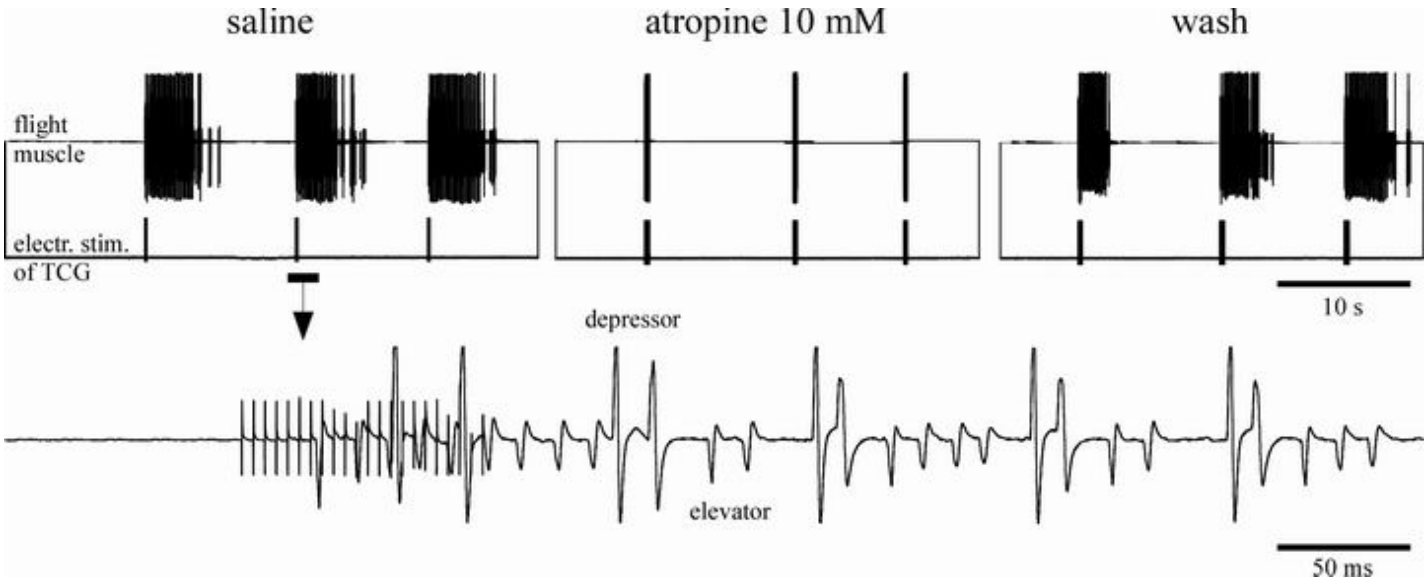
Our data question the key role long postulated for octopamine in flight initiation in locusts, and reveal the necessity of a cholinergic mechanism. Firstly, in addition to octopamine, the amines tyramine, dopamine and noradrenalin, though not serotonin, all initiate flight motor activity when applied to the denervated thoracic nervous system. Secondly, flight is similarly initiated by both acetylcholine and the muscarinic agonist pilocarpine, but not by nicotinic agonists. Thirdly, flight-initiating interneurones (e.g. the tritocerebral commissure giant interneurone, TCG) are not detected by octopamine-immunocytochemistry, and flight can still be initiated by natural stimulation (wind directed to the head hairs) as well as by pilocarpine in animals depleted of amines by reserpine treatment. Finally, flight initiated by either natural wind-stimulation, or selective electrical stimulation of the TCG-interneurone, are both reversibly blocked by the muscarinic antagonist atropine(fig), but seemingly not by the selective octopamine antagonist epinastine or the non-selective α-adrenergic antagonist phentolamine. We conclude that the central pattern generator for locust flight is naturally activated by cholinergic interneurones, though we suggest a modulatory role of octopamine and or related amines.



# Symposium

## S11: Brain Tumors

***Rainer Glass and Michael Synowitz, Berlin***

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**TS11-1B** Erucylphosphohomocholine-induced cell death in human glioma cells: Role of reactive oxygen species
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Introductory Remarks to Symposium 11

# Brain Tumors

Rainer Glass and Michael Synowitz, Berlin

Glioma are the major tumour entity of the central nervous system and they constitute a particular medical challenge since most of the patients do not survive the first year after diagnosis. Glioma are rapidly expanding tumours that receive nutrients for their growth by inducing extensive neovascularisation. These tumours can persist in the brain because they evade an effective immune reaction by secreting immunosuppressive factors. Moreover, the immune-suppressed microglia appears to support the invasion of glioma cells into the parenchyma. Glioma are thought to origin from transformed stem cells of the brain, however, recently it became evident that untransformed neural stem cells have a strong tropism for glioma and mediate anti-tumourigenic effects against these tumours. The diffuse infiltration of the tumour cells into the brain makes complete surgical removal impossible, but genetically modified neural stem cells may be used as a vehicle to track down single glioma cells and to deliver therapeutic agents.

We will give an overview on the diverse pro- and anti-tumourigenic interactions of glioma with their microenvironment in the brain and we indicate how these mechanisms may be exploited for future therapies. It will be presented how glioma manipulate the microvasulature for its own supplies, which factor is secreted by glioma to suppress immune functions, by which mechanism glioma exploit immune-suppressed microglia to boost the invasiveness of the tumour, how endogenous neural stem cells are attracted towards gliomas and how the tropism of exogenous neural stem cells can be used for glioma therapy

Peter Vajkoczy will give in vivo insights into endothelial progenitor cell (EPC) recruitment from the bone marrow towards gliomas, where EPCs induce neovascularisation. Using intravital fluorescence videomicroscopy he defines a multistep process, by which EPCs home and incorporate into "hot spots" within the tumor microvasculature. Based on these observations he will suggest novel ways to interfere with pathological neovascularization.

Michael Weller will contribute experimental and clinical data demonstrating that glioma actively secrete the immunosuppressant cytokine transforming growth factor (TGF)-beta. Firstly, he will present the complex regulation of TGF-beta bioavailability including its synthesis, processing, assembly and activation. Secondly, he will show that TGF-beta antagonists, inhibitors of TGF-beta processing , TGF-beta scavengers, but also antisense strategies are promising agents for glioma therapy.

Michael Synowitz will explain that glioma release a soluble factor that increases the metalloproteinase activity in microglial cells, which are amassed in the tumour. The glioma cells exploit this microglial proteinase activity to invade into the brain.

Rainer Glass will introduce that neural stem cells are chemoattracted from the stem cell niches of the brain towards gliomas. On the site of pathology neural stem cells release a factor, which induces glioma cell death. However, this process is age dependant and is attenuated with the decline of neurogenesis in adulthood, which may also explain why gliomas are predominant in older patients.

Gaetano Finocchiaro will present new strategies for the therapeutic targeting of glioblastoma cells with cancer stem-like properties. Glioma stem cells are emerging as the main tumour-initiating and -maintaining entity in brain cancer. Current therapies target the bulk tumour mass and potentially fail to account for the different molecular and clinical properties of the glioma stem cells.

The current discoveries made in the field of brain tumour research may be of interest to neuroscientists as well as clinicians. By presenting the interplay of glioma with their microenvironment we intend to provide a more complete view on the mechanisms, which take place during neoplastic transformation of the central nervous system.

# Anti-angiogenic therapy of brain tumors

Peter Vajkoczy

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Glioblastoma multiforme is the prototype of an angiogenic tumor and thus predestined for an antiangiogenic therapy. Over the past years, the molecular phenotype as well as the molecular mechanisms of brain tumor angiogenesis have been increasingly understood and potential therapeutic targets have been identified. Consequently, first anti-angiogenic therapies have been translated into clinical application. However, at present it is becoming clear that brain tumors may develop an unexpected resistance to anti-angiogenic or anti-vascular therapies. The current research in the field will have to focus on a better understanding of these escape strategies in order to successfully add anti-angiogenesis to the therapeutic armentarium in treating brain tumors.

# Targeting TGF-ß for the immunotherapy of glioblastoma

Michael Weller

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The limited efficacy of surgery, radiotherapy and chemotherapy in the treatment of glioblastoma calls for innovative treatment approaches targeting specific biological features of these tumors. Glioblastoma cells are known for the release of multiple immunosuppressive molecules, including transforming growth factor (TGF)-ß. The very low frequency of systemic metastases in tumors which otherwise exhibit all features of malignancy has been attributed to an efficient glioma immune surveillance outside, but not inside, the central nervous system. Promising strategies of immunotherapy include targeting the synthesis, release or activity of TGF-ß. TGF-ß may be antagonized by RNA interference, inhibition of proprotein processing or TGF-ß receptor antagonists, conferring resistance to TGF-ß-induced immune paralysis. In fact, while the biological neutralization of glioma-associated immunosuppressive molecules alone may not induce the immune rejection of these tumors, it may still be a precondition for active strategies of immunotherapy to be successful. Future experimental trials in glioma-bearing rodents and phase I/II clinical trials will have to demonstrate whether the initiation of an efficient efferent immune response against glioblastoma cells may be more easily triggered by peripheral vacccination or by efforts to create a strong immune stimulatory environment within a postsurgical tumor cavity.

# Microglia stimulate glioma invasion

Michael Synowitz[1,2], Rainer Glass[2], Kathrin Färber[2], Kronenberg Golo[2], Darko Markovic[2], Jürgen Schnermann[3], Nico van Rooijen[4] and Helmut Kettenmann[2]

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We report that experimental glioblastoma grow more vigorously in A1 adenosine receptor (A1AR)-deficient mice associated with a strong accumulation of microglial cells at and around the tumors. A1ARs were prominently expressed in microglia associated with tumor cells as revealed with immunocytochemistry but low in microglia in the unaffected brain tissue. The A1AR could also be detected on microglia from human glioblastoma resections. To study functional interactions between tumor and host cells, we studied glioblastoma growth in organotypical brain slice cultures. A1AR agonists suppressed tumor growth. When, however, microglial cells were depleted from the slices, the agonists even stimulated tumor growth. Thus, adenosine attenuates glioblastoma growth acting via A1AR in microglia.

# Therapeutic targeting of glioblastoma cells with cancer stem-like properties.

Gaetano Finocchiaro

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A lot of interest, experimental data and debate are growing on the idea that cancer stem-like cells (CSC) exist in tumors and are responsible for tumor initiation and perpetuation. Initial findings corroborating the concept were obtained in leukemias: after that, malignant gliomas were in focus. Different experimental lines have proposed that immune selection of tumor cells based on the expression of stem cell markers, isolation of neurospheres and the sorting of a side population may at least converge in suggesting that not all gliomas cells have the same tumorigenic potential. The concept of complex cell hierarchies, making tumor organization similar to that of an organ, as well as that of the cell of origin (neural stem cells, neural precursor cells, de-differentiating glial cells?) are, on the other hand, less defined and matter of considerable debate. Remarkably, this new wave of research may provide important therapeutic consequences, indicating new targets for pharmacological compounds or immunotherapy. This may in turn give intriguing insights on the biology of cancer stem-like cells as well as of glial cells and their progenitors. To investigate the potential of CSC immunotherapy we used the GL261 glioma model. By culturing GL261 murine glioma cells with EGF-bFGF, we obtained neurospheres (NS) with cancer stem-like properties (GL261-NS) producing highly aggressive and infiltrating brain tumors. Injection of dendritic cells (DC) loaded with GL261-NS cured the majority of GL261-NS tumors. On the contrary, DC loaded with serum-cultured, GL261 adherent cells were unable to contrast GL261-NS lethality. A confirmation of GL261-NS properties in NS from human gliomas would encourage new approaches for DC-based immunotherapy.

# Cell-intrinsic limitations of the neural precursor cell response to brain tumors.

Rainer Glass, Joo-Hee Wälzlein, Michael Synowitz and Helmut Kettenmann

Cellular Neuroscience, Max Delbrück Center, Robert Rössle Str 10, Berlin, Germany

Neural precursor cells (NPCs) are attracted to experimental glioblastoma and induce tumor cell death. We show that the NPCs surrounding glioblastoma originate from rapidly proliferating cells in the subventricular zone (SVZ) and are diverted from their physiological migratory path towards the olfactory bulb thereby reducing olfactory bulb neurogenesis. In young mice (30 day old) high levels of subventricular proliferation maintained a continuous supply of NPCs, while in adult mice (90 day old) glioblastoma exhausted NPC proliferation in the SVZ. Fast proliferation of NPCs depended on the abundance of D-type cyclins, of which cyclin D1 age-dependently declined, accounting for the reduced supply of NPCs towards glioblastoma in adult. Disruption of the cyclin D2 gene specifically reduced adult neurogenesis and resulted in larger tumors. We conclude that transit-amplifying cells in the SVZ maintain a steady supply of NPCs towards glioblastoma. Provision of NPCs to glioblastoma is limited by cell-autonomous and age-related mechanisms controlling precursor cell proliferation.

# Erucylphosphohomocholine-induced cell death in human glioma cells: Role of reactive oxygen species

Wilfried Kugler[1], Karen Linnemannstöns[1], Leo Veenman[2], Moshe Gavish[2] and Max Lakomek[1]

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In contrast to standard chemotherapeutics, alkylphosphocholines (APC) are membranophile agents not directly targeting cellular DNA. Erucylphosphohomocholine (ErPC3) is a promising APC member for parenteral administration. It exerts strong anticancer activity and is a potent inducer of apoptosis, even in highly chemoresistant cells. Initial mechanistic studies indicate that mitochondria are central to ErPC3-induced apoptosis. Mitochondria are one of the major sources of reactive oxygen species (ROS). Oxidative stress and the redox state of a cell are supposed to play a pivotal role in regulating apoptosis. The 18 kDa peripheral-type benzodiazepine receptor (PBR) exerts various cell functions and is involved in a functional structure designated as the mitochondrial permeability transition pore. Published data suggest that PBR is a target for ROS which induce the formation of PBR polymers modulating PBR-mediated functions. We have shown previously that the classical PBR ligands PK11195 and Ro5-4864 both prevented apoptosis induction by ErPC3 in human glioma cells. In a first attempt to analyse a possible correlation between ErPC3, PBR, and ROS we investigated the role of ROS in ErPC3-induced cell death, using A172 and U373MG cells and different ROS inhibitors. The antioxidant butylated hydroxyanisole was able to inhibit ErPC3-induced apoptosis and cytotoxicity in both cell lines whereas iron chelators (deferoxamine, phenanthroline) blocked apoptosis in U373MG cells only. Sulfhydryl reagents (dithiotreitol, glutathione), superoxide dismutase, N-acetylcysteine and Trolox had no effect on ErPC3-induced cell death. Catalase partly inhibited ErPC3-mediated apoptosis in A172 cells. These findings are consistent with the hypothesis that hydrogen peroxide induced by ErPC3 acts close to its site of generation in A172 cells, whereas hydroxyl radicals produced in U373MG cells trigger the execution of apoptosis by inducing opening of the mitochondrial permeability pores.

This work was supported by a grant from the Volkswagen-Stiftung to W.K., L.V., M.L., and M.G. (Joint Lower Saxony - Israeli Research Projects; VWZN2047).

# Symposium

## S12: Computational Models of Vision

*Laurenz Wiskott and Gustavo Deco, Berlin and Barcelona (E)*

### Slide

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*Laurenz Wiskott and Gustavo Deco, Berlin and Barcelona (E)*

**S12-2** Towards an analytical derivation of complex cell receptive field properties
*Laurenz Wiskott, Henning Sprekeler and Pietro Berkes, Berlin and London (UK)*

**S12-3** Large scale models of the V1 cortical network
*Robert M. Shapley, New York (USA)*

**S12-4** Pinwheels - A structure without a function
*Fred Wolf, Göttingen*

**S12-5** Models of invariant object recognition and global motion recognition in the ventral and dorsal visual systems.
*Edmund Rolls, Oxford (UK)*

**S12-6** "Competition and Cooperation Cortical Mechanisms in Visual Cognition"
*Gustavo Deco, Barcelona (E)*

**S12-7** A model of the ventral visual system based on temporal stability and local memory
*Peter König, Osnabrück*

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**TS12-1B** Correspondence Based Object Recognition with Networks of Cortical Columns
*P. Wolfrum and C. von der Malsburg, Frankfurt*

**TS12-2B** Centrins, gatekeepers for the light-dependent translocation of transducin through the connecting cilium of the photoreceptor cell, regulated via
calcium and phosphorylation
*A. Giessl, S. Rausch, P. Trojan, MC. Thissen, D. Wünschig, S. Klumpp, A. Pulvermueller and U. Wolfrum, Mainz, Berlin and Muenster*

**TS12-3B** The Neural Processing of Natural Stereoscopic Images. An EEG Study.
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*L. Jansen, S. Onat and P. König, Osnabrück*

**TS12-5B** Assessing stereo matching algorithms using ground-truth disparity maps of natural scenes
*R. Märtin, JM. Steger, K. Lingemann, A. Nüchter, J. Hertzberg and P. König, Osnabrück*

**TS12-6B** Integration of different features in guiding eye-movements
*F. Schumann, A. Acik, S. Onat and P. König, Osnabrück*

**TS12-7B** VISUAL GROUNDSPEED CONTROL IN FREE-FLYING FRUIT FLIES
*N. Rohrseitz, AD. Straw and SN. Fry, Zürich (CH) and Pasadena (USA)*

Introductory Remarks to Symposium 12

# Computational Models of Vision

Laurenz Wiskott and Gustavo Deco, Berlin and Barcelona (E)

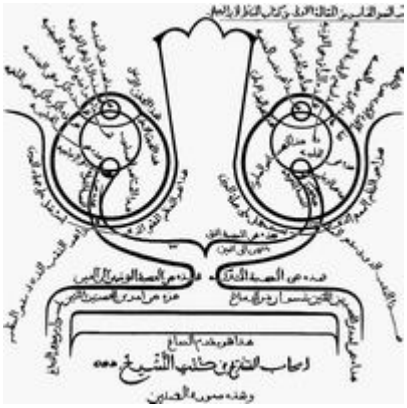

Illustration of the visual system by
Ibn al-Haythem (965-1039)

The visual system is particularly attractive to theoreticians for several reasons: A large amount of experimental results are available to inspire and constrain the models, it is a sensory system and therefore the inputs are fairly well defined, it is sufficiently complex to offer a number of interesting research questions, it is so homogeneous that one can expect approaches based on only a few principles to have large explanatory power, and any progress being made in modelling biological vision is likely to foster progress in technical vision applications as well. However, despite the considerable effort and progress that theoreticians have made in modelling vision, very basic questions are still open and subject to controversial discussions: What are optimal visual features and how can they self-organize? How are visual invariances achieved? How is visual attention implemented and controlled? How are object classes being learned? This symposium focusses on a systems level of visual processing from feature extraction to visual perception and brings together researchers with different perspectives and modelling approaches.

# Towards an analytical derivation of complex cell receptive field properties

Laurenz Wiskott[1], Henning Sprekeler[1] and Pietro Berkes[1,2]

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Slow Feature Analysis (SFA) [1] is an algorithm for extracting slowly varying features from a quickly varying signal. If applied to image sequences generated from natural images using a range of spatial transformations, SFA yields units that share many properties with complex and hypercomplex cells of early visual areas [2]. All units are responsive to Gabor stimuli with phase invariance, some show sharpened or widened orientation or frequency tuning, secondary response lobes, end/side-inhibition, or selectivity for direction of motion.
Interestingly, the results do not depend on the higher-order statistics of natural images. We get virtually identical results with colored noise images. This permits a clear formulation of the conditions under which complex cell properties emerge and makes the problem amenable to an analytical treatment. Here we show that important complex cell properties can be derived by means of variational calculus from first principles and a few basic assumptions.

[1] Wiskott, L. and Sejnowski, T.J. (2002). Slow Feature Analysis: Unsupervised Learning of Invariances. Neural Computation, 14(4):715-770. http://itb1.biologie.hu-berlin.de/~wiskott/Abstracts/WisSej2002.html

[2] Berkes, P. and Wiskott, L. (2005). Slow feature analysis yields a rich repertoire of complex cell properties. Journal of Vision, 5(6):579-602. http://journalofvision.org/5/6/9/

# Large scale models of the V1 cortical network

Robert M. Shapley

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I will describe a large-scale (order $10^4$ units) computational model of a local patch of the input layer 4Cα of the primary visual cortex (V1) of the macaque monkey. The model attempts to be realistic by including anatomical and biophysical data from many sources. The model neurons are integrate-and-fire neurons with biologically plausible synaptic conductances. This model can account for the distributions of orientation and spatial frequency selectivity across the population of V1, and also the relative prevalence of linear and nonlinear spatial summation in V1 neurons. The crucial controlling variable appears to be the relative strength of cortico-cortical inhibition relative to excitation. Indeed, strong cortico-cortical inhibition in the model is a prerequisite for cortical stability, for feature selectivity, and for spatial-summation linearity.

# Models of invariant object recognition and global motion recognition in the ventral and dorsal visual systems.

Edmund Rolls

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The ventral visual system operates to form invariant representations of faces and objects in the inferior temporal visual cortex. It has been shown that a multistage feed-forward architecture with convergence and competition at each stage is able to learn invariant representations of objects including faces by use of a Hebbian synaptic modification rule which incorporates a short memory trace (0.5 s) of preceding activity. This trace rule enables the network to learn the properties of objects which are spatio-temporally invariant over this time scale (Rolls and Deco, 2002). A new learning principle utilises continuous spatial transformations to compute invariant representations (Stringer et al, 2006). It has been found that in complex natural scenes, the receptive fields of inferior temporal cortex neurons shrink to approximately the size of an object, and are centred on or close to the fovea (Rolls et al, 2003). It is proposed that this provides a solution to reading the output of the ventral visual system, for primarily the object that is close to the fovea is represented by inferior temporal visual cortex neuronal activity. The effect is captured in models that use competition to weight the representation towards what is at the fovea. The model has been extended to account for covert attentional effects such as finding the location of a target object in a complex scene, by incorporating modules to represent the dorsal visual system, backprojections, and short term memory networks in the prefrontal cortex to keep active the representation of the object of attention, and does not require temporal synchronization to implement binding (Aggelopoulos et al 2005). The model has also been extended to a theory of how invariant global motion such as rotation is computed in the dorsal visual system.

Rolls,E.T. and Deco,G. (2002) Computational Neuroscience of Vision. Oxford University Press: Oxford.
Rolls,E.T., Aggelopoulos,N.C., and Zheng,F. (2003) The receptive fields of inferior temporal cortex neurons in natural scenes. Journal of Neuroscience 23: 339-348.
Aggelopoulos,N.C., Franco,L. and Rolls,E.T. (2005) Object perception in natural scenes: encoding by inferior temporal cortex simultaneously recorded neurons. Journal of Neurophysiology 93: 1342-1357.
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Rolls,E.T. and Stringer,S.M. (2006) Invariant global motion recognition in the dorsal visual system: a unifying theory. Neural Computation, in press.
Rolls,E.T. and Stringer,S.M. (2007) Invariant visual object recognition: a model, with lighting invariance. Journal de Physiologie Paris, in press.

# "Competition and Cooperation Cortical Mechanisms in Visual Cognition"

Gustavo Deco

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Cognitive behaviour requires complex context-dependent processing of information that emerges from the links between attentional perceptual processes, working memory and reward-based evaluation of the performed actions. We describe a computational neuroscience theoretical framework which shows how an attentional state held in a short term memory in the prefrontal cortex can by top-down processing influence ventral and dorsal stream cortical areas using biased competition to account for many aspects of visual attention. We also show how within the prefrontal cortex an attentional bias can influence the mapping of sensory inputs to motor outputs, and thus play an important role in decision making. We also show how the absence of expected rewards can switch the attentional bias signal, and thus rapidly and flexibly alter cognitive performance. This theoretical framework incorporates spiking and synaptic dynamics which enable single neuron responses, fMRI activations, psychophysical results, the effects of pharmacological agents, and the effects of damage to parts of the system, to be explicitly simulated and predicted. This computational neuroscience framework provides an approach for integrating different levels of investigation of brain function, and for understanding the relations between them. The models also directly address how bottom-up and top-down processes interact in visual cognition, and show how some apparently serial processes reflect the operation of interacting parallel distributed systems.

# A model of the ventral visual system based on temporal stability and local memory

Peter König

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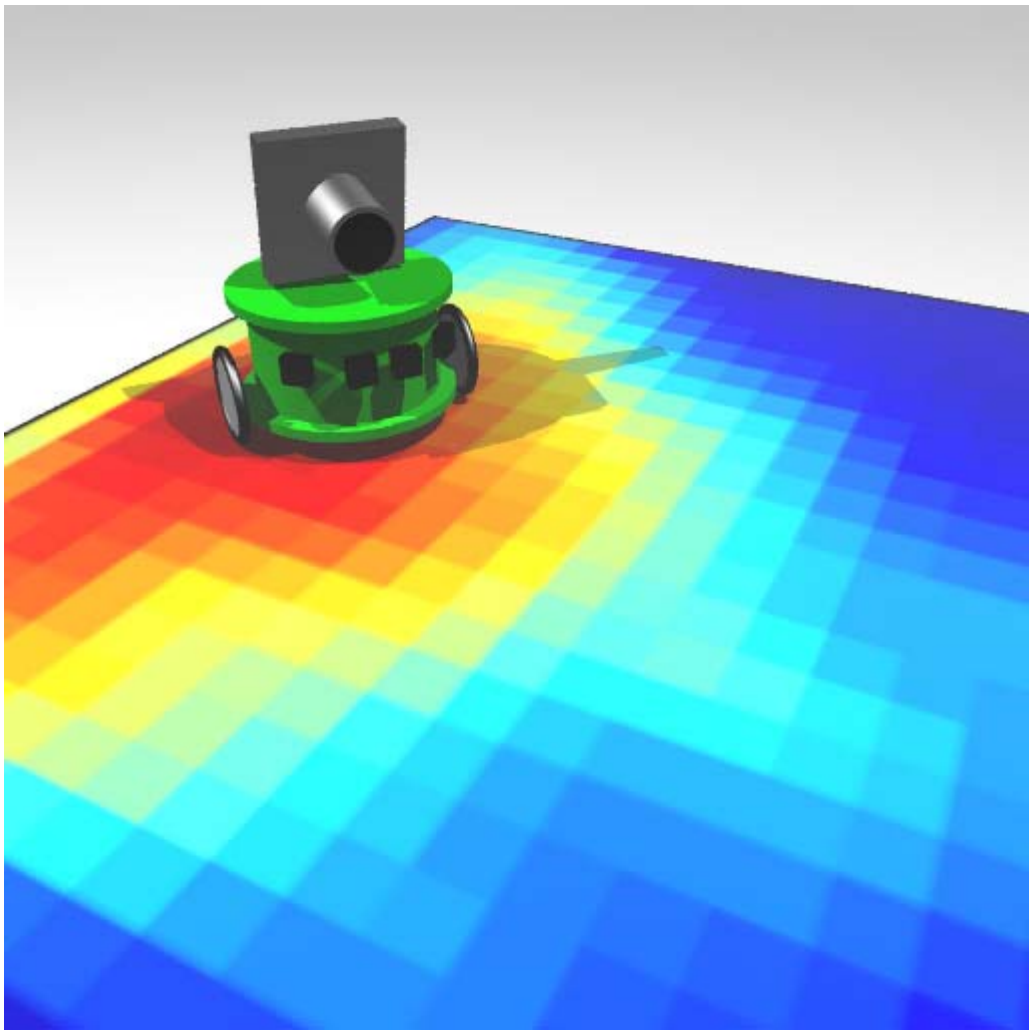
The cerebral cortex is a remarkably homogeneous structure suggesting a rather generic computational machinery. Indeed, under a variety of conditions, functions attributed to specialized areas can be supported by other regions. However, a host of studies have laid out an ever more detailed map of functional cortical areas. This leaves us with the puzzle of whether different cortical areas are intrinsically specialized, or whether they differ mostly by their position in the processing hierarchy and their inputs but apply the same computational principles. Here we show that the computational principle of optimal stability of sensory representations combined with local memory gives rise to a hierarchy of processing stages resembling the ventral visual pathway when it is exposed to continuous natural stimuli. Early processing stages show receptive fields similar to those observed in the primary visual cortex. Subsequent stages are selective for increasingly complex configurations of local features, as observed in higher visual areas. The last stage of the model displays place fields as observed in entorhinal cortex and hippocampus. The results suggest that functionally heterogeneous cortical areas can be generated by only a few computational principles and highlight the importance of the variability of the input signals in forming functional specialization.



# Correspondence Based Object Recognition with Networks of Cortical Columns

Philipp Wolfrum[1] and Christoph von der Malsburg[1,2]

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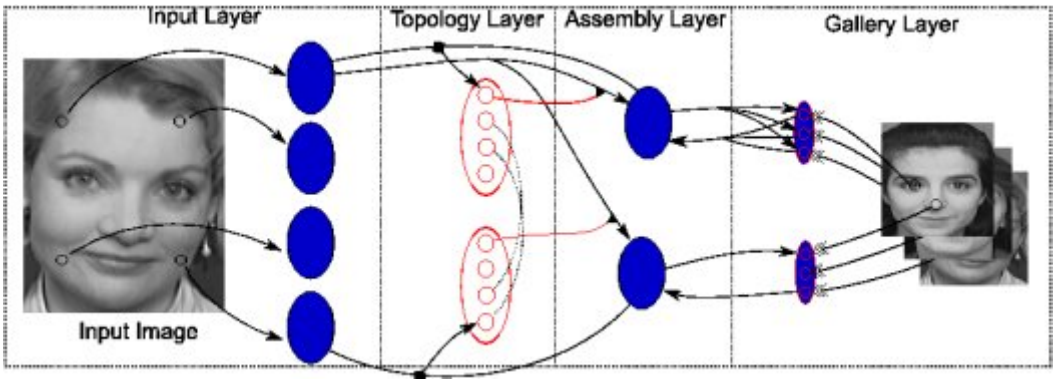
We present a fully neural model for correspondence based invariant object recognition. Its performance is demonstrated by using it to recognize faces in a position-invariant manner from a gallery of 100 stored faces.

The system is based on a model for correspondence finding [1] that makes use of a population code within cortical columns to achieve fast (~25 ms) comparison and point-to-point matching between one-dimensional images. Here we extend this model to a system that matches two-dimensional images of different geometry and can compare input images to several stored models simultaneously.

The network consists of macrocolumns [1] organized in the following layers (see figure):
*Input layer*: Represents the input image in a rectangular grid of 12x12 points. For each grid point, a macrocolumn represents 40 Gabor features extracted from the image at that position. This roughly corresponds to the complex cells in V1.
*Topology layer*: Contains control neurons connecting every input point to every point in the assembly layer. The activity of the control neurons is driven by the feature similarity of the points they connect and in the end represents a position-invariant match between input and assembly layer. At the same time, the control units modulate the connection strength between the units they connect and thus have the role of routing information correctly within the network. We assume that intermediate visual areas like V2 and perhaps V4 are involved in this routing process.
*Assembly layer*: Represents face images in a "face graph" topology, i.e. a graph containing nodes for important landmarks. At the beginning of the recognition cycle, a superposition of all faces stored in the gallery is projected to this layer. This helps to establish a match with the input. Later, the assembly layer contains the correctly positioned input image, which is compared to the gallery.
*Gallery layer*: Contains all stored faces, represented in the same topology as the assembly layer. Comparison with the assembly layer and competition among stored models leaves only the correctly recognized identity active in the end. Anatomically, the gallery would be located in higher areas of IT and in specialized cortical regions like the fusiform face area.

Competition between units of the topology and the gallery layers rises during the recognition cycle but is relatively weak. Yet a clear network decision emerges through lateral cooperation between units coding for the same percept. The network as a whole performs simultaneously position-invariant matching and identification of input images.

[1] Lücke, J. and von der Malsburg, C. : *Proc. ICANN 2006* **4131**, 668-677



# Centrins, gatekeepers for the light-dependent translocation of transducin through the connecting cilium of the photoreceptor cell, regulated via calcium and phosphorylation

Andreas Giessl[1], Sebastian Rausch[2], Philipp Trojan[1], Marie Christin Thissen[3], Diana Wünschig[1], Susanne Klumpp[3], Alexander Pulvermueller[2] and Uwe Wolfrum[1]

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Photo isomerisation of rhodopsin activates a heterotrimeric G-protein cascade leading to closure of cGMP-gated channels and hyperpolarization of photoreceptor cells (PRC). Massive translocation of the visual G-protein transducin, Gt, between the inner and outer segment contributes to long term adaptation of photoreceptor cells. Calcium-triggered assembly of a centrin-transducin complex in the connecting cilium of rod photoreceptor cells may regulate transducin movements.

Centrins are calcium-binding EF-hand phospho-proteins. In PRC of the vertebrate retina 4 centrins are differentially localized in the ciliary apparatus of the connecting cilium (CC). Previous studies revealed calcium-dependent binding of centrins to the βγ-subunits of the visual G-protein transducin (Gt). Present FRET and light scattering analyses showed binding of the Gtβγ-subunit to the N-terminus of centrins.

In vitro and ex vivo assays showed light-dependent phosphorylation of centrins in PRC. Inhibitor experiments indicated CK2 being responsible for centrin phosphorylation. We identified target sites for CK2 mediated phosphorylation. Protein phosphatase PP2Cβ was found to dephosphorylate centrins. Immunohistochemistry confirmed co-localization of centrins with CK2 and PP2Cβ in the CC. Kinetic light-scattering assays demonstrated a decreased binding affinity of phosphorylated centrins to Gt.

Centrin binding to Gt is antagonistically modulated by calcium and light-dependent phosphorylation. In PRC, these centrin modifications may regulate light-dependent movements of Gt between the inner and outer segment. The assemblies of the visual G-protein with centrins are novel aspects of the fine tuned supply of signaling proteins in photoreceptor cells, and potential links between molecular translocations and signal transduction in general.

Support: FAUN; DFG

# The Neural Processing of Natural Stereoscopic Images. An EEG Study.

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We investigate cortical mechanisms of depth perception under natural conditions. We acquired 29 high-resolution stereo images and concomitant laser range scans of natural scenes. In addition we generated 'Pink Noise' stereo images with identical second-order statistics but random phase spectra, and 'White Noise' images. However, the stereo images in both artificial categories contain the same binocular disparity as the 'Natural' images. The participants rated the intensity of their scenic 3D perception, and rated 'Pink Noise' significantly lower than the other two.

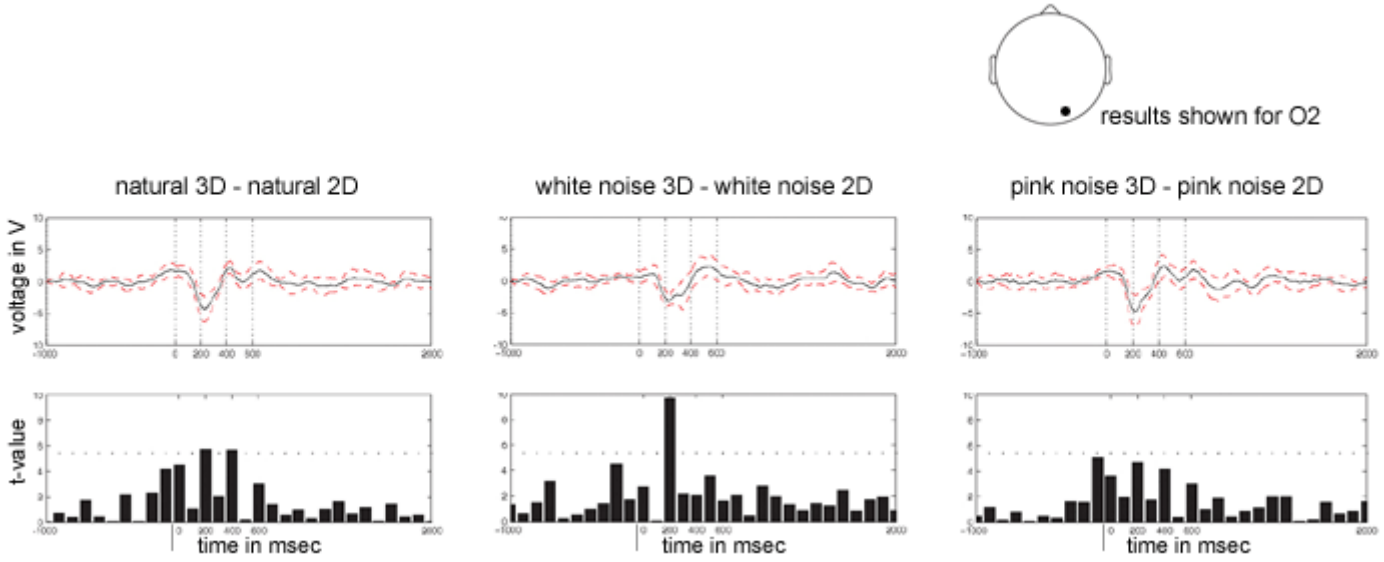
These three types of images were presented to 7 human participants in 2D and 3D conditions and, we recorded a 32-channel EEG simultaneously.

We found out that neural activation in 3D significantly differs from 2D in central occipital electrodes at around 200 ms after stimulus onset

in all categories. Activation of lateral occipital electrodes, in contrast, predicts whether the stimuli are able to induce scenic

stereoscopic perception, as there is a highly significant activation in the 'Natural' and 'White Noise' case, but solely a moderately significant activation in the 'Pink Noise' case (see figure for the case of O2 electrode). In addition, frontocentral activity is sensitive to differences in second-order statistics at about 400 ms after stimulus onset: here, 'Natural'and 'Pink Noise' scenes elicit a significantly different activation than 'White Noise' scenes.

Concluding, our results demonstrate a category-invariant effect of stereovision in occipital recording sites at 200 ms after stimulus presentation that is modulated by top-down processes in lateral occipital electrodes, as well as category-specific effects in fronto-central recording sites, at 400 ms after stimulus onset.



# Overt Attention During Free Viewing of Natural Stereoscopic Images

Lina Jansen, Selim Onat and Peter König

Institute of Cognitive Science, University Osnabrück

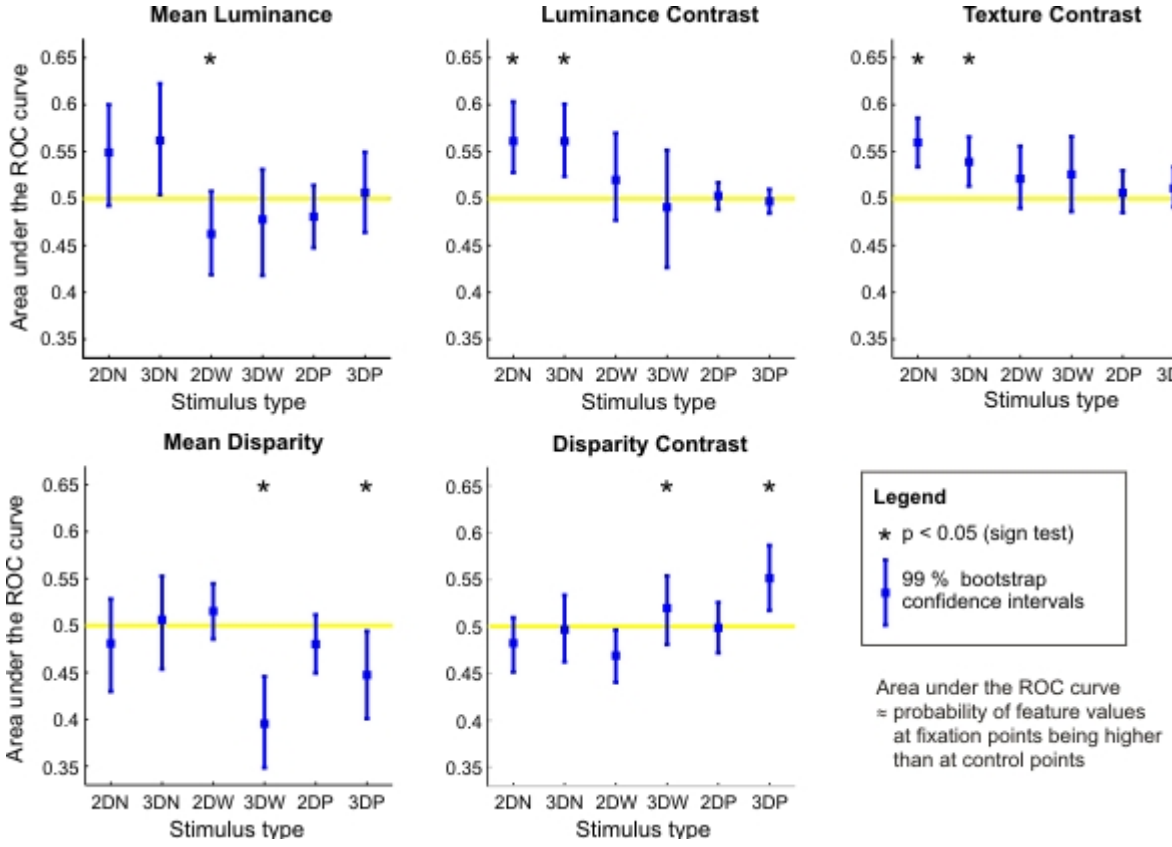
Recent models of overt attention are based on studies of the behaviour of humans watching natural images without any binocular cues. As a consequence, little is known about image statistics at the fixation points while humans are freely watching 3D natural images.

Using a laser scanner and a pair of cameras, we acquired 24 stereoscopic natural images together with their ground truth disparity information (see Märtin et al. same conference). We used the obtained disparity information to generate pink (P) and white noise (W) images that have the same binocular information as their natural versions (N). Both noise stimuli and the natural images were shown in a 2D and 3D condition making up a total of 6 conditions. On this stimulus set eye movements of human subjects were recorded in a free viewing task.

Our results show that basic eye movement properties such as mean fixation duration, number of fixations, and mean saccade length differ between conditions. In the presence of binocular cues, fixations were spread more equally across the images.

We used a ROC analysis to compare the distribution of both monocular (mean luminance, luminance contrast, texture contrast) and binocular (mean disparity, disparity contrast) features at fixations with the respective distribution at control points. Luminance contrast and texture contrast were higher at fixated than at control locations for both 2D and 3D natural images, but not in 2D and 3D pink noise images. Mean Disparity was smaller at fixations in the 3D white noise and 3D pink noise condition. Thus, in both 3D noise conditions subjects looked more often at locations that are closer. Disparity contrast also was higher at fixated locations in the two 3D noise conditions, but not in 3D natural images. However, disparity contrast at fixated regions was generally higher in the 3D than in the 2D conditions.

In summary, the presence of binocular cues changes the basic eye movement properties in all image types and the saliency of locations in noise images.



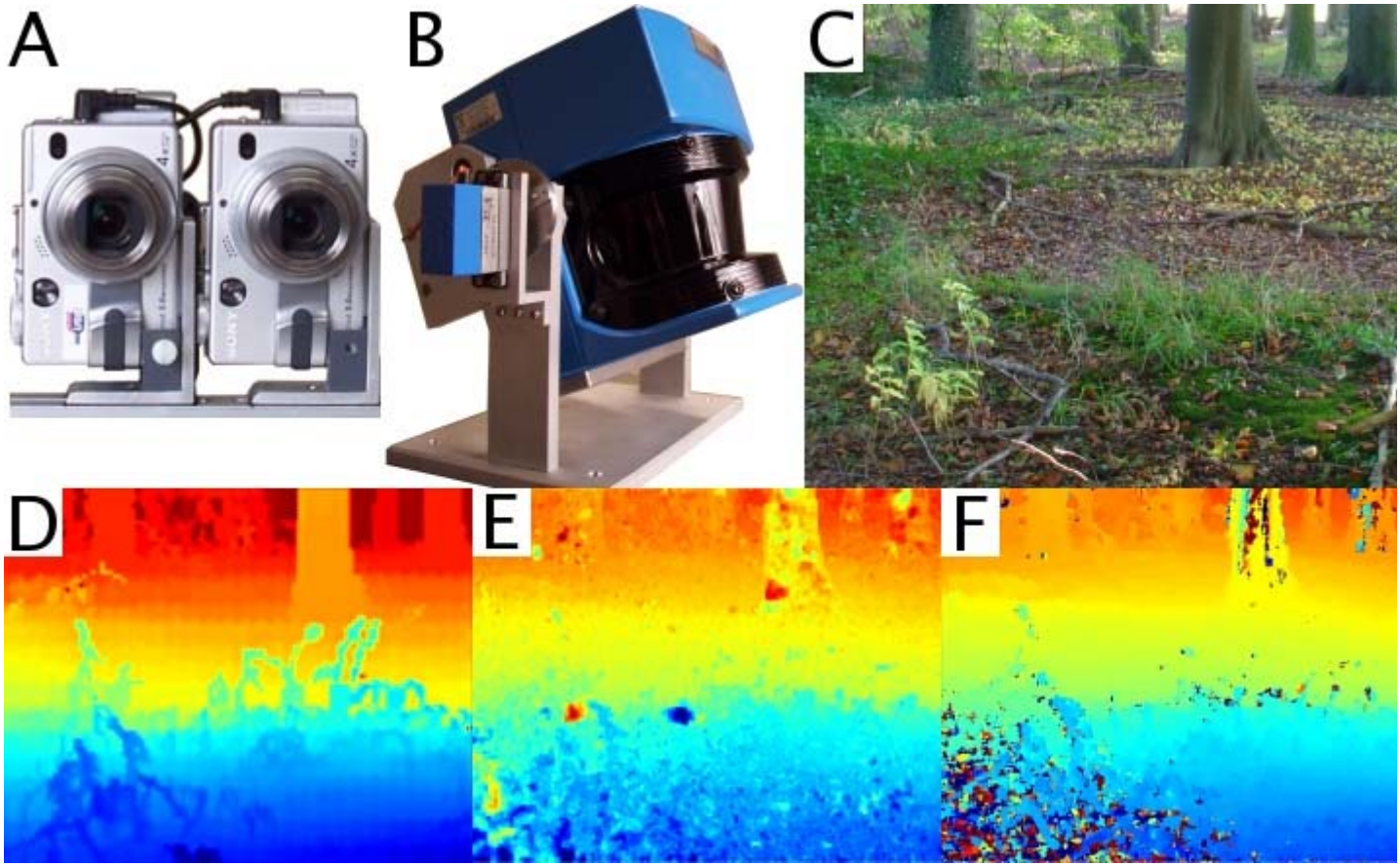
# Assessing stereo matching algorithms using ground-truth disparity maps of natural scenes

Robert Märtin[1], Johannes M. Steger[1], Kai Lingemann[2], Andreas Nüchter[2], Joachim Hertzberg[2] and Peter König[1]

[1]Institute of Cognitive Science, Universität Osnabrück
[2]Institute of Computer Science, Universität Osnabrück

Stereo matching algorithms from computer vision can advance our understanding of the fundamental problems of stereoscopic vision and, if biologically plausible, can serve as models of cortical processing. Here, we test different algorithms for their real-world validity by assessing their performance on stereo images of natural scenes. To do this, ground truth disparity maps are required. We compile a database of high resolution stereo images of natural scenes taken by two Sony DCS-V1 (Fig. A). In addition, we directly measure the depth structure of the same scenes using a 3D laser scanner (Sick Inc. LMS200, Fig B). Both, scan and stereoscopic image, are taken from the same vantage point. After appropriate calibration, we use these scans to approximate ground truth disparity for the images taken by the stereo camera.

Next, we compare different stereo matching algorithms: cross-correlation (CC) and belief propagation (BP). For the stereo image in Fig. C (only right image shown) disparity maps obtained from the laser scans, BP and CC are shown in Fig. D, E and F, respectively. After examining a total of 29 stereo images we conclude that CC is vastly outperformed by BP, as BP matches the scanned disparity maps best. The mean correlation coefficient between LS and BP is 0.61 ± 0.043 (standard error), while it is only 0.30 ± 0.037 between LS and CC. BP, performing fast inference on graphical models by passing messages between neighboring nodes, is regarded to be a biologically plausible metaphor of cortical computation within a probabilistic framework (e.g. Rao (2006), Lee & Mumford (2003)). Our results suggest that BP is also able to solve real-world stereo problems as encountered by our visual system.



# Integration of different features in guiding eye-movements

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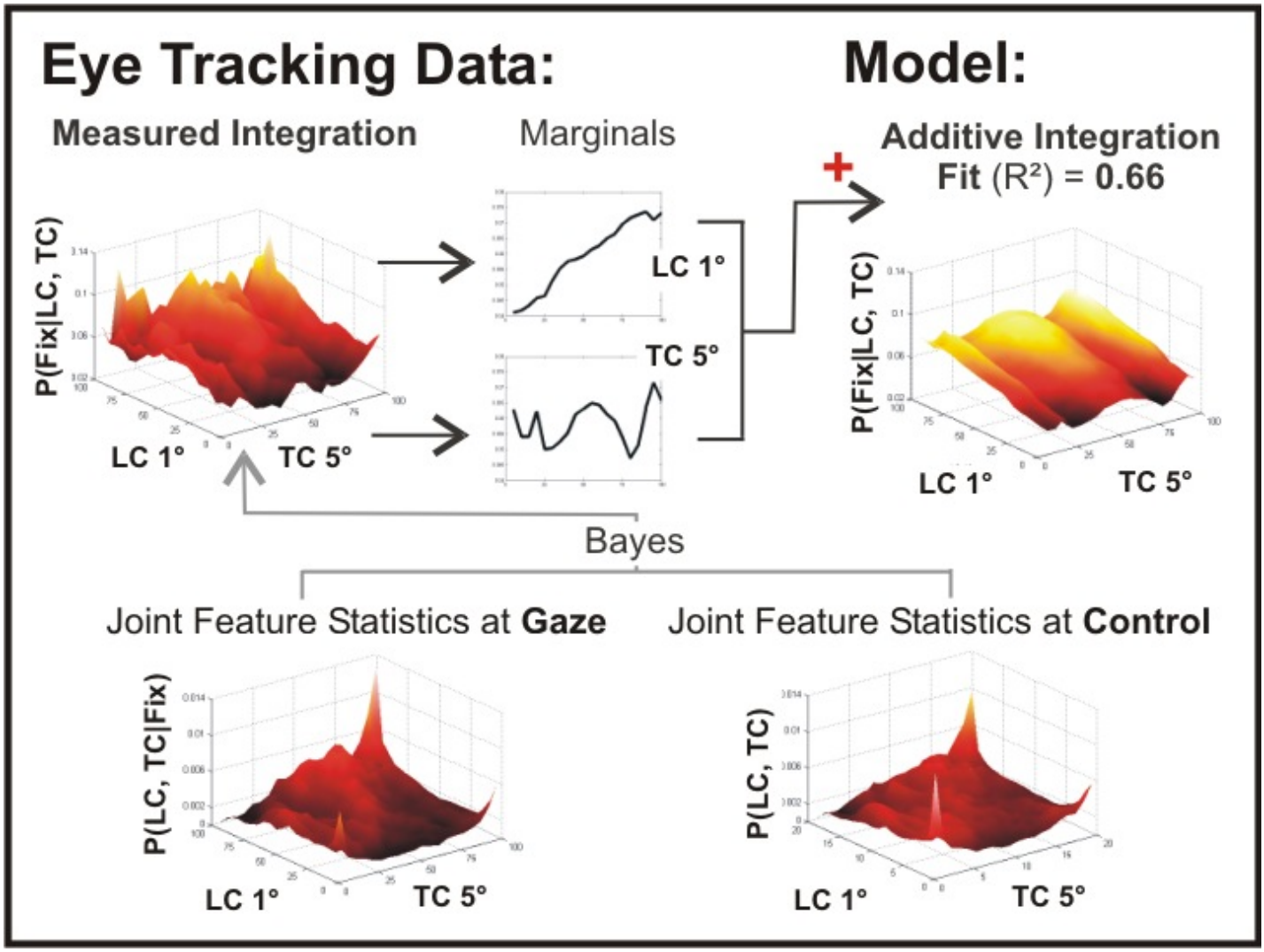
In natural behaviour we actively attend to parts of a visual scene by moving our eyes. Models of such overt attention combine different local features in a bottom-up process. Here, we study this integration process empirically and investigate the interaction of luminance, luminance contrast, texture contrast, edges and colour contrast during free viewing of natural stimuli. (1) We describe the interaction of features, how the saliency of one feature varies in the context of a second feature. We find that in general knowing the value of one feature does not give much information about the saliency of another feature, i.e. different features contribute independently to a joint saliency map. (2) We use additive and multiplicative integration processes to model feature integration. Additive integration of feature saliencies gives a good description of the data for all analyzed feature combinations within and across feature channels. However, multiplicative interaction explained the data equally good. In summary, we argue that selection of attended regions can be explained by the additive integration of independently processed single feature saliency functions.



# VISUAL GROUNDSPEED CONTROL IN FREE-FLYING FRUIT FLIES

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Despite detailed knowledge of the motion processing pathways in flies, the neural mechanisms underlying responses to translatory optic flow remain little understood. In this study, we applied a systems analysis of visual groundspeed control responses in the fruit fly *Drosophila*, in view of the underlying motion processing mechanisms.

We implemented a novel behavioral paradigm based on a wind tunnel equipped with 3D tracking and virtual reality display technology, to artificially decouple the visual feedback that a fly experiences when moving through the environment. We measured the tuning properties of the fly's visuomotor responses to vertical sine-gratings, whose temporal (TF) and spatial frequency (SF) we varied over a large parameter range. To stimulate flies with precise TF, we adjusted the pattern phase according to the flies' current body position along the wind tunnel (one parameter 'virtual open-loop' paradigm).

The response strength to the short open-loop stimulation was measured as mean acceleration, which was constant for all combinations of TF and SF. The system transfer function, represented by the measured tuning properties, reveals a linear dependence on pattern velocity within the likely range of behaviorally relevant stimuli. The responses to pattern combinations of same velocity were invariant over a large parameter range.

The measured tuning properties are difficult to explain with the present understanding of motion processing in flies and suggest the presence of alternative mechanisms and pathways, which remain to be explored. Future research will include the behavioral analysis of visual free flight behavior in transgenic fruit flies to explore the neural circuitry involved in the processing of translatory optic flow.

# Symposium

## S13: Functional Role of Nucleotide Signaling in the Nervous System

***Peter Illes and Herbert Zimmermann, Leipzig and Frankfurt/Main***

### Slide

**S13-1** Opening remarks for Symposium 13
*Peter Illes and Herbert Zimmermann, Leipzig and Frankfurt/Main*

**S13-2** Quantal release of ATP in central synapses
*Alexei Verkhratsky, Manchester (UK)*

**S13-3** The role of ATP and its metabolites for synaptic plasticity in the cerebellum
*Joachim W. Deitmer, Johannes Brockhaus and Diana Casel, Kaiserslautern*

**S13-4** Glia-neuron cross-talk via ATP in the brain
*Maria Pia Abbracchio, Milano (I)*

**S13-5** Trophic actions of extracellular nucleotides on neurons and glial cells
*Heike Franke, Leipzig*

**S13-6** Nucleotide signaling in adult neurogenesis
*Herbert Zimmermann, David Langer, Santosh Kumar Mishra, Varsha Shukla, Kristine Gampe, Ivette Grimm, Jasmin Delic, Christof Schomerus, Horst-Werner Korf, Helmut Kettenmann and Norbert Braun, Frankfurt/Main and Berlin*

Introductory Remarks to Symposium 13

# Functional Role of Nucleotide Signaling in the Nervous System

Peter Illes and Herbert Zimmermann, Leipzig and Frankfurt/Main

Signaling via extracellular nucleotides has been recognized as one of the most ubiquitous intercellular signaling mechanism in the nervous system. During the past decade, impressive progress has been made in the characterization of the molecular players involved and in the identification of essential functions initiated by nucleotides. Nucleotides may act as direct functional switches or also as mutual modulators or enhancers of additional signaling pathways. Both, neurons and glial cells carry receptors for nucleotides of which seven ionotropic (P2X) and eight metabotropic (P2Y) subtypes have been identified and characterized to date. Whereas P2X receptors respond to only ATP, P2Y receptors can be activated by ATP, ADP, UTP, UDP and nucleotide sugars. Agonist specificity varies between P2Y receptor subtypes. Recently, the orphan receptor GPR17 has been identified as a new dual uracil nucleotide/cysteinyl-leukotriene receptor. Extracellular nucleotides mediate or modulate fast signal transmission and cause short and long term changes in neural and glial functions. These include synaptic transmission, spreading of glial calcium waves, neuron-glia cross-talk and an array of trophic and proliferative actions that govern neural and glial development as well as regenerative processes. Bringing together leading experts from Germany, Great Britain and Italy, the symposium highlights essential functions of nucleotides such as quantal ATP release, initiation of synaptic plasticity, the cross-talk between glia and neurons and trophic actions such as the control of astrogliosis, differentiation, cell proliferation, and neuron survival. Most recently nucleotides have been assigned a role in the control of adult neurogenesis in the mammalian brain. All these data suggest that nucleotides are amongst the most versatile messengers in the brain.

# Quantal release of ATP in central synapses

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Adenosine triphosphate (ATP) acts as a fast excitatory transmitter in several regions of the central nervous system (CNS) including the medial habenula, dorsal horn, locus coeruleus, hippocampus and somatosensory cortex. ATP is released via several pathways, including exocytosis from presynaptic terminals and diffusion through large transmembrane pores (e.g. hemichannels, P2X7 receptors or volume-sensitive chloride channels) expressed in astroglial membranes. In presynaptic terminals, ATP is accumulated and stored in the synaptic vesicles. In different presynaptic terminals these vesicles may contain ATP only, or ATP and another neurotransmitter (e.g. GABA or glutamate); in the latter case, two transmitters can be co-released.

Somatosensory cortex layer III neurons with pyramidal shaped somata in acutely isolated transverse slices were voltage-clamped and both spontaneous miniature postsynaptic currents (mEPSCs) and postsynaptic currents (EPSCs) evoked by stimulating axons originating from layer V neurons were monitored. The mEPSCs recorded from pyramidal neurons in vitro had a bimodal amplitude distribution. Larger events were blocked by glutamate receptor (AMPA, kainate, NMDA) antagonists, whereas smaller events were selectively but partially inhibited by P2X receptor antagonists. The non-glutamatergic mEPSCs were asynchronous with those mediated by glutamate or GABA. Stimulation of single intracortical axons elicited quantal currents sensitive to P2X receptors antagonists with amplitudes corresponding to the non-glutamatergic mEPSCs. Thus, quantal events associated with activation of P2X receptors occur but with a lower probability than those of glutamate, and they evoke unitary currents about half the amplitude of those mediated through AMPA receptors. In conclusion, this work shows the co-existence of two independent postsynaptic current components, associated with activation of glutamate and P2X receptors. Our data also indicate that ATP is released from a specific set of vesicles, which are present in glutamate-containing terminals but ATP-mediated component of transmission occurs at only a subset of cortical synapses.

# The role of ATP and its metabolites for synaptic plasticity in the cerebellum

Joachim W. Deitmer, Johannes Brockhaus and Diana Casel

FB Biologie, TU Kaiserslautern, P.B. 3049, Kaiserslautern, Germany

We have found that ATP is a neuromodulator in the rat cerebellar cortex, which is endogenously released and which enhances the synaptic activity from inhibitory interneurons onto Purkinje neurons from the third postnatal week onwards (Casel et al., 2005, J. Physiol. 568.1, 111-122). The effect of ATP is increased by inhibiting ecto-nucleotidases, which degrade ATP to adenosine (Ado). Ado itself has no effect on the spontaneous synaptic activity in Purkinje neurons, but markedly modulates evoked synaptic responses during stimulation of parallel fibres (granule cells). The amplitude of evoked postsynaptic currents, as recorded in Purkinje neurons of acute brain slices in the patch-clamp whole-cell mode, decreased in two phases by ado (100 µM): in a transient phase by 60% (Dittman & Regehr, 1996, J. Neurosci., 16, 1623-1633), and in a long-lasting phase by 30%. Both phases were also induced by ATP and the Ado agonist CPado, but not by the non-degradable ATP-g-S. The effect of Ado and ATP could be inhibited by the A1-receptor antagonist DPCPX, but not by A2- and A3-receptor blockers. The second phase appeared to be mediated by a mechanism similar, if not identical, to the 'classical long term depression' (LTD), induced by simultaneous stimulation of parallel fibres and Purkinje neuron depolarisation, suggesting that LTD is mediated by the release of ATP, which is degraded to Ado. Both forms of LTD were suppressed by inhibiting PKC in the postsynaptic Purkinje neuron. When ecto-nucleotidases were blocked by ARL67156, an LTD could still be induced by Ado, but not by ATP or by the 'classical LTD' protocol. Our results suggest that ATP in the cerebellar cortex enhances inhibitory input via P2 receptors and, after being degraded to Ado, reduces evoked excitatory input to the Purkinje neurons via A 1 receptors.

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# Glia-neuron cross-talk via ATP in the brain

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ATP acts as a dominant messenger in intercellular communication in the brain via activation of P2 receptors: the seven ligand-gated P2X receptor channels, and the eight G-protein-coupled P2Y receptors. A wide array of these receptors are expressed by both neurons and glial cells. Upon mechanical or neurotransmitter stimulation, astrocytes release ATP and respond to ATP with a propagating wave of intracellular calcium increases, allowing a homotypic astrocyte-astrocyte communication, as well as an heterotypic signalling which also involves adjacent neurons and other types of glia. In previous studies, we have characterized the specific glial P2 receptors involved in this form of short-term calcium-dependent glia-neuron cross-talk (Fumagalli et al., 2003; Bianco et al., 2005). Nucleotides are also involved in the glia-neuron cross-talk contributing to the long-term changes occurring in brain upon trauma and ischemia. Under these conditions, massive amounts of nucleotides are released by both microglia and astrocytes, and contribute to neuroinflammatory damage. Thus, there is an active interest in the characterization of the P2 receptors involved in propagation of brain damage upon injury. In this respect, we recently focused our attention on GPR17, a previously orphan receptor that we have identified as a novel P2Y receptor (Ciana et al., 2006). GPR17 is phylogenetically related to both already known P2Y receptors and to CysLT1 and CysLT2 receptors responding to cysteinyl-leukotrienes (cysLTs), another family of proinflammatory molecules which are also released in the injured brain. We have shown that GPR17 is dually activated by both uracil nucleotides and cysLTs, and that, in rat cortex, GPR17 is specifically expressed in neuronal cells. Upon induction of focal ischemia, its expression is markedly increased both within and at the borders of ischemic infarcts. To confirm a role for this new receptor in ischemic injury, in vivo inhibition of GPR17 by either CysLT/P2Y receptor antagonists or anti-sense oligonucleotide technology dramatically reduced brain damage, suggesting this receptor as a common molecular target mediating injury by nucleotides and cysLTs. These findings may have intriguing implications for the development of dualistic drugs (in this case, anti-stroke agents) of previously unexplored therapeutic potential.

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Glia. 2003 43:218-03.

# Trophic actions of extracellular nucleotides on neurons and glial cells

Heike Franke

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ATP may act as a growth factor participating in differentiation, cell proliferation and survival, as well as a toxic agent mediating cellular degeneration and death. Most effects are mediated through the two cell surface receptor families, P2X and P2Y, widely expressed in the CNS.

After different kinds of "acute" CNS-injury (e.g. ischemia, mechanical stress, axotomy), extracellular nucleotides are thought to be released from damaged cells and, thereby, reach high concentrations in the extracellular space. Using an in vivo model of reactive astrogliosis, we have demonstrated that endogenous ATP is released after injury and participates in astrogliosis caused by stab wound injury in the nucleus accumbens (NAc) and cortex of rats. Microinfusion of P2X and P2Y receptor agonists into the NAc increased the number of the proliferating (glial fibrillary acidic protein/5-bromo-2'-deoxyuridine (GFAP/BrdU)-double stained) cells; this effect could be inhibited by P2 receptor antagonists. P2X and P2Y receptor-subtypes are expressed/co-expressed on astrocytes of untreated rats and the P2 receptor expression (P2X1,7 and P2Y2,6) was altered by mechanical injury. The data indicate a significant injury-induced increase in P2Y1 receptor expression on astrocytes, microglia and neurons, e.g. on vesicular glutamate transporter 3 (VGLUT3)- or tyrosine hydroxylase (TH)-immunopositive fibres and/or cells in the NAc. We assume that the P2Y receptor-mediated stimulation of different signalling pathways, e.g. the mitogen-activated protein cascade/extracellular signal regulated protein kinases (MAPK/ERK)-, the phosphoinositide 3-kinase/serine kinase (PI3K/AKT)- and the nitric oxide/nitric oxide synthase/ guanylyl cyclase (NO/NOS/GC)-pathways participate to a variable extent in the process of regeneration and/or proliferation under in vivo conditions.

Otherwise, the toxic effects of injury-evoked ATP-release may cause morphological responses, including necrosis, apoptosis and degeneration processes. We have shown that the inhibition of P2 receptors may prevent injury-induced astroglial proliferation and ischemia-induced neuronal death in the periinfarct area after permanent occlusion of the middle cerebral artery in spontaneously hypertensive rats. The possible involvement of purinergic receptors (e.g. P2X7, P2Y1) in the mediation of degenerative processes (caspase 3 activation, nuclear condensation/DNA fragmentation) was also studied.

In ex vivo experiments, a P2 receptor agonist promoted fibre outgrowth in organotypic slice co-cultures of the dopaminergic and hippocampal system, which could be inhibited by pyridoxalphosphate-6-azophenyl-2´,4´-disulphonic acid (PPADS), suggesting the involvement of P2 receptors. An early time- and region-dependent expression of different P2 receptor subtypes was observed.

In summary, these data suggest that ATP acting via P2 receptors is a potent regulator of normal physiological and pathological processes in the brain, with a specific focus on pathophysiological implications of P2 receptor functions, related to detrimental and/or beneficial effects.

# Nucleotide signaling in adult neurogenesis

Herbert Zimmermann[1], David Langer[1], Santosh Kumar Mishra[1], Varsha Shukla[1], Kristine Gampe[1], Ivette Grimm[1], Jasmin Delic[1], Christof Schomerus[2], Horst-Werner Korf[2], Helmut Kettenmann[3] and Norbert Braun[1]

[1]Biocenter, AK Neurochemie, J.W. Goethe-Universität, Max-von-Laue-Str. 9, Frankfurt am Main, Germany; [2]Medical School, J.W. Goethe-Universität, Frankfurt am Main, Germany; [3]Max Delbrück Center for Molecular Medicine, Berlin-Buch, Germany

In the adult rodent brain, active neurogenesis takes place in the subventricular zone of the lateral ventricles (SVZ) and in the dentate gyrus of the hippocampus. In both neurogenic regions, astrocyte-like cells function as precursor cells/stem cells. The cellular and molecular events driving a subpopulation of precursor cells into neurogenesis as well as the mechanisms controlling the cellular transition states to mature neurons are poorly understood. Here we provide evidence that extracellular nucleotides and nucleosides are involved in the control of neurogenesis in the adult murine brain. We show that all progenitor cells of the adult SVZ (type A, B and C cells) express the extracellular nucleotide hydrolyzing enzyme alkaline phosphatase (tissue non-specific form, TNAP) whereas the stem cells (type B cells) in addition specifically express the extracellular nucleoside triphosphate-hydrolyzing enzyme NTPDase2. Progenitor cells in the dentate gyrus (residual radial glia) express NTPDase2 but not TNAP. These two ecto-nucleotidases are also associated with specific subsets of progenitor cells during development. Ecto-nucleotidases control the availability of nucleotides and nucleosides at their respective receptors. A patch-lamp analysis of cells in hippocampal slices derived from adult mice transgenic for GFP under the control of the nestin promotor suggests that progenitor cells express P2X nucleotide receptors. Neurospheres cultured from the adult mouse SVZ in the presence of EGF and bFGF express both ecto-nucleotidases and hydrolyze extracellular ATP to adenosine. ATP, ADP and to a smaller extent UTP evoke rapid Ca2+ transients in neurospheres that are mediated by the metabotropic P2Y1 and P2Y2 nucleotide receptors that in turn activate the MAP kinase pathway. Agonists of these receptors and also low concentrations of adenosine augment cell proliferation in the presence of the mitogenic growth factors EGF and bFGF. Our results support the notion that extracellular nucleotides contribute to the control of adult neurogenesis in both, the SVZ and the dentate gyrus.

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# Symposium

## S14: Cell Intrinsic Mechanisms in the Regulation of Neural Development

***Dorothea Schulte and Dieter Engelkamp, Frankfurt/Main***

### Slide

**S14-1** Opening remarks for Symposium 14
*Dorothea Schulte and Dieter Engelkamp, Frankfurt/Main*

**S14-2** Individual cell migration serves as the driving force for optic vesicle evagination
*Joachim Wittbrodt, Felix Loosli, Richard J. Adams and Martina Rembold, Heidelberg and Cambridge (UK)*

**S14-3** Ptf1a determines GABAergic neuronal cell fate in the retina
*Muriel Perron, Mélodie Robach, Morgane Locker, Yoichiro Aoki, Yonglong Chen and Tomas Pieler, ORSAY (F) and Goettingen*

**S14-4** Control of vertebrate visual system development by MEIS homeodomain proteins
*Dorothea Schulte, Peer Heine, Zsuzsa Agoston and Keely Bumsted-O'Brien, Frankfurt/Main and Auckland (New Zealand)*

**S14-5** Identification of intrinsic determinants of midbrain dopamine neurons
*Johan Ericson, Stockholm (S)*

**S14-6** E(Spl) transcription factors and neurogenesis control in the zebrafish midbrain
*Laure Bally-Cuif, Prisca Chapouton, Christian Stigloher, Christoph Leucht and Christina Lillesaar, Neuherberg*

**S14-7** A novel approach to selectively target Pax6 expressing neurons
*Dieter Engelkamp, Karsten Benzing and Andreas Schedl, Frankfurt and Nice (F)*

### Poster

**TS14-1C** Pax6-Mediated Induction of Glutamatergic Neurons in Embryonic Stem Cell Cultures
*C. Keller, P. Koch, M. Hack, M. Goetz and O. Bruestle, Bonn and Munich*

**TS14-2C** A conserved role for the related protein Meis2 during vertebrate eye development
*P. Heine, KM. Bumsted-O`Brien, D. Engelkamp and D. Schulte, Frankfurt/Main and Auckland (New Zealand)*

**TS14-3C** Early patterning along the dorso-ventral axis of the midbrain: transcriptional regulation of ephrinB1 expression
*Z. Agoston, A. Badde and D. Schulte, Frankfurt/Main*

**TS14-4C** Divergent roles of ApoER2 and VLDLR in neuronal migration
*I. Hack, S. Weinelt, B. Brunne, D. Junghans, S. Zhao and M. Frotscher, Freiburg*

**TS14-5C** Orthopedia homeodomain protein is essential for diencephalic dopaminergic neuron development
*S. Ryu, J. Mahler, D. Acampora, J. Holzschuh, S. Erhardt, A. Simeone and W. Driever, Freiburg and Naples (I)*

**TS14-6C** Transport of HCN1 channels to presynaptic compartments: novel plasticity that may contribute to establishment of connectivity in developing hippocampus
*RA. Bender, O. Kretz, H. Beck, M. Frotscher, TZ. Baram and T. Kirschstein, Hamburg, Freiburg, Bonn, Irvine (USA) and Rostock*

**TS14-7C** PRESENCE OF DOUBLE-STRAND BREAKS DURING RETINAL NEUROGENESIS.
*J. Baleriola and E. de la Rosa, Madrid (E)*

Introductory Remarks to Symposium 14

# Cell Intrinsic Mechanisms in the Regulation of Neural Development

Dorothea Schulte and Dieter Engelkamp, Frankfurt/Main

During embryogenesis a pool of proliferating progenitor cells gives rise to all neural cell types of the central nervous system. Neural progenitor cells change continuously through development. Such progenitor cell changes are the basis of neural tube patterning and regionalization of the developing CNS, they regulate the balance between cell proliferation and cell cycle exit, cell fate decisions and cell migration. Cell intrinsic mechanisms include the differential expression of transcriptional regulators, components of the cell cycle machinery or signal transduction pathways.

The idea for a symposium on "Cell intrinsic mechanisms in neural development" is to provide a platform to present and discuss recent advantages in the field. The speakers will address a representative cross section of related topics from the recent observation that cell cycle exit and cell fate determination are coupled to the genetic basis of circuit formation. Emphasis has been given to researchers, who approach these questions by using a broad range of different model organisms, from mouse and chicken to Xenopus, zebrafish and Medaka.

Three of the talks will focus on the embryonic neural retina, a widely used model for the study of neural development: Jochen Wittbrodt (EMBL) uses Medaka fish to investigate the developmental mechanisms governing oculogenesis; Muriel Perron (Université Paris XI) will report on the generation of GABA-erig neurons in the retina and Dorothea Schulte (MPIH, Frankfurt) on the function of transcriptional co-factors in early eye development.

Additional three talks will focus on the development of more posterior regions in the central nervous system: Johan Ericson (Karolinska Institute, Stockholm) will address the role of a transcriptional code in the specification of distinct neuronal subtypes in the ventral nervous system; Laure Bally-Cuif (GSF, Munich) uses zebrafish to investigate the molecular mechanisms underlying progenitor cell maintenance and cell type specification in the embryonic midbrain and Dieter Engelkamp (MPIH, Frankfurt) will report on the genetic programs that control progenitor cell migration at the rhombic lip.

# Individual cell migration serves as the driving force for optic vesicle evagination

Joachim Wittbrodt[1], Felix Loosli[1], Richard J. Adams[2] and Martina Rembold[1]

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The cellular mechanisms underlying organ formation are largely unknown. Here we visualize early vertebrate eye morphogenesis at single cell resolution by *in vivo* imaging in medaka. Prior to optic vesicle evagination, retinal progenitor cells (RPCs) modulate their convergence in a fate specific manner. Presumptive forebrain cells converge towards the midline, whereas medial RPCs remain stationary, predetermining the site of evagination. Subsequent optic vesicle evagination is driven by active migration of individual RPCs. Mutant analysis demonstrates that the retina specific transcription factor *rx3* determines both the RPC-characteristic convergence and migration behaviour. Hence, migration of individual cells mediates essential steps of organ morphogenesis.

# Ptf1a determines GABAergic neuronal cell fate in the retina

Muriel Perron[1], Mélodie Robach[1], Morgane Locker[1], Yoichiro Aoki[1], Yonglong Chen[2] and Tomas Pieler[2]

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The molecular machinery governing glutamatergic-GABAergic neuronal subtype specification in the developing retina is largely unknown. We show here that the primary defect of a loss of Ptf1a function is a specific inhibition of GABAergic neuron production in the retina, resulting in secondary and almost complete loss of horizontal cells. Conversely, overexpressing Ptf1a in retinal precursors leads to a dramatic increase of neurons with a GABAergic phenotype at the expense of non GABAergic neurons. This results in an almost complete loss of photoreceptor cells and Müller glia cells, associated with a dramatic increase of amacrine and horizontal cells. The scattered expression of Ptf1a in the retinal neuroepithelium led us to propose that Ptf1a-expressing precursors define a sub-population dedicated to the generation of GABAergic retinal neurons, i.e. all horizontal cells and some amacrine cells. Altogether, our results suggest that Ptf1a is involved in driving retinal precursors to differentiate into GABAergic neurons.

# Control of vertebrate visual system development by MEIS homeodomain proteins

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Although the structure and developmental origin of the vertebrate lens-eye and the invertebrate compound eye are dramatically different, several components of the regulatory network that directs eye development are conserved across the animal kingdom. The transcription factor eyeless/Pax6, for instance, is of essential importance for eye development in vertebrates and invertebrates, and atonal and its vertebrate homologue Ath5 are important regulator of retinal neurogenesis in both systems. However, examples of non-conservation are also known, suggesting that the molecular network that controls ocular differentiation is only partially conserved.

The TALE-homeodomain transcription factor homothorax (hth) plays multiple roles during Drosophila eye development. It is involved in establishing the territories for eye and ventral head capsule at early stages of development, promotes rapid cell proliferation of retinal progenitor cells prior to neurogenesis during 3rd larval instar, when photoreceptors and accessory cells of the eye are generated in the wake of the morphogenetic furrow, and is required for the specification of the DRA (dorsal rim area) photoreceptors, a specialized class of photoreceptors that detect polarized light. Although structural homologues of hth are known in vertebrates, their contribution to vertebrate retina development has remained largely elusive up to now. I will present our recent data on the role of the hth related proteins Meis1 and Meis2 during vertebrate visual system development and will discuss aspects of evolutionary conservation and non-conservation of hth / Meis function.

# Identification of intrinsic determinants of midbrain dopamine neurons

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Dopamine neurons in the midbrain degenerate in patients with Parkinson's disease, and the prospect of using cell replacement strategies to treat Parkinson's has intensified research aimed at understanding the normal generation of these cells during embryonic development. We have found that the transcription factors Lmx1a and Msx1 play central roles in the specification of dopamine neurons in the developing midbrain. When introduced into embryonic stem cells, Lmx1a alone can effectively direct the differentiation of cells into dopamine neurons with a correct midbrain identity. Moreover, after transplantation of Lmx1a-induced cells in rodent models of Parkinson's disease, we observe survival of cells and an extensive re-innervation of TH+ axons in the striatum of host animals. These data suggest that intrinsic determinants can be used as powerful tools to control the differentiation of stem cells into clinically relevant neuronal subtypes.

# E(Spl) transcription factors and neurogenesis control in the zebrafish midbrain

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Neurogenesis within the embryonic and adult vertebrate CNS is a spatio-temporally tightly controlled process: it permits the generation of the right amount and subtypes of neurons to establish a functional circuitry, but concomitantly spares pools of progenitor cells for later events of growth, adaptation or repair. Within the zebrafish embryonic CNS, progenitor pools can be identified by their absence of expression of proneural genes (ngn1, zash1, coe2) and their long-lasting proliferation. Previous results from our laboratory and others suggest that the maintenance of these pools is the result of an active process of neurogenesis inhibition, mediated by a subclass of E(Spl) factors that do not respond to Notch (Bae et al., 2005; Geling et al., 2003; Geling et al., 2004; Hans et al., 2004; Ninkovic et al., 2005).

We recently observed that the midbrain-hindbrain boundary (MHB) progenitor pool is under control of four E(spl) genes, her3, 5, 9 and 11. Expression of these genes is dynamically regulated, and progressively switched off in precursors that will then exit the pool and contribute neurons of various subtypes to the mid- and hindbrain (Tallafuss and Bally-Cuif, 2003). We are currently taking conditional approaches to address whether, in this location, the temporal regulation of E(Spl)-mediated neurogenesis inhibition also influences neuronal identity.

In the vertebrate adult brain, the factors functionally involved in attributing the long lasting progenitor (stem) cell state remain largely unknown. We tested the potential implication of Notch-independent E(Spl) factors in this process. We observed that, in the zebrafish adult brain, her5 is selectively expressed in a small population of ventricular midbrain cells that display stem cell-like properties (Chapouton et al., 2006). Conditional gain- and loss-of function experiments during adulthood support a role for Her5 in preventing differentiation of these cells. Further, blocking Notch activity in the adult brain enhances neuronal differentiation from fast amplifying progenitors abutting the her5-population, but spares the her5-population itself. Together, our observations suggest that Notch-independent E(Spl) factors might be part of a shared mechanism encoding stem cell properties in the vertebrate embryonic and adult CNS.

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# A novel approach to selectively target Pax6 expressing neurons

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Cell migration is essential for development. We have developed a novel mouse model that enables the visualization and manipulation of a defined subpopulation of migrating neurons. To demonstrate the power of our system we show that Pax6 has multiple functions in rhombic lip derived tangentially migrating neurons. In Pax6 mutant mice these neurons migrate along ectopic routes, to ectopic positions and settle disorganized. Moreover, we show that several gene cascades control midline crossing behavior of these neurons. One of these involves Pou4f2 which induces a prolonged midline arrest of growth cones to influence the proportion of ipsilaterally versus contralaterally settling neurons. Altogether, these results demonstrate that our approach serves as a versatile tool to study the function of genes involved in cell determination, cell migration and axonal pathfinding processes. Furthermore, our model should facilitate the analysis of Pax6 target genes in any Pax6 positive tissue.

# Pax6-Mediated Induction of Glutamatergic Neurons in Embryonic Stem Cell Cultures

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Results from recent studies indicate that Pax6 efficiently promotes neuronal differentiation in primary neural precursors, radial glia and postnatal astrocytes. We explored whether this potent neurogenic effect can be used to induce neuronal fates in differentiating embryonic stem (ES) cells. Murine ES cell-derived neural precursors (ESNPs) proliferating in the presence of FGF2 and EGF were transduced with a retroviral vector encoding the Pax6 and EGFP genes. Pax6-transduced cells showed a pronounced decrease in proliferation even in the presence of growth factors compared to control-transduced cells. Upon growth factor withdrawal, they differentiated almost exclusively into neurons (99,6 ± 0,2%). In contrast, ESNPs transduced with an EGFP control vector differentiated into neurons, astrocytes and oligodendrocytes. At a clonal level, Pax6-transduced cells generated purely neuronal clones, whereas control cells gave rise to neuronal, non-neuronal and mixed clones. Remarkably, Pax6 also influenced the neurotransmitter phenotype of the newly generated neurons. Whereas EGFP-transduced cells generated only few vGlut1-positive glutamatergic and mostly GAD67-positive GABAergic neurons (8,4±2,1% vs. 44.3±1,2%), Pax6-transduced cells yielded 91.7±2,9% glutamatergic neurons. RT-PCR data on the expression of other region-specific transcription factors indicate that this transmitter shift coincides with a suppression of ventral and a promotion of dorsal telencephalic fates. Our data suggest that in ESNPs Pax6 instructs an exclusive neuronal fate and promotes a cortical glutamatergic phenotype.

# A conserved role for the related protein Meis2 during vertebrate eye development

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The proper balance of cell proliferation and differentiation is of essential importance to animal development. For each organ to develop correctly there has to be enough cell proliferation to ensure the proper size of the final structure, yet proliferation has to be tightly balanced with differentiation to avoid disproportional growth. The photoreceptors and accessory cells of the fly compound eye are generated in the wake of the morphogenetic furrow (MF), which sweeps across the eye imaginal disc during 3rd larval instar. Cells furthest away from the MF express the TALE-homeodomain protein homothorax (hth), which acts together with the Pax6 homologue eyeless (ey) and the zinc-finger protein teashirt (tsh) to promote rapid cell proliferation and to prevent premature differentiation. When hth is repressed by signals from the MF, cells transit to more committed states and subsequently differentiate. Progenitor cells of the Drosophila eye thus progress from a highly proliferative, immature to a more mature, neurogenic state, which is accompanied by the repression of hth. It seems likely that similar changes in progenitor cell competence also take place in the vertebrate eye, when retinal progenitor cells switch from generating exclusively proliferating progeny to the production of mitotic and postmitotic daughter cells. The molecular events that trigger such changes, however, are still largely unknown. Here we describe the role of the hth related protein Meis2 in the vertebrate eye anlage. We show that Meis2 is a component of the network of transcription factors that regulate eye development, is required for the rapid proliferation of early retinal progenitor cells and discuss the degree of evolutionary conservation of hth / Meis2 function during vertebrate and invertebrate eye development.

# Early patterning along the dorso-ventral axis of the midbrain: transcriptional regulation of ephrinB1 expression

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The optic tectum is the visual centre in non-mammalian vertebrates and receives retinal optic fibers in a precise topographic manner. The development of the retinotopic map is mainly guided through a class of cell/cell-adhesion molecules, the Eph-receptors, and their ligands, the ephrins. Eph-receptors and ephrins are expressed in specific graded patterns on retinal optic fibers and along the optic tectum. These complex patterns convey positional identity between tectal neurons and retinal ganglion cell axons, which navigate across the tectal surface towards their correct target zones. The mechanisms that govern the formation of the retinotopic map, therefore, require the precise tempo-spatial expression of Eph-receptors and ephrins on retinal ganglion cell axons as well as on tectal cells (McLaughlin and O´Leary, 2005). This specific expression of Eph/ephrin molecules is regulated by transcription factors, which are expressed in distinct tempo-spatial patterns and thereby regionalize the retina and the midbrain early in development. The transcription factors En-1/2, for example, control the expression of ephrinA2 and -A5 and consequently play a key role in the formation of the retinotopic map along the anterior-posterior axis of the optic tectum (e.g. Logan et al., 1996). EphrinB1 is involved in retinotopic map formation along the dorso-ventral axis of the optic tectum (McLaughlin and O´Leary, 2005). However, the mechanisms underlying the regulation of tempo-spatial ephrinB1 expression in the tectum have not yet been elucidated. Here we describe the expression and function of a transcription factor, which is necessary and sufficient for ephrinB1 expression in the embryonic midbrain and provide evidence for a regulatory network of transcription factors involved in tectum differentiation and development along its dorso-ventral axis.

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# Divergent roles of ApoER2 and VLDLR in neuronal migration

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The mammalian neocortex consists of six layers of neurons with distinct functional and morphological identities. These layers are generated in an inside-out sequence, with early born cells in the deep layers and later born cells in the outer layers. To generate this pattern, cells migrating to the cortical plate must stop at their appropriate destinations. The molecular mechanisms that regulate the interaction of the migrating neurons with their environment, the signalling cues they receive during migration and subsequent differentiation, are complex and as yet poorly understood. One major signalling pathway which regulates the migration of neurons during formation of the cortex involves the extracellular matrix protein Reelin. The target cells of Reelin express the Reelin receptors very low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2) and the cytoplasmic adaptor protein disabled 1 (Dab1), which binds to these receptors. The reeler mouse lacking Reelin, shows an inversion of cortical layers. Mice defective in both ApoER2 and VLDLR, and dab1 mutants show a phenotype reminiscent of reeler. Milder phenotypes are found if only one of the two lipoprotein receptors for Reelin is absent. However it is unclear whether both Reelin receptors have similar functions, during cortical development. To address this question, we combined bromodeoxyuridine (BrdU) labelling of newly generated neurons with staining using specific markers of individual cortical layers and co-culture experiments on VLDLR-/- and ApoER2-/- mutant mice. We present evidence for divergent roles of the two Reelin receptors with VLDLR mediating a stop signal for migrating neurons and ApoER2 being essential for radial glia-guided migration during late phases of cortical layer formation.

# Orthopedia homeodomain protein is essential for diencephalic dopaminergic neuron development

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Neurons that produce dopamine as a neurotransmitter constitute a heterogeneous group important for the control of various behaviors and physiology. In mammals, dopaminergic (DA) neurons are found in distinct clusters mainly located in ventral midbrain and caudal forebrain. Ventral mesencephalic groups are involved in movement control, cognitive and reward-associated behaviors and their degeneration and dysfunction are implicated in Parkinson's disease, drug addiction, and depression. Most of caudal forebrain or diencephalic groups are present in hypothalamus and ventral thalamus and regulate neuroendocrine functions. Lastly, a small number of DA neurons are located in the posterior regions of the dorsal hypothalamus and the periventricular gray of the central thalamus (so called A11 group). Very little is known about the function of these neurons, but they form long spinal cord projections and are implicated in Restless legs syndrome (RLS), a common neurological condition affecting 2 to 15% of the general population. A wealth of information exists on signaling molecules and transcription factors important for the development of mesencephalic DA groups. In contrast, surprisingly little is known about the development of diencephalic DA neurons.

In order to identify molecules and pathways important for DA development, we have taken a forward genetics approach in zebrafish. DA neurons are found in distinct clusters both in telencephalon and diencephalon of zebrafish. Here we report a mutation, m866, which results in a severe reduction of DA neurons in the diencephalic posterior tuberculum and hypothalamus. We show that the gene affected in m866 is orthopedia a (otpa), which encodes a homeodomain transcription factor. We demonstrate that otp is expressed in the majority of posterior tubercular and hypothalamic DA neurons and is required for their development in zebrafish. Zebrafish devoid of Otp completely lack diencephalospinal DA projections. Mouse Otp is important for the development of neuroendocrine cells in the hypothalamus, but its role in the development of other types of neurons was unknown. We show that in mouse, Otp is expressed in A11 DA group and is required for their specification. We provide evidence that Otp can induce ectopic tyrosine hydroxylase positive cells in zebrafish. Thus, Otp is one of the few known transcription factors that can determine aspects of the differentiated DA phenotype, and the first factor to control the development of the diencephalospinal DA system. Together our data establish Otp as the first intrinsic determinant for the diencephalospinal dopaminergic system conserved through evolution.

# Transport of HCN1 channels to presynaptic compartments: novel plasticity that may contribute to establishment of connectivity in developing hippocampus

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Developing neuronal networks evolve continuously, requiring that neurons modulate their intrinsic properties and responses to synaptic input. Increasing evidence supports roles for the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel-mediated current (Ih) in these functions. Here we describe a novel developmental plasticity involving transient HCN channel expression in axonal and presynaptic compartments. Using immunohistochemistry, electron microscopy and in vitro pathway ablation, we show that HCN1 channels are expressed within axon terminals of the medial perforant path (mPP) of immature rats, yet disappear with maturation. Using electrophysiology, we show that these presynaptic HCN channels modulate the efficacy of perforant path synapses in age- and frequency-dependent manner: Blockade of Ih using ZD7288 (10 µM) or zatebradine (20 µM) increased short-term depression (STD) when mPP was stimulated with 20 Hz, but not after stimulation with 1, 5, or 10 Hz. Consistent with the anatomical data, this effect was age-dependent, and observed in slices from immature but not adult rats. In addition, whereas STD was less pronounced in immature compared to adult mPP when HCN channels were functional, blockade of Ih increased STD of immature mPP to values indistinguishable from the adults. These data suggest that presynaptic HCN channels contribute to age-dependent regulation of short-term plasticity at medial perforant path-granule cell synapses that could be important for the maturation of medial perforant path-granule cell connectivity.

# PRESENCE OF DOUBLE-STRAND BREAKS DURING RETINAL NEUROGENESIS.

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Programmed cell death affecting projecting neurons during the development of the central nervous system (CNS) is a highly regulated and physiological phenomenon necessary for the proper formation of the CNS. However, in stages previous to neuronal differentiation, proliferative cells and young neuroblasts also undergo apoptosis in a regulated fashion. This programmed cell death during neurogenesis might have different porpuses: to fit the number of neural precursors, selection of neural phenotypes and elimination of genetically abnormal cells. On the other hand, injuries at the DNA level are a significant apoptotic stimulus for immature neurons in the developing CNS. The phenotype seen in mice lacking proteins implicated in homologous recombination (HR) and the non-homologous end joining (NHEJ) repair pathways, indicate that double strand breaks (DSBs) are specially deleterious for young neuronal cells if they are not correctly repaired. Our objective is to determine if there is a relation between DSBs, early neurogenesis and apoptosis during the development of the vertebrate retina.

# Symposium

## S15: Microglia: Role in Neurodegeneration and Repair

***Harald Neumann and Marco Prinz, Bonn and Göttingen***

### Slide

**S15-1** Turnover of bone marrow-derived microglia in the normal and diseased brain
*Ingo Bechmann, Frankfurt/Main*

**S15-2** Innate immunity in the inflamed or injured CNS
*Trevor Owens, Odense C (DK)*

**S15-3** Communication pathways between microglial cells and neurons
*Helmut Kettenmann, Berlin*

**S15-4** Effects of microglia and macrophages in brain lesions
*Sebastian Jander, Düsseldorf*

**S15-5** Imaging neuro-immune interactions in the living nervous system
*Martin Kerschensteiner, Munich*

**S15-6** The active role of resting microglia
*Gennadij Raivich, London (UK)*

### Poster

**TS15-1C** Differentiation of mouse embryonic stem cells to microglia
*K. Kierdorf, O. Chechneva, I. Napoli and H. Neumann, Bonn and Rio de Janeiro (Brazil)*

**TS15-2C** Lipoteichoic acid-induced loss of cerebellar granule cells in mixed neuronal-glial cultures is prevented by minocycline and the phagocytosis inhibitor, cytochalasin D
*JJ. Neher and GC. Brown, Cambridge (UK)*

**TS15-3C** The therapeutic time frame of Interleukin-1 receptor antagonist after neuronal damage in organotypic hippocampal slice cultures (OHSC)
*C. Vogt, NP. Hailer and F. Dehghani, Frankfurt and Uppsala (S)*

**TS15-4C** Neural elements transform into CD11c+ dendritic cells
*I. Bechmann, J. Bunse, A. Zimmermann and C. Brandt, Frankfurt/Main and Berlin*

**TS15-5C** In vivo laser scanning microscopy of transgenic mice expressing EGFP in microglial cells to study spinal cord diseases
*C. Neusch, H. Steffens, J. Hirrlinger and F. Kirchhoff, Göttingen and Leipzig*

**TS15-6C** Aberrant microglial activation and degeneration in rats expressing a mutant Cu/Zn superoxide dismutase gene
*WJ. Streit and SE. Fendrick, Gainesville, FL (USA)*

**TS15-7C** α-tocopherol-succinate induces apoptosis in the microglial cell line, BV-2, but not in primary microglial cells
*B. Sacha, S. Zierler, S. Lehnardt, JR. Weber and HH. Kerschbaum, Salzburg (A) and Berlin*

**TS15-8C** Cyclic adenosine 3',5'-monophosphate (cAMP) mediates ammonia-induced programmed cell death in the microglial cell line, BV-2.
*N. Svoboda and HH. Kerschbaum, Salzburg (A)*

Introductory Remarks to Symposium 15

# Microglia: Role in Neurodegeneration and Repair

Harald Neumann and Marco Prinz, Bonn and Göttingen

Microglia activation is a common feature of most nervous system diseases including Alzheimer's disease, Parkinson's disease, traumatic or ischemic brain injury and multiple sclerosis. Recent new developments elucidated that microglia are bone marrow derived and are also replaced during adulthood. Innate immune receptors such as toll-like receptors (TLR) and triggering receptors expressed on myeloid cells (TREM) are now characterized as sensors for environmental changes. Furthermore, functional neurotransmitter receptors such as dopamine and noradrenaline receptors are expressed on microglia and sense local changes in the neurotransmitter levels.

The symposium will highlight the participation and molecular mechanism of microglia in neurodegeneration as well as neural regeneration. Microglial and macrophage-derived molecules such as osteopontin inhibit axonal outgrowth. Direct imaging of axonal spinal cord lesions allows the elucidation of the role of microglia in axonal degeneration and regeneration. Furthermore, it will be discussed whether resting microglia already show prominent activity and might permanently clear the normal brain tissue.

In summary, the symposium will highlight the emerging research of microglial function on neuronal survival and regeneration.

# Turnover of bone marrow-derived microglia in the normal and diseased brain

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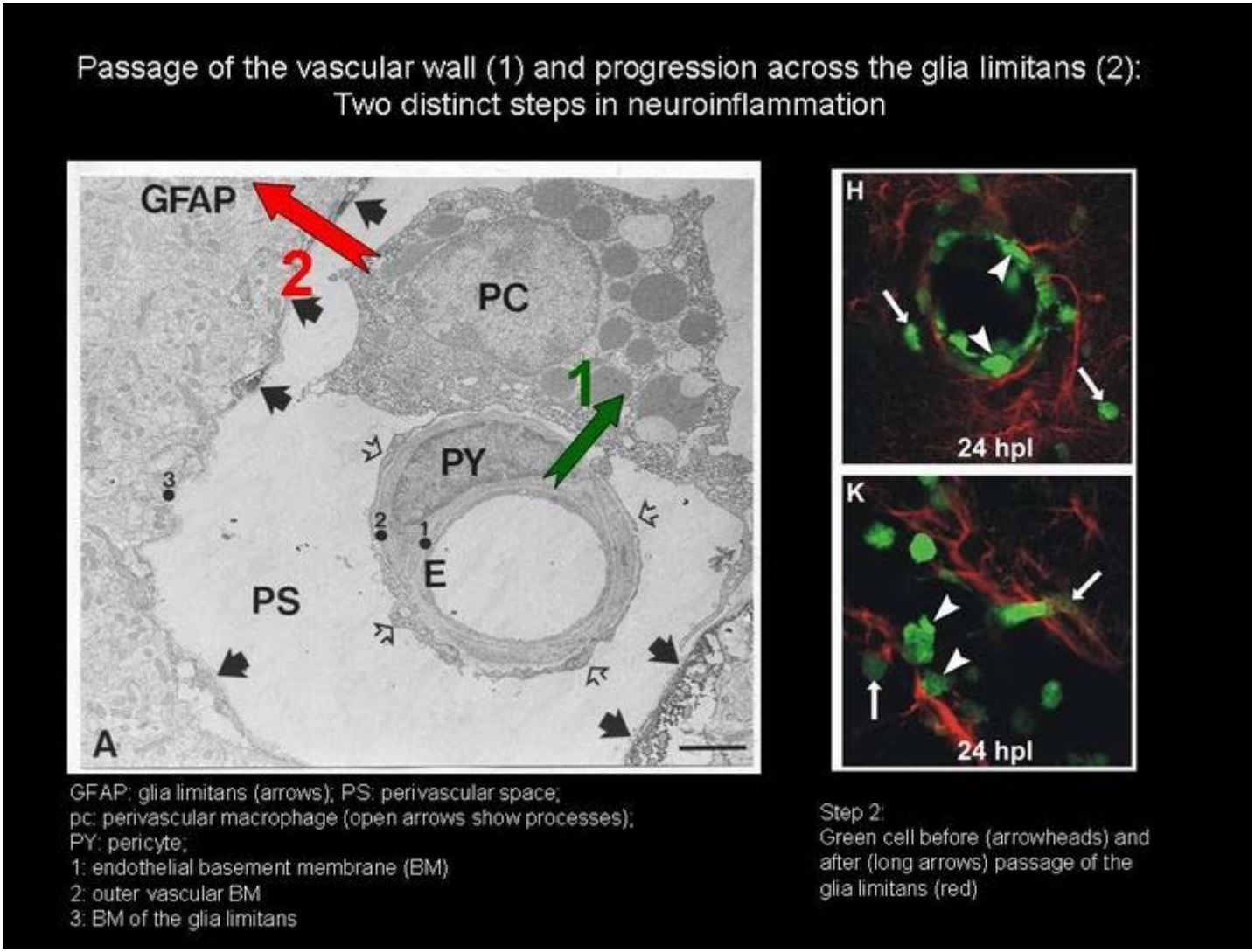
For long microglia has been regarded as a population of exclusively intrinsic brain cells. Using chimeric mice with GFP expressing bone marrow and injection of tracers into spleen and brain, we have shown that haematogenous cells pass the vascular wall to reside in perivascular spaces under normal conditions, but progress across the glia limitans to reach the neuropil in pathologies. Once inside the neuropil, they transform into ramified microglia-like elements.

Recently, myelin epitopes have been found in cervical lymph nodes of MS patients raising the intriguing possibility that dendritic cells may carry them from brain to lymphoid organs. In mice expressing GFP under the CD11c promoter, we found green cells with microglial morphologies in peri- and juxtavascular location. In slice preparations from these animals, the number of green cells strongly increased over time showing that their appearance is not dependent on recruitment from the blood. Apparently, there is an intraneural (microglial) precursor. Thus, monocytic cells not only enter, but may also leave the brain to present CNS-antigens in lymphoid organs.



# Innate immunity in the inflamed or injured CNS

Trevor Owens

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Inflammatory demyelinating diseases such as multiple sclerosis (MS) are characterized by infiltrates of leukocytes within the central nervous system (CNS). It is assumed that the immune cells in MS, mostly T lymphocytes and macrophages, contribute to demyelinating pathology and axonal damage, or may play a beneficial role, under certain circumstances. In either event, the mechanism by which leukocytes are attracted to and enter the CNS is of interest. We study the entry of leukocytes to the CNS, and their activation to effector function. We are most interested in signals from the CNS and the immune system that drive or regulate cellular entry. This presentation will describe recent work on activation of glial cells in the mouse brain, in response to stereotactic axonal injury, chemical demyelination and viral infection. Glial activation in response to these experimental stimuli is initiated independently of immune involvement, and results in secretion by glia of chemokines and cytokines. Injury-induced glial-derived chemokines promote the entry of macrophages and T cells to the affected region. However, experimentally-induced or transgenic chemokine production is by itself insufficient to induce leukocyte entry, but signals that can be provided through recognition of pathogens or endogenous (innate) triggers are also required. Microglial Toll-like receptor-2 signals may play such a role, and in response to injury can act act as upstream on-switches for glial cytokine and chemokine responses, and for proliferative expansion of microglial cells. Cross-talk between glial cells is also mediated by cytokines, with characteristic intracellular signals. Activated microglia and macrophages show antigen-presenting cell surface phenotypes, and cells with such phenotype can activate T cells in culture. However, the T cells that enter the CNS, in the systems that we study, require signals additional to chemokines and cytokines for functional activation. Our findings identify a complex of signaling events that must combine for immune pathology in the CNS.

# Communication pathways between microglial cells and neurons

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Microglial cells are the major immunocompetent cells in the brain and express many features of monocytes. This includes signalling cascades well described in the immune system involving chemokines and cytokines and their receptor systems. We have addressed the question whether microglia would also express receptors to sense neuronal activity. We have recently developed an *in situ* model which allows to study the physiological responses of resting and activated microglia. This enables us to characterize the functional receptors and the physiological phenotype of microglia in situ. Using this approach, we could identify microglial receptors for GABA, the major inhibitory transmitter of the CNS. Activation of the $GABA_B$ receptors suppressed indicators of microglial activation such as the release of Il-6 (Kuhn et al., 2005). A similar reduction in proinflammatory mediators was found with activation of purinergic receptors which are important signalling molecules for astrocyte activity. Chronic dopamine receptor stimulation enhanced migratory activity and attenuated the lipopolysaccharide (LPS)-induced NO release similar as by stimulation of adrenergic receptors. While, however, noradrenaline attenuated the LPS induced release of TNF alpha and IL-6, dopamine was ineffective in modulating this response. Thus microglia express dopamine receptors which are distinct from adrenergic receptors (Färber et al., 2006). In conclusion, microglial cells express a variety of the classical neurotransmitter receptors. These findings support the hypothesis that microglial cells are less prone to activation when they sense normal neural activity.

# Effects of microglia and macrophages in brain lesions

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CNS injury of any kind induces long-lasting inflammatory reactions that may contribute to lesion progression as well as beneficial tissue remodeling. Lesion-induced neuroinflammation is dominated by the innate, antigen-nonspecific immune system, with microglia/macrophages as most important cell type involved. Osteopontin (OPN) is a strongly expressed secretory product of activated microglia/macrophages which acts through a variety of matrix receptors such as αvβ3/β5 integrins and CD44. We have recently studied the potential role of OPN in axotomy lesions of optic and sciatic nerve as well as focal stroke models. Gene expression studies showed that lesion-associated macrophages strongly express OPN after crush injury of the optic, but not sciatic nerve and that OPN-coated matrices constitute a nonpermissive substrate for axon outgrowth in vitro. We therefore postulate that the CNS-specific OPN expression by macrophages may contribute to the failure of axon regeneration observed in the adult CNS. On the other hand, experiments with OPN knock-out mice in focal ischemia models revealed neuroprotective effects of OPN that were mediated via the inhibition of microglia and downregulation of inducible nitric oxide synthase expression. Thus, current evidence suggests pleiotropic effects of OPN on neurons and inflammatory cell populations that may provide a basis for novel therapeutic strategies.

# Imaging neuro-immune interactions in the living nervous system

Martin Kerschensteiner

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Multiple sclerosis (MS) is an inflammatory disease of the central nervous systems and the most common neurological cause of disability in young adults. Disability in MS is caused by immune-mediated damage to axonal connections. However, how immune cells damage axons in MS is unresolved.

In this presentation I want to introduce a recently developed imaging approach that allows us to follow the interaction of immune cells and axons in the living nervous system. The approach is based on double-transgenic mice in which immune cells, for example macrophages and microglial cells, are labelled with green fluorescent protein, whereas neurons and their processes are labelled with cyan fluorescent protein. Neurons and immune cells can then be co-visualized in the central nervous system using either two-photon or widefield in vivo microscopy. Currently we are using this approach to characterize the interactions of macrophages/micoglial cells and axons in an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis. We hope that this approach will help us to understand if and how immune cells damage axons in neuroinflammatory disease like multiple sclerosis.

# The active role of resting microglia

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Every tissue appears to employ its own somewhat specialized watchdogs to keep order and to remove dysfunctional debris. These must also deal with potentially infectious agents, and be able to signal for help from the professional immune system, including T- and B-lymphocytes, natural killer cells, and other circulating white blood cells such as dendritic cells, macrophages and granulocytes.

The brain and spinal cord parenchyma, the central nervous system proper, this function is taken by a highly branched and monocyte & dendritic cell related-cell type, the microglia, that acts as a first line of defence against neural infection (1). These specialized cells are professionals that remove dead cells and other forms of debris such as axons and myelin, and play a pivotal role in the early recruitment of T-cells following even minor forms of injury. They also exhibit surface expression of immunoglobulin, and receptors for complement and apoptotic cell surface markers, in line with their local surveillance role. In the normal brain, these resting microglia are highly ramified cells, with an elaborate tertiary and quaternary branch structure, staking a claim to a brain area 30-50 μm across, with rarely any overlap with the branches of even nearby sister cells.

As shown by two recently published papers using in vivo two-photon laser-scanning microscopy (2,3), it is these fine branches that are highly mobile and provide extensive and continuous surveillance of their cellular environment, that depends on purinergic receptors and connexin hemi-channels and takes cues from surrounding astrocytes. In the injured organism and injured brain, additional signals also enjoin the neuroglial activation cascade, including markers of infection such as endotoxin, and inflammation-associated cytokines. My talk will centre on putting these studies in perspective and dissecting those molecules, addressing their role in immune surveillance and post-traumatic neurological outcome.

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# Differentiation of mouse embryonic stem cells to microglia

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Microglia play a detrimental as well as a beneficial role in many neurological diseases like multiple sclerosis and Alzheimer's disease. They can act by regulating pro-inflammatory cytokines production and inflammatory reactions, eliminating damaged cells by phagocytosis or supporting the survival of cells by release of numerous growth factors. There is recent evidence that microglia derived from embryonic stem (ES) cells can migrate into the central nervous system after intravenous injection in healthy mice (Tsuchiya et al., 2005).
We aimed here to differentiate mouse C57/BL6 ES cells to microglia and to establish an ES-cell-derived microglial precursor cell-line for further use as a therapy of neurological diseases. For differentiation of ES cells to microglia a modified standard protocol for differentiation of ES cells into neurons was applied (Lee et al., 2000; Tsuchiya et al., 2005). At different stages of differentiation the cultures were analyzed by immunocytochemistry and flow cytometry. At the beginning of differentiation embryonic bodies (EBs) were formed from ES cells. Thereafter Nestin+ neuronal precursors were selected by cultivation of EBs for 6 days in DMEM/F12 medium supplemented with Insulin-Transferrin-Selenin-Fibronectine. Expansion of Nestin+ cells in N2 medium with addition of bFGF for 6 days resulted in increased number of neuronal precursor cells and formation of neuronal network. At this stage IBa1+ cells were detected. These IBa1+ cells incorporated into the neuronal network and were found between neuronal processes. In cultures which were kept in N2 medium, IBa1+ cells developed a ramified microglia-like morphology and it was possible to isolate microglia-like cells out of these cultures. Flow cytometry analyses demonstrated constitutive high percentage of CD11b and CD45 positive cells in the ES cell derived microglia precursor cell cultures, as well as a high amount of CXCR4 positive cells in early passages. In vivo injection of that microglia- like cells in the animal model of multiple sclerosis showed that they have the ability to migrate into the brain of these mice. Migration of the ES cell- derived microglia into the central nervous system was also found in mice challenged intraperitoneally with lipopolysaccharides (LPS).
Thus, microglial cells can be differentiated from mouse ES cells to be available for cell and gene therapy of neuroinflammatory and neurodegenerative diseases.

* equal contribution; References: Tsuchiysa et al. (2005) J Neuroimmunology 160: 210-218 ;
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# Lipoteichoic acid-induced loss of cerebellar granule cells in mixed neuronal-glial cultures is prevented by minocycline and the phagocytosis inhibitor, cytochalasin D

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Bacterial meningitis retains high rates of morbidity and mortality, and survivors often sustain debilitating neurological sequelae due to neuronal cell-death resulting from the infection and the ensuing inflammatory response. As bacterial autolysis occurs *in vivo*, the pathophysiology of gram-positive bacterial meningitis can be simulated *in vitro* by application of the major cell-wall component lipoteichoic acid (LTA). LTA-treatment has been shown to result in loss of cerebellar granule neurons in mixed neuronal-glial cultures - an effect that was mediated by glial cells.[1] Similar to the original study, stimulation of mixed cultures with LTA here resulted in a gradual loss of neurons (up to 30%) over 7 days. Also in correspondence with the original experiments, neuronal loss occurred without an accumulation of dying cells. In fact, dying neurons were virtually absent in LTA-treated cultures after 7 days *in vitro*.

The specific mechanism of neuronal cell-loss has not been previously investigated. Therefore, in order to test whether phagocytosis was involved in LTA-induced loss of neurons, cytochalasin D, a compound that blocks F-actin polymerization, was used as an inhibitor of phagocytosis. Cytochalasin D prevented neuronal loss for up to 7 days after LTA-stimulation. Additionally, in an assay for phagocytotic capacity, cytochalasin D partially inhibited an LTA-induced increase in the number of polystyrene microspheres that were ingested by microglia. These results suggest that LTA-stimulation enhances the phagocytotic capacity of microglia - an effect that is opposed by cytochalasin D and which might be involved in the loss of neurons observed in this model of bacterial meningitis.

The semi-synthetic tetracycline, minocycline, has been shown to exert pleiotropic effects on neurons and glial cells in many models of neurodegenerative disease. Therefore its effect on LTA-induced loss of cerebellar granule cells was also investigated here. To simulate treatment after an initial infection, minocycline was added to the cultures 24 hours after LTA-stimulation. Continuous addition of minocycline every 24 hours resulted in full neuroprotection for the duration of the experiment (7 days). Interestingly, minocycline did not affect microglial proliferation, which is induced in response to LTA-stimulation in mixed cerebellar granule cell cultures. This is in contrast to many other investigations, which most commonly reported that minocycline exerts its neuroprotective effect through the inhibition of microglial activation.

These data suggest that gram-positive bacterial infection induces an increase in phagocytotic activity of microglial cells. This effect might contribute to neuronal cell loss, since treatment with cytochalasin D was found to prevent elimination of neurons in LTA-treated cultures. In addition, the widely used drug minocycline exerted long-term protection against LTA-induced neurodegeneration and thus indicates a potential treatment for preventing neuronal demise in patients suffering from gram-positive meningitis.

[1] Kinsner *et al.*, J. Neurochemistry, 2005, 95, 1132-1143.

# The therapeutic time frame of Interleukin-1 receptor antagonist after neuronal damage in organotypic hippocampal slice cultures (OHSC)

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The proinflammatory cytokine Interleukin (IL)-1 is an important mediator of neuronal demise and glial activation after acute central nervous system (CNS) damage such as spinal cord injury or stroke. IL-1 receptor antagonist (IL-1ra) has been shown to improve neuronal survival in different lesion models. We first investigated the effect of IL-1ra on neurons and microglial cells in organotypic hippocampal slice cultures (OHSC): OHSC derived from rats were excitotoxically lesioned after 6 days in vitro (div) by application of N-methyl-d-aspartate (NMDA; 50µM) and treated with IL-1ra (100 ng/ml) for 4 h or up to 9 div. Degenerating neurons in OHSC were labelled with propidium iodide, microglial cells stained with fluorescent isolectin B4, and the preparations quantitatively were analyzed by confocal laser scanning microscopy. Treatment of NMDA-lesioned cultures with IL-1ra significantly attenuated NMDA-induced neuronal damage and reduced the number of microglial cells.

As to determine the therapeutic time frame of IL-1ra we attempted a delayed application of IL-1ra. OHSC were treated with IL-1ra (100 ng/ml) at 4, 8, 16, 24, 36 or 48 h after induction of neuronal injury respectively. We found that delayed application of IL-1ra potently suppressed neuronal damage by 55.1% if it was initiated up to 16 h, and less potent but nevertheless significant neuroprotection was observed up to 24 h after the onset of excitotoxic injury ($p<0.05$). IL-1ra also suppressed the increase in the number of microglial cells by 46.3% if its application was delayed up to 24 h ($p<0.05$), and a significant but less pronounced decrease in the number of microglial cells was found up to 36 h after the initiation of excitotoxicity.

Our findings indicate that a) IL-1ra, even when only applied for short periods of time, reduces neuronal cell death and induces a dose-dependent decrease in the number of microglial cells after excitotoxic damage and b.) IL-1ra exhibits significant neuroprotective effects even if its application is considerably delayed, supporting the use of this compound as a neuroprotective treatment after acute CNS pathologies.

# Neural elements transform into CD11c+ dendritic cells

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CD11c+ dendritic cells (DC) are not normally found in the brain parenchyma, but appear under inflammatory conditions, e.g. in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). At present, it is unclear whether these DC are recruited from the blood or derive from intrinsic (microglial) precursors. Here, we excluded supplementation by hematogenious cells by studying the appearance of DC in organotypic brain slice cultures, which were obtained from mice expressing the green fluorescent protein (GFP) under the CD11c promoter. We found little or no GFP+ cells in slices of p3 and adult animals at 1h after incubation, but their number strikingly increased between 12 and 36 h in culture. Confocal analyses revealed ramified morphologies and co-expression of microglia/macrophage markers (IBA-1, MAC-1) on intraparenchymal GFP+ cells. Thus, expression of the DC marker CD11c is induced de novo in precursor cells present in the neural tissue.

# In vivo laser scanning microscopy of transgenic mice expressing EGFP in microglial cells to study spinal cord diseases

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Introduction: In vivo visualization of individual cells in the intact or diseased spinal cord will help understanding acute and degenerative neurological diseases. The advances in transgenic mouse technology expressing celltype-specifically fluorescent proteins as well as in laser-scanning microscopy allow identifying individual cells in situ or in vivo. In a combinatorial approach, we were using two-photon microscopy and transgenic mouse technology to visualize single microglial cells expressing EGFP in the intact and acutely injured spinal cord. Methods: Adult transgenic mice with microglial-specific EGFP expression were anaesthetized. Vital functions were closely monitored and could be maintained for a period of at least 9 h. During anaesthesia, a laminectomy was performed at the main input region of the hind leg, at the spinal cord segment L4. After surgery, the mouse was placed underneath the objective of the laser-scanning microscope (two-photon excitation). To minimize movements in the region of interest, the adjacent spines were rigidly fixed. To monitor in vivo responses of microglial cells to acute injury, we applied a stab wound using a needle with a 250µm diameter. Results: EGFP-positive microglial cells could be easily identified at high resolution using two photon microscopy in the spinal cord in vivo. Following the surgical procedure, we were able to monitor individual microglial cells with time-lapse imaging for a maximum of ~6 hours. In the intact animal, resting microglial cells displayed highly motile branches that continuously surveyed the microenvironment as has been observed in the cortex in a previous study (Nimmerjahn et al., 2005). According to their typical morphology, cells of the monocytic lineage were additionally identified that moved across several 100 µm during a 20 min observation period. Following a stab wound at spinal cord segment L4, we observed morphological changes of microglial cells located nearby the lesion site as early as 120 min postlesion by transformation of ramified to amoeboid microglial cells. Conclusion: The visualization of individual cells using laser-scanning microscopy in the intact mouse spinal cord enables us to study acute spinal cord lesions on a single cell level. Resting microglial cells of the intact spinal cord display highly motile branches that continuously survey the microenvironment. In contrast to a cortical injury, microglial activation following an acute spinal cord trauma is observed as early as 2-3 h postlesion. This technique will allow us to study microglial activation in acute lesion models as well as in neurodegenerative paradigms of the spinal cord involving microglial cells, e. g. amyotrophic lateral sclerosis. Furthermore, our system enables use to investigate pharmacological modulation of resting or activated microglial cells under an in vivo paradigm.

# Aberrant microglial activation and degeneration in rats expressing a mutant Cu/Zn superoxide dismutase gene

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We have performed a histopathological analysis of microgliosis in transgenic rats modeling a familial form of amyotrophic lateral sclerosis (ALS). Multiple levels of the CNS from spinal cord to cerebral cortex were studied in SOD1G93A transgenic rats at three stages of natural disease progression, including presymptomatic, onset of symptoms, and end stage, using immuno- and lectin histochemical markers for microglia, such as OX-42, OX-6, and Griffonia simplicifolia isolectin B4. Studies during disease progression revealed abnormal aggregates of microglia forming in the spinal cord as early as the presymptomatic stage and involving the brainstem as disease progressed. During the symptomatic stages there was formation of multinucleated giant cells comprised of fused microglia. These were found along with other microglia that showed frank cytoplasmic degeneration (ctyorrhexis), as well as nuclear pyknosis, particularly in end stage animals. However, few TUNEL-positive microglia were identified. In one rat, a nidus of bacillus bacteria was observed in the brainstem. To assess functional responsiveness of microglia to neuronal injury, we performed facial nerve axotomies in presymptomatic transgenic and normal wildtype rats. While there was no evidence of facial motor neuron degeneration in either group, transgenic animals were found to have dramatically reduced numbers of apoptotic microglia when compared to wildtypes on day 14 after axotomy. In summary, our findings reveal severe abnormalities in microglia, including cell fusions, cytorrhexis, and dysregulated apoptosis, which are likely the result of expression of mutant SOD1 in these cells. These microglial changes, which are distinct from those that accompany microglial activation, suggest that degeneration and malfunctioning of microglia contributes to development and progression of motor neuron disease.

# α-tocopherol-succinate induces apoptosis in the microglial cell line, BV-2, but not in primary microglial cells

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α-tocopherol-succinate, an esterified member of the vitamin E family, inhibits cell proliferation and induces cell death in tumour - but not in normal cells. Whereas α-tocopherol, the most popular analogue of vitamin E, terminates oxidative stress by scavenging of free radicals, α-tocopherol-succinate is redox-inactive. In the present study, we compared the impact of α-tocopherol-succinate and α-tocopherol, respectively, on induction of apoptosis in the highly proliferative immortalized microglial cell line, BV-2, (Blasi et al., 1990, J. Neuroimmunol. 27, pp. 229-37) and primary murine microglial cells.

Primary microglial cells as well as BV-2 cells showed similar nuclear structures. Specifically, intact nuclei showed an ellipsoid shape, and dense spots of heterochromatin throughout the nucleus. In response to staurosporine, primary microglial cells underwent apoptosis, characterized by hypercondensation of chromatin, accumulation of DAPI-labelled chromatin at the periphery of the nucleus, and nuclear fragmentation. α-tocopherol-succinate (100μM) did not significantly increase cell death in primary microglial cells, but induced apoptosis in most BV-2 cells (24 hours). BV-2 cells exposed to α-tocopherol-succinate for 12 hours showed already nuclear signs of apoptosis, including hypercondensation and accumulation of chromatin at the periphery of the nucleus. Primary microglial cells as well as BV-2 cells treated with α-tocopherol for 24 hours did not show nuclear features of apoptosis. Nuclear signs of α-tocopherol-succinate-induced apoptosis in BV-2 cells were accompanied by an increase of NO-production. NO production was monitored by measuring nitrite levels in the culture media using the Griess assay. Preliminary experiments indicate that primary microglial cells fail to produce NO upon exposure to α-tocopherol-succinate (100μM).

In conclusion, our studies demonstrate that (1) the highly proliferative microglial cell line, BV-2, response to α-tocopherol-succinate by induction of apoptosis, whereas primary microglial cells are insensitive to α-tocopherol-succinate and (2) that α-tocopherol is not the apoptosis-inducing metabolite in BV-2 cells.

# Cyclic adenosine 3',5'-monophosphate (cAMP) mediates ammonia-induced programmed cell death in the microglial cell line, BV-2.

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Neuropathological diseases associated with hyperammonemia, such as hepatic encephalopathies and congenital urea cycle disorders, are primarily caused by increased entry of ammonia into the brain. Hypotheses concerning the pathogenic mechanisms of ammonia toxicity include energy metabolism failure, neurotransmitter disturbances, and oxidative stress. Among the glial elements, astrocytes are thought to be the main target of ammonia. Ammonia-induced pathological changes in astroglia may cause deficits in their ability to take up glutamate, thereby leading to abnormal glutamatergic neurotransmission and decreased detoxification of ammonia by the brain. In contrast, little is known about the responsiveness of microglial cells to increased levels of ammonia, despite the hypothesis that these cells are the first elements that respond to pathological conditions.

In the present study, we observed in the microglial cell line, BV-2, that ammonia at low concentrations enhanced the number of mitotic cells but at high concentrations dose-dependently promoted programmed cell death. Whereas many cells showed classical hallmarks of apoptosis, including accumulation of condensed chromatin at the nuclear envelope, blebbing of the plasma membrane, and formation of apoptotic bodies, other cells exposed to ammonia showed signs of mitotic catastrophe. Mitotic catastrophe is a type of cell death occurring during mitosis or resulting from mitotic failure, due to the deficiency of different cell cycle checkpoint mechanisms. Together, our findings indicate that ammonia promotes mitosis, but at high concentrations the mitotic program does not proceed properly, finally leading to programmed cell death.

To elucidate the intracellular signaling cascades involved in the effects of ammonia on BV-2 cells, we evaluated the impact of the intracellular second messenger, cyclic adenosine 3',5'-monophosphate (cAMP), which plays a role in both mitosis and apoptosis. In further studies we found that in BV-2 cells (1) ammonia dose-dependently elevated intracellular cAMP concentration, (2) blockade of the adenylyl cyclase by SQ-22536 suppressed ammonia-induced apoptosis, (3) the cAMP analogues, 8-Br-cAMP (8-Bromoadenosine 3',5'-cyclic monophosphate) and Sp-cAMP (Sp-adenosine 3',5'-cyclic monophosphorothioate), mimiced the effect of ammonia and promoted apoptosis, and (4) inhibition of cAMP-degrading phosphodiesterases by IBMX and rolipram induced apoptosis.

Taken together, our results suggest that intracellular cAMP mediates ammonia-induced programmed cell death in microglia. The clarification of signaling pathways leading to microglial cell death may be critical for a better understanding of the pathogenesis of hyperammonemia.

# Symposium

## S16: Active Sensing: How Nervous Systems Explore the External World

*Martin C. Göpfert and Harald Luksch, Cologne and Aachen*

### Slide

**S16-1** Opening remarks for Symposium 16
*Martin C. Göpfert and Harald Luksch, Cologne and Aachen*

**S16-2** Active vision: Strategies and neuronal mechanisms of spatial orientation in blowflies
*Martin Egelhaaf, Roland Kern and Lindemann Jens, Bielefeld*

**S16-3** TRANSDUCER-BASED AUDITORY INPUT AMPLIFICATION IN THE DROSOPHILA EAR.
*Jörg T. Albert and Martin C. Göpfert, Cologne*

**S16-4** Sound processing in singing crickets
*James Poulet, Lausanne (CH)*

**S16-5** Neural mechanisms of audio-vocal control in bats: no echo without call
*Gerd Schuller, Planegg-Martinsried*

**S16-6** Feedback loops for target selection in the visual midbrain
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**S16-7** Active sensing - interaction of behavior and input in various modalities
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### Poster

**TS16-1C** Biomechanical characteristics of an active tactile sensor
*V. Dürr and JH. Dirks, Bielefeld*

**TS16-2C** Active vision strategy during flight in *Eristalis tenax*
*BRH. Geurten, E. Braun, R. Kern and M. Egelhaaf, Bielefeld*

**TS16-3C** On the Segregation of Object and Background by a neuron most sensitive to small-field motion
*J. Heitwerth, R. Kern and M. Egelhaaf, Bielefeld*

**TS16-4C** Optic flow generated by active saccadic flight strategy of blowflies
*R. Kern, JP. Lindemann and M. Egelhaaf, Bielefeld*

**TS16-5C** Active tactile sampling patterns during insect walking and climbing
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**TS16-6C** Simulated blowfly tangential cells control virtual flight behaviour
*JP. Lindemann, R. Möller and M. Egelhaaf, Bielefeld*

**TS16-7C** The impact of optomotor stimulation on the pursuit system of male blowflies
*C. Trischler, R. Kern and M. Egelhaaf, Bielefeld*

**TS16-8C** A sparking fish that counts - transformation of a latency code to a rate code in weakly electric fish
*J. Engelmann, M. Metzen and J. Bacelo, Bonn and Gif-sur-Yvette (F)*

**TS16-9C** Object perception in actively sensing electric fish
*G. von der Emde and S. Fetz, Bonn*

**TS16-10C** What homing wasps see
*N. Boeddeker, W. Stürzl, J. Hemmi and J. Zeil, Canberra, ACT (AUS)*

**TS16-11C** TRP-DEPENDENT MECHANICAL GATING OF AUDITORY TRANSDUCERS IN THE DROSOPHILA EAR
*JT. Albert and MC. Göpfert, Cologne*

**TS16-12C** Correlates of mechanical input amplification in the electrical output of the *Drosophila* ear
*T. Effertz and M. Goepfert, Cologne*

**TS16-13C** Regulation of mechanical input amplification in *Drosophila* hearing
*M. Goepfert and P. Treskes, Cologne*

**TS16-14C** Active transduction model explains the performance of the *Drosophila* ear
*B. Nadrowski and M. Goepfert, Cologne*

**TS16-15C** Spatial and temporal activity characteristics in primary auditory cortex investigated with current source density analysis under pharmacological manipulation
*MF. Happel, M. Jescke, M. Deliano and FW. Ohl, Magdeburg*

**TS16-16C** Disentangling the contribution of intracortical and thalamo-cortical projections to the generation of subthreshold spectral receptive fields in the auditory cortex
*M. Jeschke, M. Deliano and FW. Ohl, Magdeburg*

**TS16-17C** Effects of iontophoretical application of GABA and gabazine remote from the application site in gerbil primary auditory cortex
*C. Möller, S. Kurt, H. Scheich and H. Schulze, Magdeburg and Ulm*

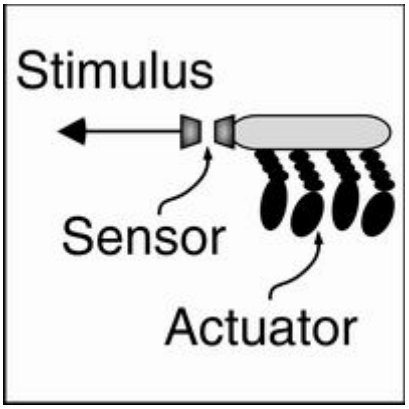
**TS16-18C** Representation of texture information in the barrel cortex of the rat
*PM. Itskov, M. Vonheimendahl and ME. Diamond, Trieste (I)*

**TS16-19C** The Functional Architecture of the Shark's Dorsal-Octavolateral Nucleus: an *In-vitro* Study
*N. Rotem, E. Sestieri, D. Cohen, M. Paulin, H. Meiri and Y. Yarom, Eilat (Israel), Ramat Gan (Israel), Dunedin (New Zealand) and Jerusalem (Israel)*

Introductory Remarks to Symposium 16

# Active Sensing: How Nervous Systems Explore the External World

Martin C. Göpfert and Harald Luksch, Cologne and Aachen




The conventional view of sensory systems as intricate, yet passive collectors of external information does not capture the full complexity of how sensation works. Sensation is active. It relies on the nervous system actively sampling the stimulus world. By directing behaviours such as eye movements, sniffing, or touching, the nervous system controls its sensory input, thereby exploring the stimulus environment and grabbing relevant information.

Active sensation relies on sensory-motor loops: sensory input shapes motor output to facilitate sensation. These loops can take place on various levels, ranging from molecular feedback between motor proteins and adjacent sensory transducer channels (Albert) to feedback between body-movements and visual flow (Egelhaaf). Active sensation can involve complex signalling pathways that control the responsiveness to external signals (Poulet), feedback loops for target selection (Luksch), and remote sensing such as electro- and echolocation (Schuller). By bringing together neuroscientists working on different systems, this symposium aims to explore the implications of active sensing on sensory processing, its investigation, and its fruitfulness for technical implementation (Koenig) by looking into the general constraints imposed by active sensation and the advantages it yields under natural stimulus conditions.

# Active vision: Strategies and neuronal mechanisms of spatial orientation in blowflies

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It may appear plausible to assume that the control of complex behaviour requires a correspondingly complex brain. Our studies on blowfly visually guided orientation behaviour show that this assumption is not generally justified. We investigate by behavioural, neuronal and modelling analysis which brain mechanisms enable blowflies to move in their environments without colliding with obstacles. The blowfly is an excellent model system to analyse how this vital task can be solved. With its miniature brain, the fly is able to control highly aerobatic flight manoeuvres and, in this regard, outperforms any man-made autonomous system. To accomplish this extraordinary performance, flies employ a clever trick: they shape actively by the specific properties of their own movements the dynamics of the image sequences on their eyes ('optic flow'). Instead of steering smooth curves by slow turns, flies actively shift their gaze by brief turns of body and head while keeping the gaze rather constant between these saccades. As a consequence, flies separate the rotational and translational components of their trajectory on the level of basic behaviour. This locomotion strategy results in visual information that can be processed by the brain with relatively little computational effort.

By utilising the intervals of stable vision between saccades, an ensemble of motion sensitive neurons in the blowfly visual system is able to extract from the optic flow information about different aspects of the spatial layout of the environment. This extraction is possible because the retinal image flow evoked by translation, containing information about object distances, is confined to low frequencies and the residual intersaccadic head rotations are small and encoded at higher frequencies. Information about the spatial layout of the environment can thus be extracted in a computationally parsimonious way. The detectability of environmental objects is even enhanced by adaptation mechanisms in the visual motion pathway.

The consistency of our experimentally established hypotheses is tested by modelling the motion vision system. This model can reproduce the main features of the neuronal responses. Using this model to control the locomotion of a 'Cyberfly' moving in virtual environments we could show that the translational optic flow information coded in the motion sensitive neurons is sufficient to control basic saccadic obstacle avoidance behaviour.

On first sight, our experimental and modelling results on neuronal function based on naturalistic, behaviourally generated optic flow seem to be in stark contrast to conclusions based on conventional visual stimuli.

# TRANSDUCER-BASED AUDITORY INPUT AMPLIFICATION IN THE DROSOPHILA EAR.

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Ears are active sensors that use mechanical feedback to amplify sound-induced vibrations. The source of this feedback resides in motile mechanosensory cells. One candidate actuator that may promote this amplification is the mechanotransducer machinery proper. Recent research confirms that in Drosophila transduction and amplification are molecularly linked. Investigation of the auditory mechanics in mutant flies revealed that amplification requires the transient receptor potential NompC (=TRPN1) which is also required for the sensitive mechanical gating of auditory transducer channels. The gain of amplification, in turn, was found to depend on the heteromultimeric cation channel formed by the TRPVs Nan and Iav. Nan/Iav function seems to adjust the ear's sensitivity by modulating NompC dependent mechanotransduction. These findings show that transduction and amplification depend on the same molecular components, whereby the existence of related TRP channels in vertebrate hair cells suggests that TRP-dependent transduction and amplification may not be restricted to fruit flies.

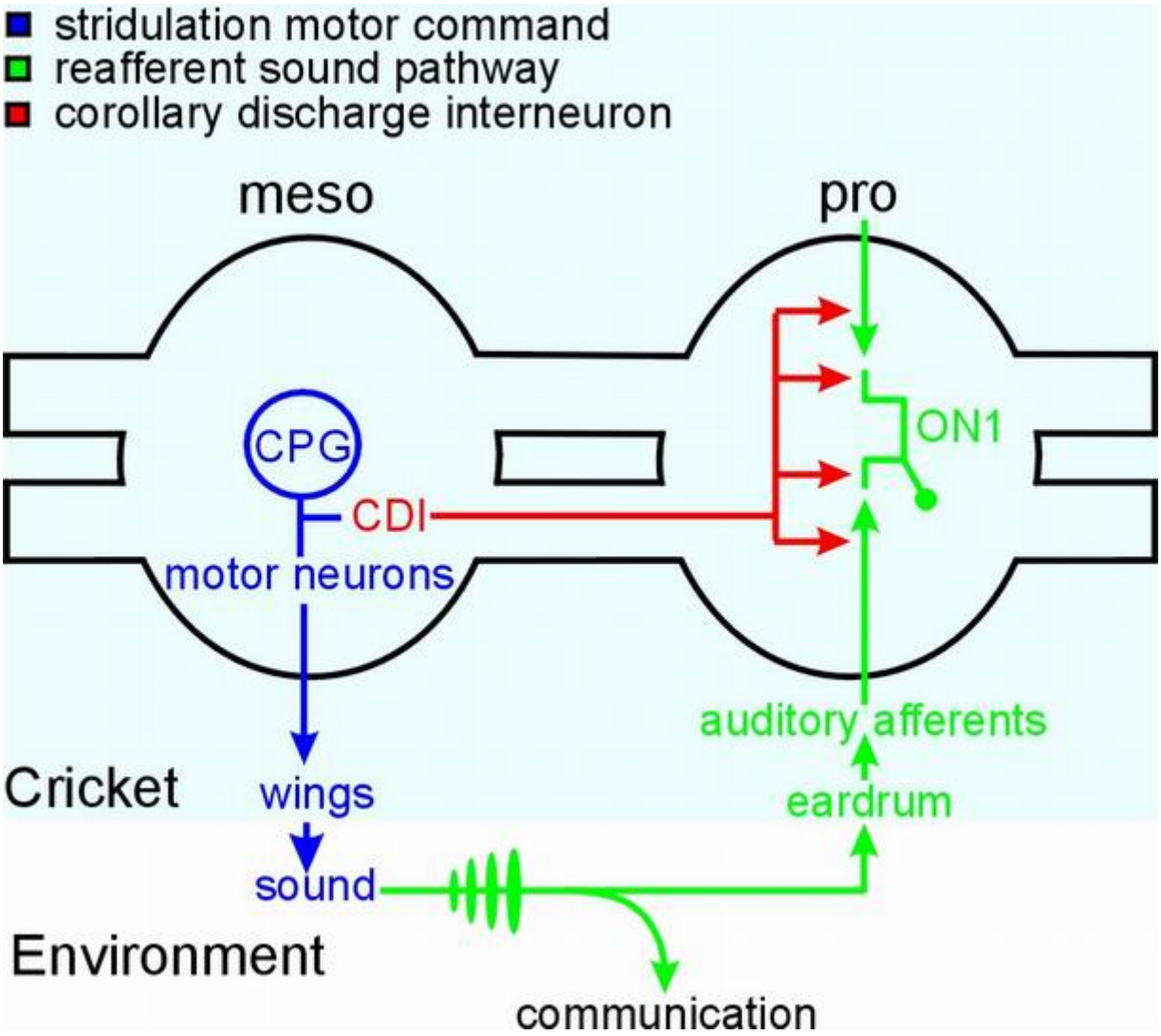
Supported by the VolkswagenFoundation

# Sound processing in singing crickets

James Poulet

Ecole Polytechnique Federale de Lausanne, Brain Mind Institute, Station 15, Lausanne, Switzerland

Stridulating male crickets are able to hear sounds from the environment despite generating loud songs for hours on end. In this talk I will summarise recent work examining how they achieve this feat of sensory processing. While the responsiveness of the crickets' peripheral auditory system (tympanic membrane, tympanic nerve, state of the acoustic spiracle) is maintained during sound production, central auditory neurons are inhibited a corollary discharge precisely timed to coincide with the auditory neurons' maximum response to self-generated sound. The corollary discharge inhibition is mediated by the bilateral, multisegmental corollary discharge interneuron. The central nervous system can therefore control the amount of sensory input during sound production and prevent desensitisation of the crickets' auditory pathway.



# Neural mechanisms of audio-vocal control in bats: no echo without call

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Bats interact with their environment using sophisticated echolocation systems for orientation in space and for prey capture. Ultrasonic signals are transmitted over short, medium or long ranges via airborne vibrations and constitute highly flexible carrier signals. They carry information about the reflecting and reverberating surroundings imprinted as spectral and temporal features on the echoes. As emitter and receiver coincide in the bat, the neuronal control interferes at two stages: first on the motor side controlling the emitted vocalizations and second on the receiving side modulating auditory processing. The characteristics of the acoustic signal can be neurally adjusted in order to optimize acoustical imaging of the reflecting targets or in order to stabilize the acoustical echo parameters in the optimal processing range of the hearing system (e.g. Doppler Shift compensation). The effect of echolocation call emission on the processing characteristics of the hearing system can either be acoustical self stimulation that influences subsequent echo analysis or a temporary bias and alteration of echo processing linked to the onset of vocalization.

The functional involvement of several brain stem sites in the vocal pathway and the auditory system have been identified in bats with different approaches like micro-stimulation, tract tracing, recordings and reversible or permanent lesioning. The periaqueductal gray, the paralemniscal area, the Nucleus of the brachium of the inferior colliculus and the pretectal area were shown to be good candidates for a differential functional involvement in vocal control and/or sensory-motor interfacing. The findings suggest that the vocally active areas are part of a distributed network governing vocal shaping, mediating sensory feedback and coordinating further motor actions accompanying echolocation.

Interestingly, some vocal areas participate in the control of echolocation calls but are not necessarily involved in the control of communication calls. It is hypothesized that the special requirements of the echolocation system of bats as an active sensing system have led to a functional accentuation of sensory-motor circuitry of echolocation call control.

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# Feedback loops for target selection in the visual midbrain

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To survive in a complex world, animals have to exploit all available information to avoid predators, to detect prey or mating partners, and to orient in their environment. Since many of these tasks involve spatial orientation, vertebrates construct multimodal maps of space that include all sensory afferents that can be organized along spatial coordinates. The visual midbrain (superior Colliculus in mammals and optic Tectum in non-mammals) is a prime example of such a multimodal map and receives input from visual, auditory and somatosensory projections, with additional afferents from specialized sensory systems if present (e.g., infrared, lateral line etc.). The multimodal map of space constructed in the midbrain is organized along visual coordinates, with non-visual afferents aligning to this master during development. In essence, any stimulus perceivable to the animal will thus lead to excitation in the visual midbrain and can be transferred to the motor map which may elicit a coordinated movement of body, head and sense organs to the stimulus.

While the organization and the development of the midbrain multimodal map of space are well studied, less is known about the computation of input in actively behaving animals. In a given environment, most animals have concepts of the appropriate behaviours that will be executed upon stimulation. However, to account for unpredictable events, animals also have to rely on a bottom-up analysis of their sensory surround, a feature that appears to be accomplished by the midbrain. Thus, the task is not only to generate a faithful representation of the sensory surrounds, but also to weigh information from the various senses to pick out the most relevant in a given situation, regardless of the sensory channel this information is contained in. Given the multitude of sensory input that is represented in the surround, the system has to incorporate a fast and reliable mechanism that transfers the excitation map into a non-ambiguous focus of excitation and thus to a singular motor output signal which is necessary to avoid a motor deadlock.

In the midbrain of birds, feedback loops have been worked out that may fulfil the task of the "winner-takes-all" mechanism described above. The loops consist of reciprocal connections from the optic Tectum to subdivisions of the isthmic nuclei group: an excitatory homotopic feedback to the upper and medial tectal layers, a second excitatory homotopic feedback to the lower tectal layers, and a heterotopic inhibitory feedback to the lower tectal layers. In addition, the isthmic nuclei themselves are interconnected with inhibitory projections. Several findings are striking about the system: the input to the isthmic nuclei from the optic Tectum stems from an unusual cell type with anatomical and physiological specializations; the isthmic nuclei have almost no additional input or output projections except for the one with the Tectum; specialized ion channels suggest that timing might be important, while anatomical specializations appear to be ill suited for precise timing. We have recently investigated various aspects of the tecto-isthmic feedback loop and will report the status quo of our understanding of its connectivity and its role in target selection.

Supported by DFG

# Active sensing - interaction of behavior and input in various modalities

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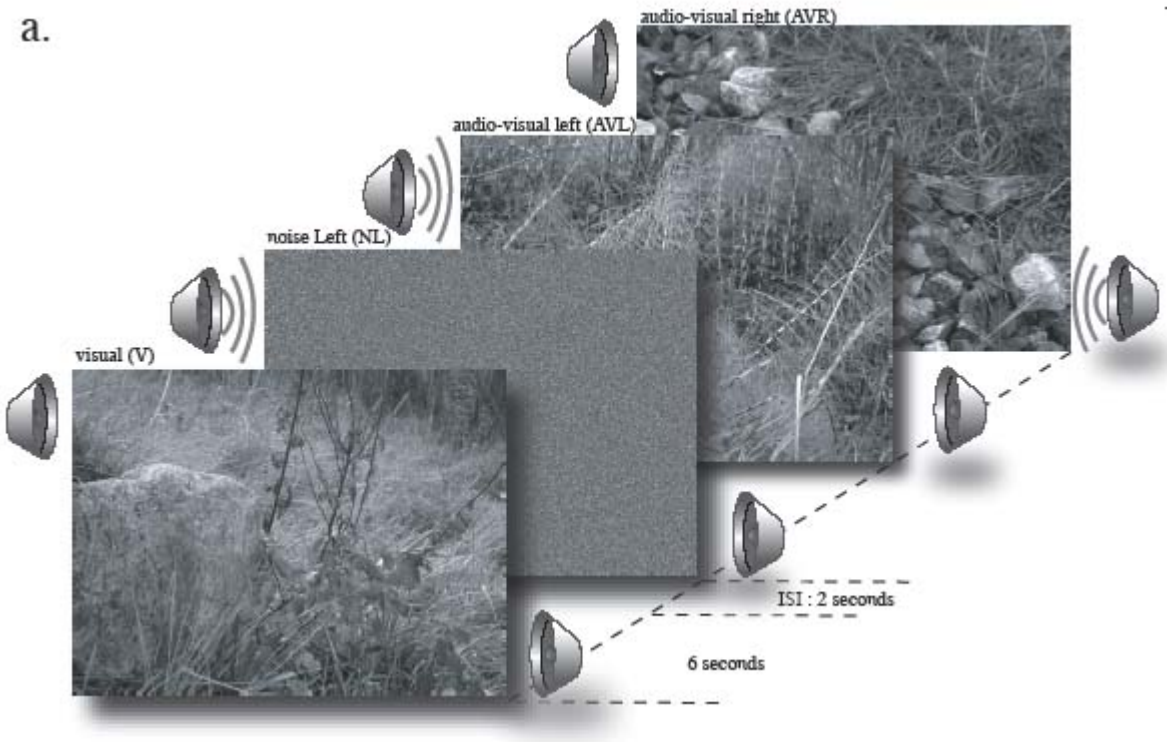
During free exploration of the environment, different sources of information may possibly interact in the control of overt attention. In this work, by recording the eye movements of human subjects during free viewing of natural multimodal stimuli, we investigated these interaction mechanisms. We presented natural images together with lateralized natural sounds in a variety of conditions. We demonstrate that the selection of fixation points during multimodal conditions is dependent on both unimodal stimuli. Further analysis shows that a linear combination of both unimodal signals provides a good model for this integration process. Hence our results support a functional joint saliency map which integrates different unimodal saliencies before any decision is taken about the subsequent fixation point. These results provide boundaries for the performance and architecture of any overt attention model performing in multimodal conditions.



# Biomechanical characteristics of an active tactile sensor

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In contrast to the whisker of a mammal, the arthropod antenna not only transmits but also senses mechanosensory cues of various sub-modalities, including contact location, bending and vibration. Many arthropods actively move their antennae to sample the space ahead. Whereas the sensilla of insect antennae are fairly well documented, very little is known about the biomechanical transfer characteristics of the flagellum. The latter is of prime importance for the assessment of sensory tuning of the antenna, cross-talk between mechanosensory sub-modalities, and the neural separation of external signals from self-stimulation effects.

The stick insect *Carausius morosus* is a good organism for the study of active tactile sensing in exploratory locomotor behaviour. It continuously moves its antennae during locomotion and adapts the movement pattern to the walking direction and to the actual behavioural context (see Krause et al., this vol.).

The prime objective of the present study was to describe the characteristics of the stick insect flagellum that give rise to its remarkable combination of high flexibility and stiffness. The second objective was to determine damping and bending properties and their possible role for information coding and transmission.

The flagellum of the antenna is very light, long and thin, with a length:diameter ratio of approximately 100:1. It has a tapered shape, the diameter decreasing from 0.32 to 0.15 mm from base to tip. Average material density increases from 1.06 to 1.32 mg/mm³ from base to tip. Azan-stained cross sections of the cuticle reveal decreasing endocuticle thickness, ranging from 15.5 µm at the base to 1.6 µm near the tip. At the same time, the ratio of soft endocuticle over sclerotised exocuticle decreased from 84% at the base to 20% at the tip. Thus, a tapering wedge of soft endocuticle lines the inside of a stiff cone of exocuticle.

This gradient matches the local damping properties of the flagellum. Time constants of return time courses were measured after step deflection, while clamping the flagellum at different locations. We found that damping is overcritical anywhere along the flagellum, but is much stronger at the base ($\tau$ = 20-30 ms) than in the middle of the flagellum ($\tau$ = 10-15 ms). Dried flagella were undercritically damped, revealing two major oscillation frequency components (approx. 240 Hz and 1020 Hz). This and other findings suggest the presence of two coupled mechanical subsystems.

Bending stiffness decreased exponentially from 1 N/m at the base to 0.01 N/m near the tip, giving rise to characteristic bending shapes for distinct combinations of contact distance and deflection magnitude. With increasing contact distance the location of the curvature maximum steadily shifts distally, while increasing deflection magnitude leads to an increase of curvature. Thus, there is a unique curvature maximum for any contact point location in 2D space. We propose that a small feed-forward neural network, receiving bending information from few campaniform sensilla along the flegellum, would be most convenient to code head-centred coordinates of the contact point.

This work is supported by BMBF grant PTJ-BIO BNK2-061 to VD

# Active vision strategy during flight in *Eristalis tenax*

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Orientation is essential for purposive movement in space. In many animals orientation tasks are solved with visual sensory systems. The neuronal basis of motion vision has been well studied in insects, especially in the blowfly *Calliphora* (Egelhaaf, 2006). Recent studies have shown that *Calliphora* uses an active sensing strategy during flight to shape the perceived optic flow and, thus, to facilitate extraction of meaningful information about the outside world (Kern et al., 2006).

The main objective of this project is to elucidate if the difference in flight behaviour between hoverflies (Syrphidae) and blowflies (Calliphoridae) reflects differences in their active sensing strategies and eventually in the tuning of the neuronal mechanisms of optic flow processing. Since hoverflies and blowflies have partly homologous visual interneurons, the project will allow us to draw conclusions about how the behaviour of an animal is reflected in its specific neuronal tuning and the way it perceives the environment.

In a first step we observed the Syrphidae *Eristalis tenax* in behavioural experiments to search for its basic behavioural components. It was possible to get pictures with sufficient resolution of the head during characteristic flight manoeuvres as for example hover flight and backward thrust, using high speed cameras at 500 fps. The huge amount of data creates a need for an automated analysis tool. This is done with a new high performance custom made object detection software package.

The head and body trajectories will allow us to derive head movements occurring in these typical flight periods. Also fixation behaviour involving conspecifics could be observed. The trajectories will be segmented into its basic action components which can then be categorized and analysed. Yaw and roll compensation of the head was observed in different types of turns during flight. Also the pitch angle between thorax and head changes for different flight types, such as translatory flight and hover flight. It will be investigated how the fly shapes the optic flow it perceives by movements of the head and thereby exercises active sensing.

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# On the Segregation of Object and Background by a neuron most sensitive to small-field motion

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Flies are able to land on small object and to pass obstacles without a collision. It has concluded that the FD cells (figure detection cells) play a role in the detection of objects, since they respond stronger to small-field motion, i.e. movement of an object in their receptive field, than to large-field motion (Egelhaaf, 1985; Kimmerle & Egelhaaf 2000a & b).

So far predominantly experimenter-defined stimuli were employed to characterise FD-cells, which are much simpler in their spatio-temporal properties than the optic flow experienced by a free-flying fly when passing an object in front of its background. Here we analyse the performance of one of the FD-cells, the FD1-cell, under naturalistic stimulus conditions, i.e. under conditions which come close to what a free-flying fly might have seen in a real three-dimensional environment.

The FD1 cell strongly responds to the naturalistic optic flow in bouts of activity nearly during the entire flight. Especially if the background is close to the fly the FD1 cell responds with a firing rate of up to over 300 Hz independently of the presence or absence of an object. Thus by the firing rate itself it is not possible to detect the presence or absence of an object when the background is close. Nonetheless, when an object is in the receptive field of the FD1 cell there are even slightly higher firing rates. When the background is far, and no object is in the receptive field then the response decreases considerably. However, if the object passes through the receptive field of the FD1-cell the response increases considerably. These results suggest, that the FD1 cell may signal the nearness of environmental structures rather than specifically the existence of small objects, although it responds better to relatively small objects than to the background when stimulated with simple experimenter-defined stimuli. To assess the significance of this small-field tuning under natural stimulus conditions, the performance of FD1 will be compared with he performance of motion sensitive cells which do not show small-field tuning, but have otherwise similar response properties..

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# Optic flow generated by active saccadic flight strategy of blowflies

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Blowflies do not turn in a gradual way when flying a curve. Rather, they change their direction of flight by rapid turns, so-called body saccades, which go along with saccadic head movements. Even in situations where the blowfly could, at least in principle, fly straight they show a continual sequence of saccadic turns. This saccadic flight and gaze strategy has been interpreted as an active sensing strategy by which the rotational and translational changes of gaze and, thus, the corresponding rotational and translational optic flow components are largely separated. This separation is likely to be functionally significant, because only the translational optic flow component contains information about the three-dimensional layout of the environment, and separation by behavioural means greatly facilitates the neuronal representation of this behaviourally relevant information [1-3]. Although the relationship between translational optic flow and distance information is well understood, in principle, the optic flow generated by free-flying animals has not yet been documented systematically. Moreover, it is still not clear what features of the optic flow evoke saccadic turns. Therefore, the poster addresses for environments with different spatial layout, such as straight flight tunnels of different width or flight tunnels with inserted obstacles, the flight characteristics of free-flying blowflies (e.g. properties of saccades and velocity), on the one hand, and the characteristics of the resulting actively generated optic flow, on the other hand. On this basis, it is investigated by which features of the time-dependent optic flow patterns across both eyes saccades are evoked and how the saccade characteristics (e.g. their amplitude) depend on the optic flow characteristics.

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# Active tactile sampling patterns during insect walking and climbing

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Many insects continuously move their antennae during locomotion. In the nocturnal stick insect *Carausius morosus*, the antennae are of equal length as the front legs, so that any antennal contact information is relevant to ongoing leg movements. During walking, the movement patterns of the antennae are rhythmical, coordinated with the stepping patterns of the legs, and oriented towards the walking direction.

Here we report results from motion capture experiments on unrestrained walking *C. morosus* with and without the presence of obstacles. Animals were marked with small retroreflective markers on their thorax and antennae, and tracked with video cameras at 50 fps (no obstacles) or 100 fps (with obstacles). Obstacles used were a vertical rod of 6 mm diameter or steps of various heights (19 to 66 mm). Joint angles were calculated for both the head-scape (HS) and scape-pedicel (SP) joint according to Krause & Dürr (2004, Biol. Cybern. 91: 168-181).

During straight walking, the two antennal joints were strongly coupled to each other with a mean phase lag of 30°, resulting in elliptic movements relative to the head. The azimuth of head and prothorax were strongly coupled to the step cycle of the front legs, adding approximately 30° of horizontal movements to antennal orientation. Thus, rhythmic head and prothorax movements may induce coupling of antenna and front leg, even if the antennal joint rhythm itself drifts relative to the stepping rhythm.

In all trials with obstacles, head and prothorax were immobilised to simplify the kinematic analysis. When touching the vertical rod with an antenna, stick insects responded with an aimed reaching-movement towards the rod, followed by climbing behaviour. During the reaching movement, up to three further antennal contacts occurred (median duration: 90 ms), each of which was above the previous contact. Linear regression analysis revealed that the location of the last antennal contact predicts leg contact location in a highly significant manner. When the first antennal contact occurred during a swing movement, the tarsus was redirected towards the contact location with a mean delay of 40 ms.

Upon the first contact with the rod, the rhythmic antennal movement pattern showed a marked increase of the cycle frequencies in both joints. The prevalent movement pattern after antennal contact was a switch to HS joint levation followed by SP joint abduction (med. delays: 22.5 and 50 ms, respectively), resulting in an upward movement. HS depression and SP adduction followed with median delays of 110 and 140 ms, respectively. After the first antennal contact, inter-joint coupling of HS and SP joints switched from pronounced coupling of downward movements (SP adduction couples to HS depression) to pronounced coupling of upward movements (SP abduction couples to HS levation).

Preliminary results on climbing of steps suggest an increase in antennal joint cycle frequency and an increase in bilaterally symmetrical levation of both antennae.

We conclude that stick insects alter their active tactile sampling behaviour after the first obstacle contact by an increase of cycle frequency and by changes in inter-joint coupling.

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# Simulated blowfly tangential cells control virtual flight behaviour

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Blowflies seem to employ an active saccadic flight and gaze strategy to separate the rotational from the translational components of the optic flow resulting from egomotion. As a consequence, during the translatory segments of flight, wide-field motion sensitive neurons encode information about the spatial layout of the environment (Kern et al. 2005, van Hateren et al. 2005). It was not clear so far, whether and how this information can be decoded and used for obstacle avoidance.

We present a closed-loop simulation of a virtual fly avoiding walls in its virtual environment by saccadic turns. The timing and amplitude of these turns is controlled on the basis of the responses of simulated wide-field motion sensitive neurons.

The sensory model used for the simulations has previously been shown to account for all major features of the graded responses of the real cell to behaviourally generated optic flow (Lindemann et al. 2005). The responses of these model neurons are coupled to a generator of saccadic turns in a simple feed-forward way. The dynamics of the turns generated by the sensory-motor interface closely match the time course of saccadic turns observed in free blowfly flight behaviour.

The rotational movement of a typical saccadic turn is limited to a duration of 50 ms. The sideward drift translation following this saccadic turn was observed to diminish only gradually over the intersaccadic time interval of another 50 ms. The optic flow components caused by such drift movements are coded in the tangential cell signals. To evaluate the consequences of such drifting, the closed-loop model was tested with different amounts of sideward drift.

Despite the simplicity of the control scheme our model simulations show that a saccadic flight strategy helps to avoid collisions with the wall of the flight arena, whereas this is not possible with a smooth controller. The information on the spatial layout of the environment encoded by wide-field motion sensitive tangential cells between the saccadic turns was found to be sufficient to generate this behaviour.

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# The impact of optomotor stimulation on the pursuit system of male blowflies

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Much is known about the flight behaviour of blowflies. Especially two aspects of flight behaviour, course stabilization by optomotor responses and visually guided chasing, have been analysed thoroughly. Optomotor responses are triggered by image motion across extended parts of the retina (wide-field stimuli): the animal follows the image motion by head and body movements and thus reduces stimulus strength (e.g. Hausen and Egelhaaf, 1989). Chasing flights are triggered by the presence of small moving objects (small-field stimuli) and are performed by male blowflies in the context of mating (e.g. Land and Collett, 1974). Object motion as well as self-motion induce retinal image flow which activate the optomotor system and the pursuit control system requiring a balanced interaction of both. So far, both behavioural components have been studied separately, leaving open the question of how chasing flies deal with conflicting optomotor stimuli which inevitably arise during chases.

Here we challenge male flies by presenting optomotor stimuli during their chase after a dummy target (fly-sized black spheres) moving on a circular track (speed 850°/s or 910°/s). Chases took place in a perspex tube (diameter 40cm, height 70cm). Vertical gratings, surrounding the tube, were held stationary or were moved either clockwise or counter-clockwise at two different speeds (45°/s, 365°/s). The scenes were filmed with two digital high-speed cameras. From the recordings the trajectories of both dummy target and pursuer were reconstructed. Data analysis so far yields that the presence of optomotor stimuli impairs chasing performance: When compared with the success rate of catches before a stationary background, the success rate of catches before a moving background decreased by 33% and by 37% when moving at 365°/s in the same or the opposite direction of the dummy, respectively. The success rate of catches decreased by 6% and increased by 2% during background motion of 45°/s moving in the opposite or the same direction of the dummy, respectively.

Details of chasing flights under the different stimulus conditions, i.e. time courses of both forward velocity and angular velocity, retinal position of target, angle of approach, and the presence and characteristics of saccades are evaluated in order to obtain more detailed insight how the coaction of optomotor responses and chases is mediated.

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# A sparking fish that counts - transformation of a latency code to a rate code in weakly electric fish

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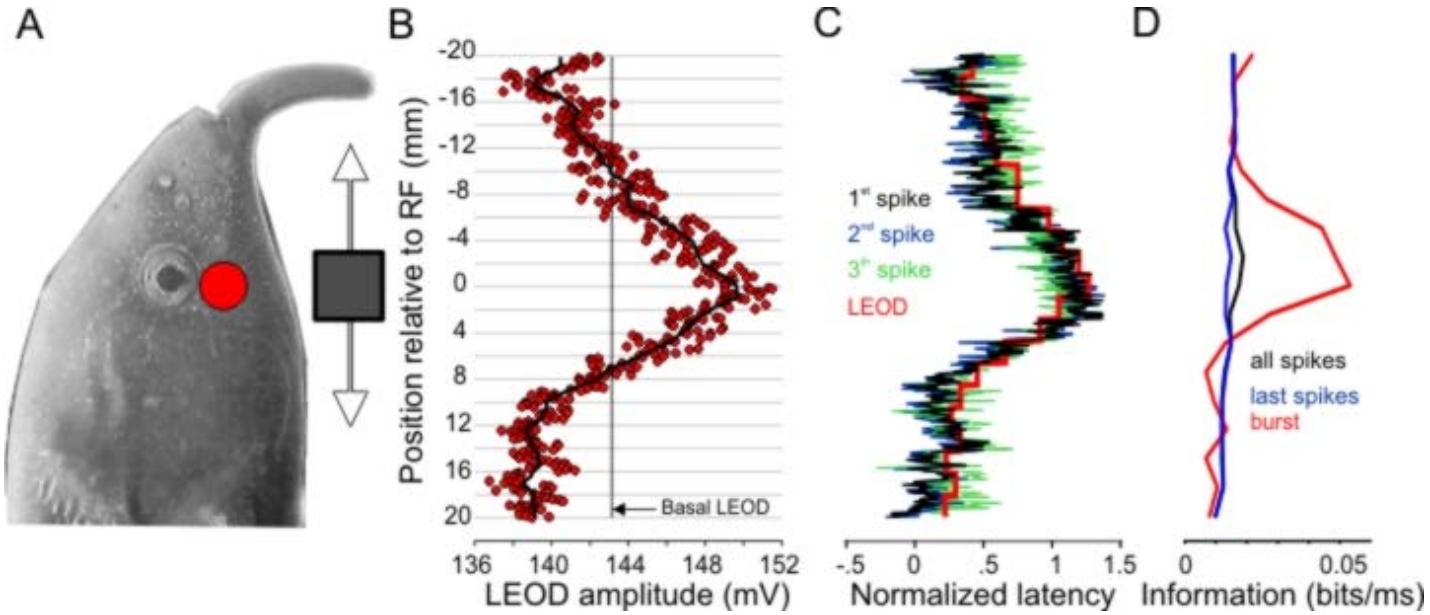
*Gnathonemus petersii*, a member of the pulse-type weakly electric fish, are well studied animals with respect to their unique electric sense. They possess three different peripheral receptors devoted to separate aspects of their environment. These aspects include active/passive electro location and electro-communication. Active electrolocation is based on the generation of a short electrical pulse (EOD) that builds up a field around the fishes body. The currents associated with the EOD flow through the skin of the fish and are sensed by specialised electroreceptors. These so-called mormyromasts respond either to changes in the amplitude of the EOD and/or the temporal structure of the EOD. The spatial distribution of the changes in the electric field that are due to objects are called electric images and the fish can extract various parameters from such images. We studied how mormyromasts code electric images and how this coding is transformed at the first central projection level, the ELL. In contrast to older data, which suggested that rate- and latency code are used in the receptors, our results show that the mormyromasts code the properties of electric images using a latency code only. In contrariety to previous experiments we analysed the responses in the presence of the natural EOD and we did not use artificial stimulation to evoke responses. In the ELL the pure latency code is transformed to a latency and/or rate code. Mechanisms and functions of the use of both coding schemes as opposed to the latency code used in the periphery are discussed.



# Object perception in actively sensing electric fish

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In the absence of light, the weakly electric fish *Gnathonemus petersii* actively senses its environment and objects within it through active electrolocation. The fish emit weak electric signals with an electric organ in their tail and perceive them with an array of electroreceptors distributed over almost their entire skin surface. Objects in the vicinity of the fish project an electric image onto the fish's skin and are thus electrically detected, localized and analysed according to their geometrical and material properties. Here we test which object features are learned when fish are trained to discriminate between two objects in a two-alternative forced-choice procedure. Using only their electric sense, fish learn to discriminate successfully between two objects of different shapes and volumes, different material, or both. Even when shape is reduced to just the contour of an object, fish continue to choose a wire-model of the previously learned S+. When in non-rewarded test trials new object combinations are offered interspersed with training trials, fish continue to discriminate between them based on their previous learning experience. There is an inborn tendency to avoid metal objects and to choose the smaller one of two objects, even when S+ and S- have been both metallic and of identical volumes. Before deciding between two objects, fish always compare both objects and base their decision on the relative differences between them. When learning about object properties several object parameters are used. In addition to volume and material, fish attend to object shape and to a lesser degree to height. Fish also attend to additional parameters, such as the possession of corners or rounded edges. When presented with a novel object combination, fish decide between them according to their relative resemblance to the S+ and S- objects used during training. Properties in common with the S- lead to avoidance of an object, while resemblance to S+ leads to object choice. Fish weigh positive and negative properties of each of the novel objects and base their decision on the outcome of this comparison. Our results suggest that during electrolocation, fish perceive objects according to the feature extraction theory, i.e. they recognize special features of an object rather than recognizing an object as a whole.

# What homing wasps see

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Ground-nesting wasps memorize the spatial location of their nest using a visual representation of their nest environment. When leaving their nests for the first time in the morning, or when they had difficulties finding the nest during the previous return, they turn to face the nest entrance and move away from it flying sideways and backwards in a series of arcs during which they gain distance from the goal at about the same rate as height above ground (reviewed by Zeil et al. 1996). The structure of learning flights suggests that they serve to record the scene around the goal from defined vantage points and to acquire distance information through motion parallax. The aim of this study is to understand the functional significance of learning flights, and how their organization relates to the homing ability of wasps. To investigate the problems involved in view-based homing under natural conditions, where the saliency of landmarks is hard to predict, we reconstructed what ground-nesting wasps see during learning and homing by recording the three-dimensional flight paths of the insects together with the orientation of their head, using two high-resolution and high-speed digital video cameras. We found that wasps move their head in a saccadic fashion during learning. Sections of flight in which gaze is held constant are linked by rapid head movements that change gaze. The insects thus do not generate a pivoting parallax field as had been deduced previously from an analysis of their body axis orientation alone, but a sequence of translational parallax fields. Both these fields of behaviourally shaped optic flow would allow the insects to segregate objects from background and to judge the relative and possibly absolute distance of landmarks. We then moved a panoramic imaging device along the same paths with a robotic gantry, recording images sequences from the perspective of the wasp during learning and homing flight manoeuvres. This method of reconstruction allowed us to determine the image transformations that the wasps create by their particular flight pattern, and how the views correlate with the ones the wasp sees during her return. We discovered a number of properties of learning and return flights that had been difficult to recognize before and that challenge some of the current ideas about the function of learning flights. During the return flight, the insects experience varying patterns of small and large image differences, relative to what they had seen during learning. Small image differences occur when the orientation of the returning wasp is similar to the orientation she had while fixating the nest entrance laterally during learning. Areas of small image differences are linked in time during a return flight and some form "valleys" that lead towards the goal, both for image differences above the horizon, which are mainly affected by orientation, and below the horizon, which are mainly affected by location in space.

Reference:
Zeil J., Kelber A. and Voss R. 1996. Structure and function of learning flights in bees and wasps. J. Exp. Biol. 199: 245-252.

# TRP-DEPENDENT MECHANICAL GATING OF AUDITORY TRANSDUCERS IN THE DROSOPHILA EAR

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The mechanotransducer (MET) channels underlying the sense of hearing are directly activated by the forces they are meant to transduce, namely the sound-induced vibrations themselves. This peculiar mode of activation crucially depends on accessory structures involved in force transmission to the channels, a feature of auditory METs that has substantially complicated their molecular identification until this day. Trying to make a virtue out of necessity, we devised an experimental paradigm that allows to probe MET function in the ears of intact flies. Exploiting the inherent reciprocity of force transmission between the auditory METs and their external receiver structure, the Drosophila antenna, we found that the gating of METs introduces a transitory compliance into the receiver's force-displacement characteristics. This 'gating compliance' was virtually absent in flies mutant for the TRP channel NompC (=TRPN1) but more prominent in flies mutant for the TRPVs Nan and Iav. These findings show that the direct mechanical gating of auditory METs in the Drosophila ear depends on members of the TRP superfamily of ion channels.

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# Correlates of mechanical input amplification in the electrical output of the *Drosophila* ear

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*Drosophila* hearing profits from the motility of mechanosensory neurons. This cellular motility introduces a compressive nonlinearity into the ear's mechanics that selectively boosts the ear's mechanical sensitivity to low intensity sounds. To test for electrical correlates of this mechanical amplification, we simultaneously measured sound-induced vibrations of the fly's antennal receiver and compound action potential (CAP) responses in the afferent nerve. Pure tone stimulation at best frequency (BF) revealed that sound-induced displacements of the tip of the receiver by as little as ± 15 nm are sufficient to elicit CAPs. The sensitivity gain due to nonlinear amplification was found to be around ten, with the nonlinear regime in the receiver's mechanics matching the dynamic range of the CAPs. At frequencies >> BF, the sign of the nonlinear effect reversed, now attenuating the receiver's vibrations. The frequency response of CAPs closely paralleled that of the receiver's velocity and followed the nonlinear shift in the receiver's tuning. Genetic disruption of the NompC channel abolished both nonlinear amplification at frequencies around BF and high frequency attenuation. At BF, receiver displacements required to elicit CAPs were ca. 10 times higher than in controls, consistent with the ca. 10 fold gain in sensitivity contributed by nonlinear amplification. Hence, mechanical amplification in the fly's auditory system is expressed in the ear's electrical output, which parallels the amplified mechanical input of the ear.

Supported by the Volkswagen-Stiftung.

# Regulation of mechanical input amplification in *Drosophila* hearing

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Mechanical feedback amplification improves hearing. The strength of this feedback specifies the sensitivity of the ear. Excess in this feedback, in turn, can lead to self-sustained oscillations, causing ringing in the ear. In the interest of gaining insights into the molecular mechanisms ears employ to adjust their sensitivity to external stimuli, we have started to genetically dissect the regulatory pathway that controls the gain of mechanical amplification in the auditory system of the fly. Examination of the auditory mechanics in *Drosophila* mutants revealed that the gain of amplification is negatively controlled via TRPVs: genetic disruption of TRPV channel proteins causes excessive amplification and self-sustained oscillations. TRPVs form calcium-permeable channels, suggesting that amplification is calcium-controlled. In effect, excess amplification and self-sustained oscillations similar to those observed in TRPV mutants result from mutations in calmodulin (*Cam*). *Cam* mutants are shown to display hypersensitive hearing, as judged from both the receiver's mechanics and the electrical response in the antennal nerve. Our findings indicate that amplification in *Drosophila* hearing is calcium-regulated, analogous to the calcium-dependent, transducer-based amplificatory process known from many vertebrate ears. Whether genetic defects in TRPV channels and/or calmodulin can cause ringing in vertebrate auditory systems remains to be seen.

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# Active transduction model explains the performance of the *Drosophila* ear

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In *Drosophila*, hearing is mediated by the antenna. Stimulus forces acting on the antennal receiver are coupled to dedicated neurons that comprise the molecular machinery for mechanosensory transduction, adaptation and amplification. Because the action of this machinery is reflected in the receiver's mechanics, the latter can be used to probe the molecular mechanisms that bring about hearing in an intact ear. These mechanisms are now shown to closely resemble those that are at work in hair cells in vertebrate ears. Based on the gating-spring model of transduction in vertebrate hair cells, we have developed an extended, symmetric gating-spring model that reflects the anatomy of the fly's auditory system and describes the physical processes that make flies hear. The model explains the active behaviour of the fly's auditory system by coupling the transduction channel complex to a set of molecular motors. The motors are described by a linear force-velocity relation, with parameters depending on the open probability of the transducer channels. We show that this model comprehensively explains the ear's nonlinearity and activity, quantitatively capturing the receiver's mechanics and the electrical response in the afferent nerve. These findings suggest that while insect and vertebrate auditory anatomies are vastly different, the physical mechanisms that bring about hearing are the same.

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# Spatial and temporal activity characteristics in primary auditory cortex investigated with current source density analysis under pharmacological manipulation

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Understanding the mesoscopic spatial and temporal organisation of cortical responses in terms of different synaptic subpopulations (thalamocortical and intracortical) in the cortex requires the elucidation of the laminar activity distribution. The combination of laminar field potential measurements and parallel application of selective transmitter agonists and antagonists is a tool to unravel the different contributions of thalamocortical and intracortical inputs to the laminar organisation of responses.

Anesthetised Mongolian gerbils (Meriones unguiculatus) were stimulated acoustically and electrically while the laminar distribution of intracortical potentials was measured with a multichannel shaft-electrode in the central region of primary auditory cortex, field AI. Current source densities (CSDs) were calculated from the depth profiles of the LFPs. The $GABA_A$ agonist muscimol (1µg muscimol / µl saline) was applied locally through a small cylinder placed onto epidural surface of the cortex.

Acoustical stimulation of a cortical site with its best frequency elicited a different CSD pattern as when stimulated with non-best frequencies. This suggests that different synaptic subpopulations are activated by best frequency and non-best frequency stimulation, respectively. Increasing the contribution of inhibition to the evoked activities by topical applications of muscimol resulted in the disappearance of different sinks and sources. In all cases only an early source-sink-source-triplet remained. This pattern is indicative of an activation of thalamocortical afferents which should still be excitable. It will be discussed to which extent synaptic subpopulations might receive differential thalamic or intracortical input with best- and non-best frequency stimulation.
CSD patterns evoked by electrical stimulation were highly similar to the acoustically evoked patterns, but on a faster time scale. This finding suggests the recruitment of largely similar synaptic subpopulations by acoustical and electrical stimulation (Jeschke, diploma thesis, 2006).

Generally, the analysis demonstrates that the combination of CSD analysis and pharmacological manipulation of cortical responses facilitates a mechanistic understanding of cortical response components.

**Acknowledgements**
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# Disentangling the contribution of intracortical and thalamo-cortical projections to the generation of subthreshold spectral receptive fields in the auditory cortex

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The receptive field of neurons is a classical concept in the neurosciences that describes the responses of neurons to the variation of stimulus parameters. This concept has been further developed based in studies of the visual system that could show that the responses of cortical neurons can be modified by stimuli outside the receptive field. These experiments led to the distinction of classical receptive fields from subthreshold receptive fields. Recent studies in the somato-sensory and visual system are putting the role of intracortical interactions for the generation of the subthreshold receptive field into the focus of research. In the auditory domain it has been hypothesized that intracortical connections contribute especially to the border areas of spectral receptive fields. Other researchers have demonstrated that the preference for spectral and temporal modulations can not be inherited from the thalamus and must therefore be created at the level of the cortex itself or via thalamo-cortical loops or cortico-cortical connections.

Hence our goal was to investigate the contribution of different projection systems to subthreshold receptive fields using one-dimensional current source density analysis in anesthetized Mongolian gerbils *(Meriones unguiculatus)* to gain further insights into the generation of such fields. As has been hypothesized, best frequency and non best frequency evoked current source densities exhibit a different laminar pattern. By taking advantage of the fact that neuronal activities that occur along the recording axis must lead to a balanced current source density pattern we could show that the additional sink after non best frequency stimulation can only be explained by activities outside the recording axis. To directly test this explanation we cut the auditory cortex along the isofrequency axis. This procedure led to the total abolishment of the additional sink after non best frequency stimulation and also to a more balanced current source density pattern. Preliminary data indicate that the tuning of the initial sink around layer 3 and 4 did not change after cutting the cortex. This finding is in alignment with the general assumption that the early layer 3 and 4 sink is caused by the activity of thalamo-cortical projections. However, the overall amplitude of the evoked current source density was decreased. This result suggests that intracortical processing e.g. by local feedback loops is necessary to generate the observed macroscopic current source density pattern under normal conditions.

For the elucidation of the nature of involved excitatory or inhibitory processes see the companion poster by Happel et al. (2007).

# Effects of iontophoretical application of GABA and gabazine remote from the application site in gerbil primary auditory cortex

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Throughout the literature the effects of iontophoretically applied neurotransmitter-agonists or antagonists on the local activity of neurons are typically studied at the site of drug application. Here we report the effects of the inhibitory neurotransmitter GABA and the $GABA_A$-antagonist gabazine (SR 95531) on neuronal activity at locations that are remote from the application site, that is, that are beyond the diffusion radius of the applied drug.

Neuronal responses to pure tone stimulation were recorded from a total of 250 single or multi-units in primary auditory cortex (AI) of 13 adult male Mongolian gerbils (1) at the application site and (2) at four additional recording sites, in distances between 300 and 1350 µm from the application site. We found that whereas application of GABA in general led to a decrease and gabazine to an increase in neuronal activity at the application site, a certain number of units at remote recording sites showed effects opposite to these local, drug-induced effects: After application of GABA, 21% of the units recorded at sites remote to the application site showed increases in neuronal activity. Such an opposite effect to the direct drug effect was even more pronounced after application of gabazine: Here, the majority of units recorded at remote sites (56%) showed a decrease of neuronal activity. These effects were seen both in spiking activity and amplitudes of local field potentials.

As a diffusion of the applied drug to the remote recording sites could be excluded, these data demonstrate the existence of long range, inhibitory interactions within gerbil AI. These interactions could be realized either by long range inhibitory projections or by long range excitatory projections to local inhibitory interneurons.

# Representation of texture information in the barrel cortex of the rat

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Rats possess superb tactile capacities using their facial whiskers. As nocturnal animals, albino rats depend on their vibrissa for object localization, judgment of the roughness or texture of surfaces, and the size and shape of small objects. Roughness discrimination is the key issue in the present study.

We trained albino Wistar rats on a texture discrimination task. Rats had to touch the texture with their whiskers and then dependent on the roughness of the texture turn left or right to get water reward. Rat could perform 100-300 trial in a session. It took rats about 2000 trials to learn the task in the current modification. Once learned the performance is high (above 75%) and stable.

When rats reached this level of performance they were implanted with the microdrive with 8-14 independently movable sharp tungsten electrodes in the barrel cortex and CA1 region of hippocampus. Together with the neuronal activity we monitored rats behavior with 2 high speed cameras.

# The Functional Architecture of the Shark's Dorsal-Octavolateral Nucleus: an *In-vitro* Study

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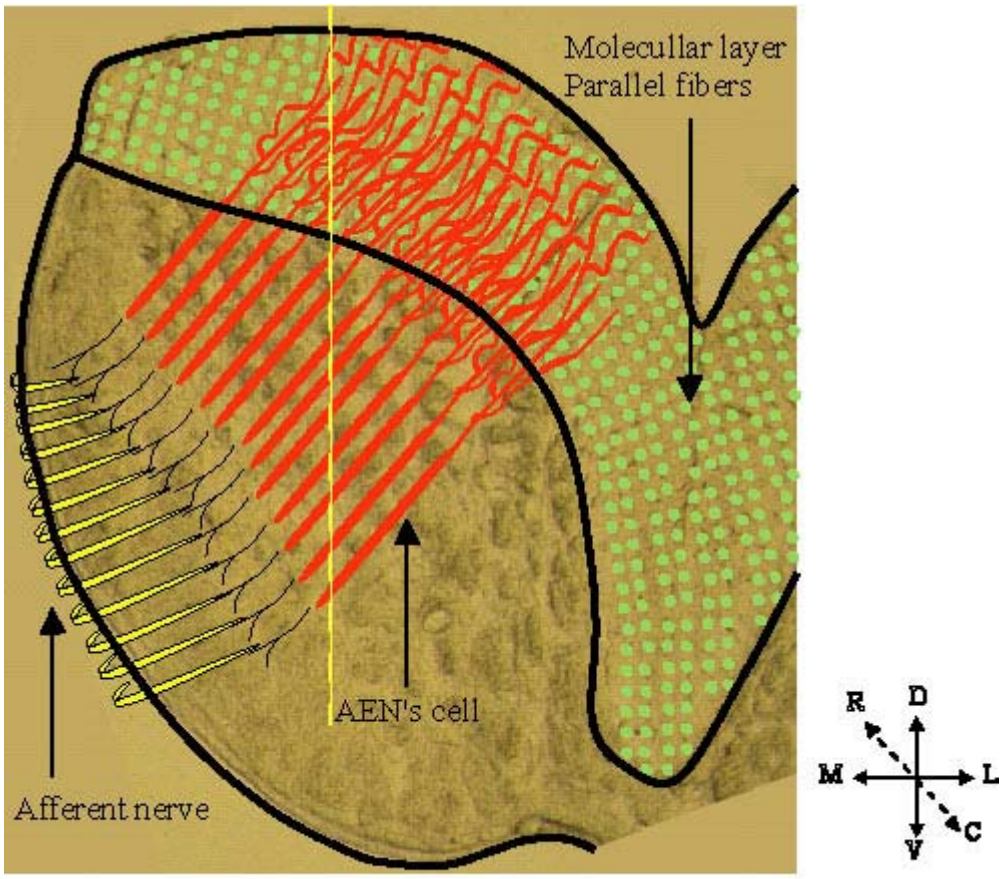
Learning to predict what part of the sensory information results from the organism's own activity enables it to respond appropriately to unexpected stimuli. One example of such mechanism is the elasmobranch dorsal octavolateral nucleus (DON) which apparently can extract unexpected components from incoming electrosensory signals. Here we introduce a novel and unique experimental approach that combines the advantages of in vitro preparations with the integrity of in vivo conditions in order to study the cellular and network mechanisms that underlie cancellation of expected sensory input.

Using this experimental approach we characterized the spatiotemporal organization of the afferent and parallel fiber inputs to the DON. The afferent nerve has a low threshold, and high conduction velocity; a small number of fibers is sufficient to activate the DON. The synapses, activated by the afferent nerve fibers, have fast kinetics that efficiently activates the DON's neurons. In contrast, the parallel fibers have low conduction velocity, high threshold and extensive convergence into the DON's principal neurons. The synaptic transmission has slow kinetics that provides a wide time window for integration of inputs.

These observations do not support the "adaptive filter" hypothesis of the DON, which asserts that parallel fibers signals are a "negative image" of expected sensory inputs. Rather, these findings indicate that filtering in the DON may be mediated by parallel fibers signals that via secondary neurons adjust the sensitivity to afferent inputs.



# Symposium

## S17: Genetics and Molecular Mechanisms of Parkinson's Disease

***Marius Ueffing and Thomas Gasser, Neuherberg and Tübingen***

### Slide

**S17-1** Genetics of Parkinson's disease: an overview
*Thomas Gasser, Tübingen*

**S17-2** Molecular characterization and functional analysis of LRRK2
*Marius Ueffing, Christian Johannes Glöckner, Norbert Kinkl, Andrea Meixner, Annette Schumacher, Matthias Bauer and Thomas Meitinger, Neuherberg*

**S17-3** Functional characterization of genes underlying Morbus Parkinson in mouse models
*DM Vogt Weissenhorn, TT Pham, T Floss, S Hoelter, M Kallnik, A Roethig, H Prokisch, U Ahting, P Kahle, K Goerner and Wolfgang Wurst, Munich*

**S17-4** MOLECULAR MECHANISMS OF SYNUCLEINOPATHES
*Philipp Kahle, Tübingen*

**S17-5** Molecular mechanisms of selective cell death in Parkinson's disease
*Birgit Liss, Marburg*

**S17-6** PARK6: The role of mitochondria and kinases in Parkinson pathogenesis
*Georg Auburger, Frankfurt/Main*

### Poster

**TS17-1C** The biochemical characterization of neuromelanin granules from the human substantia nigra
*F. Tribl, T. Müller, E. Asan, T. Arzberger, HE. Meyer, M. Gerlach, P. Riederer and K. Marcus, Bochum, Würzburg and Munich*

**TS17-2C** The *Drosophila* homolog of Parkinson's disease associated LRRK activates ERK signaling and reduces dopamin in the fly
*A. Voigt, B. Falkenburger and JB. Schulz, Göttingen*

**TS17-3C** Molecular mechanisms and neuroprotection in cellular models of Parkinson's disease
*CP. Dohm, A. Esposito, J. Liman, JC. Reed, M. Bähr, F. Wouters and P. Kermer, Göttingen and La Jolla (USA)*

**TS17-4C** Absence of alpha-synuclein from the nervous system reduces axonal degeneration
*H. Siebert, PJ. Kahle, OM. Schlüter and W. Brück, Göttingen and Tübingen*

**TS17-5C** Proteasome inhibition failed to act as a new animal model of Parkinson' s disease in Wistar rats, but led to changes in tyrosine hydroxylase contents of olfactory bulb and adrenal medulla
*A. Hawlitschka, S. Haas, O. Schmitt and A. Wree, Rostock*

**TS17-6C** Influence of short-term deep brain stimulation of the subthalamic nucleus on Homer1 gene expression in rats with unilateral 6-hydroxydopamine lesion.
*J. Henning, A. Rolfs, R. Benecke and U. Gimsa, Rostock*

Introductory Remarks to Symposium 17

# Genetics and Molecular Mechanisms of Parkinson`s Disease

Marius Ueffing and Thomas Gasser, Neuherberg and Tübingen

Parkinson disease (PD) is the second most common neurodegenerative disorder affecting 1-2% of the population aged 65 and older. The presence of -synuclein-positive Lewy body pathology is traditionally used to distinguish Parkinson's disease from parkinsonism, for which a broader spectrum of neuropathologies, including tau-immunopositive neurofibrillary tangles and ubiquitin inclusions, might accompany nigral neuronal loss. Considerable advances made in defining the genetics, aetiology, pathogenesis and pathology of PD have resulted in novel hypotheses on how this disease develops on the molecular level and how neuronal cell death is executed.

This symposium will present the current understanding of the genetic basis and molecular mechanisms involved in PD. It will have its main emphasis on genes and pathways associated with the disease and will further present experimental approaches as well as models to study it.

# Genetics of Parkinson's disease: an overview

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Recent genetic findings in rare inherited forms of Parkinson's disease (PD) have greatly contributed to our understanding of the molecular pathogenesis of this disease. The discovery that point mutations and amplifications of the SNCA-gene, which encodes a-synuclein, cause an autosomal-dominantly inherited form of PD has focussed research on the aggregation of this protein into intracellular inclusions (Lewy-bodies) as a potential crucial step in the degenerative process. There is accumulating evidence that subtle changes of a-synuclein processing, possibly caused by polymorphisms in regulatory regions of the SNCA-gene, may contribute to the common sporadic form of the disease.

The finding that mutations in an E3-ubiquitin ligase (parkin) cause a relatively common form of autosomal-recessive early-onset PD has drawn attention to the proteasomal protein degradation pathway as another key player. Recently, two additional recessive forms of PD have been identified, caused by mutations in the genes for DJ-1 and PINK1. The function of these proteins is still largely unknown. Most recently, mutations in the gene for LRRK2 were found to cause an autosomal-dominant form of PD, which is responsible for a sizable portion of all familial and even sporadic late onset PD in some populations. The encoded protein belongs to the ROCO protein family, and includes a protein kinase domain of the MAPKKK class and several other major functional domains.

The study of the gene products of those monogenic forms of the disease will provide additional important insight into the molecular pathogenesis of nigral degeneration and Lewy-body formation.

# Molecular characterization and functional analysis of LRRK2

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Leucine-rich repeat kinase 2 (LRRK2) is genetically associated with late-onset autosomal dominant Parkinson disease (PD). Mutations are found throughout the gene including the kinase domain, making LRRK2 the most common cause of inherited Parkinson disease, accounting for 1-6 % of cases including many apparently sporadic cases. Interestingly, mutations in LRRK2 lead to pleiotropic phenotypes: besides nigral loss, Lewy bodies, tau neurofibrillar tangeles and/or ubiquitin inclusions were found, dependent on the type of mutation. The LRRK2 protein consists of multiple domains and belongs to the Roco family, a novel group of the Ras/GTPase superfamily. Besides the GTPase (Roc) domain, it contains a predicted kinase domain, with homology to MAP kinase kinase kinases. We show that LRRK2 acts as an active protein kinase and that mutations in the kinase domain increase kinase activity. Data will be presented further characterizing this activity. Kinase activity is likely to be required for the toxic effect of mutated forms of LRRK2, predicting that kinase inhibitors will be useful therapeutic agents in patients with LRRK2. Data from our own studies will be discussed within the context of current knowledge on LRRK2.

# Functional characterization of genes underlying Morbus Parkinson in mouse models

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Parkinson'disease (PD) is a neurodegenerative movement disorder characterized by the loss of dopaminergic neurons in the substantia nigra. In recent years several genes have been associated with the etiology of this disease amongst which are Pink-1, LRRK-2 and DJ-1. The precise and detailed functions of these genes in PD is, however, still unkown. Therefore analysis of the phenotype of animal models carrying mutations in these genes is of uttermost importance for understanding their role in the etiology and course of the disease. In order to do so we started out to generate a DJ-1 knock-out mouse.

Loss-of-function mutations in the DJ-1 (PARK7) gene are linked to familial early-onset parkinsonism. The DJ-1 mRNA is ubiquitously expressed during mouse embryonic development. In the adult brain it can be found at high levels in the cortex, hippocampus, amygdala, substantia nigra, locus coeruleus, and the nuclei of the cranial nerves. Furthermore in other tissues such as kidney, pituitary, gonads, and muscle DJ-1 is also strongly expressed. Within the framework of a large insertional mutagenesis screen we obtained an ES cell clone habouring a gene trap vector pGT1xlf integration into the DJ-1 gene. The insertion of the genetrap vector led to a fusion transcript and a truncated protein lacking the carboxy-terminal domain required for dimerization and stability of the protein. The DJ-1 deficient mice are viable, fertile, and show no gross anatomical or neuronal abnormalities. DAT and TH immunoreactivity revealed that the numbers of dopaminergic neurons in the ventral midbrain of mutant mice are reduced about 10%-20%, whereas noradrenergic neurons in the locus coeruleus were normal. Other markers of dopaminergic neurons such as Nurr1, Lmx1b, and other neurotransmitter systems such as GABAergic and serotonergic ones were unaffected in the DJ-1 mice. Studies of the mitochondrial electron transport chain in different tissues of the DJ-1 deficient mice showed that the activities of complex I and II were unchanged whereas complex IV activity was slightly but significantly increased. This indicates that the missing protective function of DJ-1 against oxidative stress in these mice may be compensated by a tighter coupling of electron transport. Behaviourally the DJ-1 deficient mice showed only a mild reduction of spontaneous locomotor activity, but had a clear impairment in social memory and cognitive functions, which may represent early indicators of PD.

# MOLECULAR MECHANISMS OF SYNUCLEINOPATHES

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a-Synuclein fibrils comprise Lewy bodies, the neuropathological hallmark lesion of Parkinson's disease and dementia with Lewy bodies . The clinical symptoms (locomotor disturbance and cognitive impairment) appear to correlate with the anatomical distribution of a-synuclein lesions in the brain stem and cortex. Idiopathic Parkinson's disease patients have Lewy bodies in the brain stem, while patients suffering from dementia with Lewy bodies show cortical Lewy pathology. It is a matter of debate if the formation of amyloid fibrils is cytotoxic or on the contrary the visible end point of a cellular defense mechanism to remove toxic pre-fibrillar aggregates. To elucidate the consequences of a-synuclein aggregation, this protein was expressed in a variety of experimental organisms (yeast, worms, flies, mice and rats). In all these systems, a-synuclein was found to aggregate and cause adverse reactions. We have investigated in detail transgenic mice expressing human mutant [A30P]a-synuclein driven by the pan-neuronal Thy1 promoter. In these mice a-synuclein aggregates develop in an age-dependent manner that resembles human Lewy pathology microscopically, histologically, and ultrastructurally. Certain areas in the spinal cord, brain stem, mesencephalon and cortex showed a predilection to convert a-synuclein into pathological fibrils. Interestingly, in mid-aged mice formation of a-synuclein pathology in the central nucleus of the amygdala coincided with cognitive impairment particularly in fear conditioning tasks, which are known to depend on the amygdala. It took further aging until the a-synuclein pathology in brain stem and spinal cord apparently caused a lethal locomotor phenotype. Thus, it is clear that a-synuclein fibril formation is tightly linked to neuronal dysfunction, neurodegeneration, and specific behavioral defects. Further studies in transgenic animals and viral models will elucidate the relationship between amyloid fibril formation and cytotoxicity.

# The biochemical characterization of neuromelanin granules from the human substantia nigra

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Neuromelanin (NM) granules are pigmented organelles underlying the characteristic pigmentation of several catecholaminergic neurons in the primates' substantia nigra (SN) and the locus coeruleus (1). There is increasing evidence that NM granules are generated by a genetic program to exert specific functions in these brain areas. Currently, a known function of NM granules is the storage of iron, which is severely impaired, e.g., in Parkinson's disease (PD). In PD NM granules significantly accumulate iron, which is detectable via transcranial ultrasonography and may represent a biomarker for PD progression (3). Additionally, α-synuclein, the key protein of Lewy bodies, deposits onto NM granules in PD, but not under physiological conditions (2). *In vitro*, isolated NM decreases the activity of the proteasome, induces mitochondrial oxidative stress (4, 5), and thereby may contribute to neurodegeneration and PD progression.

Despite the fundamental role of NM granules, knowledge about their molecular composition was lacking due to the general absence of a functional model system. To characterise NM granules and to clarify their origin we thus applied subcellular proteomics, including organelle isolation from human SN tissue followed by 1D-SDS-PAGE, in-gel tryptic digestion and nano-LC-ESI-MS/MS for protein identification (6).

Our results provide the first biochemical description of human NM granules. We identified 72 proteins, which are mainly found in lyosomes and in lysosome-related organelles (LRO's), in particular enzymes involved in macromolecule degradation, traffic, prevention of oxidative stress or pigmentation. Nevertheless, some proteins are not found in lyososomes, but in LRO's. LRO's, including osteoclast granules, platelet dense granules or melanosomes, are constituents of highly specialized cell types and exhibit functions different from lysosomal macromolecule turnover, such as bone remodelling, blood clotting or pigmentation.

For the first time we uncovered the biogenesis of NM granules to originate from the endosomal system. Furthermore, these data strongly support the hypothesis of a physiological role of NM granules in the human brain that gets severely altered in PD.

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# The *Drosophila* homolog of Parkinson's disease associated LRRK activates ERK signaling and reduces dopamin in the fly

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Parkinson's disease (PD) is a common neurodegenerative disorder pathologically characterized by the loss of dopaminergic neurons and the presence of Lewy bodies. Although sporadic in most cases, various hereditary forms of PD have been identified. Among the inherited forms of PD mutations in the Lucine Rich Repeat Kinase 2 (LRRK2/Dardarin) seem to represent the most common one affecting up to 1-6% of all PD patients. LRRK2 is a conserved gene with homologs found in vertebrates, insects and worms. The encoded protein of 2527 amino acids harbors several functional domains: N-terminal leucine-rich repeats, a Ras in complex proteins domain that belongs to the Ras/GTPase superfamily, a C-terminal of Roc domain, a tyrosine kinase catalytic domain and a WD40 domain. PD associated mutations have been found in all functional domains of the protein. Due to the dominant effect of these mutations on degeneration of dopaminergic neurons they are most likely gain-of-function mutations.
We identified the *Drosophila* LRRK2 homolog and analyzed its biological function. We found that *Drosophila* Lrrk activates the ERK signaling cascade *in vivo*. In addition we show that neuronal expression of *Drosophila* Lrrk is toxic to dopaminergic neurons of the fly.

# Molecular mechanisms and neuroprotection in cellular models of Parkinson's disease

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Cytoplasmatic protein oligomerisation and aggregation are a hallmark of several neurodegenerative diseases and have been suggested to be causative for neuronal cell death. α-synuclein is a natively unstructured 140-aa protein that can adopt a β-stranded conformation in aggregated, fibrillar forms that accumulate in ubiquitinated protein inclusions and dystrophic neurites in Parkinson's disease. Several mutations and multiplications of the α-synuclein gene have been shown to cause hereditary forms of Parkinson's disease. On a molecular level, neuronal cell death in neurodegenerative diseases has been linked to chronic inhibition of mitochondrial complex I and the dysfunction of cellular 'quality control mechanisms'. These include dysfunction of the cellular folding machinery and protein degradation through the ubiquitin-proteasome pathway.

Here we introduce assays and biosensors that allow the visualization and quantification of α-synuclein oligomerisation, ubiquitination and chaperone function. α-synuclein oligomerisation was measured by the use of fusion constructs with optimised fluorescent proteins and linker sequences by quantification of 'Förster Resonance Energy Transfer'(FRET). Ubiquitination of α-synuclein was quantified by an imaging-based screening paradigm that measures FRET between fluorescently tagged α-synuclein and a novel, optimised ubiquitin fusion construct.

Recently, we demonstrated that BAG1-mediated neuroprotection is dependent on its interaction with Hsp70, resulting in an increased Hsp70 foldase activity. Stable overexpression of BAG1 in the neuroblastoma cell line SH-SY5Y protected the cells from dopamine and 6-hydroxydopamine induced cell death. Furthermore, utilising a transient transfection model, BAG1 also protected SH-SY5Y cells from α-synuclein induced cell death.
In summary, we present BAG1 as a potential molecule for neuroprotection in aggregopathies like PD.

# Absence of alpha-synuclein from the nervous system reduces axonal degeneration

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Synucleins are cytosolic proteins widely expressed in the brain and are located in nerve terminals. In particular, alpha-synuclein is known to be a major component of Lewy bodies (e.g. Parkinson's disease) and in the amyloid plaques of Alzheimer's disease.
As an experimental approach we used a model for Wallerian degeneration in order to investigate the outcome of either the absence or overexpression of alpha-synuclein in the peripheral nervous system. Wallerian degeneration in the peripheral nervous system is characterized by rapidly collapsing axons, degrading myelin sheaths and invading macrophages. After transection of sciatic nerves in mice of different strains, we examined the remaining myelin sheaths and axons in the distal nerve part. Further, we counted the typically invading macrophages, their size and the amount of phagocytozed myelin. All data were evaluated on knockout mice* for alpha-synuclein, on transgenic mice** overexpressing a four-fold amount of normal protein and on appropriate littermate controls. Further controls were C57/Bl6 mice from different suppliers which had been used for background breeding of the transgenic or knockout mice. In addition, we examined a C57/Bl6 mouse strain (Harlan) with a spontaneous deletion of the alpha-synuclein gene.
Five sciatic nerves of each strain were evaluated and all mouse strains showed normal signs of Wallerian degeneration, such as invading macrophages and degraded myelin sheaths. We could find significantly higher numbers of macrophages in C57/Bl6 mice of Harlan (displaying no alpha-synuclein in their genome). Low numbers of macrophages were detected in transgenic animals and knockouts. In comparison to the latter, the macrophages of Harlan-B6 mice are significantly smaller and phagocytozed lower amounts of myelin. Like in transgenic mice, we found low numbers - but of large-sized - macrophages in knockout animals. These findings make it likely that deletion of this protein from the genome does not evoke the same effects as the spontaneous mutation. Therefore we conclude that the presence or absence of alpha-synuclein has no direct effect on the migration of macrophages.
Further we found a significantly higher amount of preserved myelin in knockout mice as well as in Harlan-B6 mice but lower myelin rates in transgenic mice and the two control B6-strains. Evaluation of preserved axons even revealed a double number of axons in alpha-synuclein knockouts and also in HarlanB6 mice, in contrast to overexpressing animals.
Our data suggest that neither the presence nor absence of alpha-synuclein prevents the symptoms of Wallerian degeneration in peripheral nerves in general. However, the absence of this protein from the nervous system seems to have beneficial effects resulting in moderate but significant axonal preservation and survival. Therefore these data may contribute new insights into the mechanisms of alpha-synucleinopathies or Lewy body disease.

* OM Schlüter et al., Neurosci. 118: 985-1002
** PJ Kahle et al., J. Neurosci. 20(17): 6365-6373

# Proteasome inhibition failed to act as a new animal model of Parkinson' s disease in Wistar rats, but led to changes in tyrosine hydroxylase contents of olfactory bulb and adrenal medulla

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The existing animal models of Parkinson disease (PD) are characterized by several drawbacks, like unphysiological progress and a lack of specificity of cytotoxicity.
Recently McNaught et al. (2004, Ann Neurol, 56: 149-162) suggested that the systemic administration of proteasome inhibitors to rodents and the following disturbance of the ubiquitin dependent protein degradation could be a new model with typical progredient ongoing dopaminergic cell loss in the substantia nigra (SN) and behavioural deficits without the disadvantages above mentioned.
For own proteomic approaches and transplantation studies we intended to establish this new PD model. According to McNaught et al. (2004) we injected the synthetic proteasome inhibitor Z-lle-Glu(OtBu)-Ala-Leu-al (PSI 6x 3mg/kg over 2 weeks) to 8 Wistar-rats. Sham-rats (n=8) were treated with the vehicle (70% ethanol) only. Body weight was monitored over the whole experimental time. Analysis of locomotor dysfunctions was performed by footprint analysis for PSI-, EtOH-, totally untreated control rats (n=9) and unilaterally 6-OHDA lesioned rats (n=9, classical PD-model). Serial brain sections of PSI- and EtOH-rats were stained immunhistochemically against tyrosine hydroxylase (TH, TH-ICC), neuronal nuclei antigen (NeuN), glial fibrillary acidic protein (GFAP) and activated microglia (OX42). Standard histological stainings (HE and Nissl) were also performed to detect Lewy-body like structures and loss of dopaminergic neurons. Moreover, we examined the effect of PSI on the peripheral catecholaminergic organs adrenal medulla and the carotid body by TH-ICC, HE-staining and quantitative analysis. Densitometric measurement of TH-ICC was evaluated in the caudate putamen complex (CPu), SN, olfactory bulb and adrenal medulla. Volumetric measurements of the whole adrenal gland were performed.
Animals showed no significant differences in bodyweight. In the footprint analysis PSI-treated rats showed no significant differences compared to untreated and EtOH-treated animals, whereas all these animals performed significantly wider footsteps than 6-OHDA lesioned rats. Densitometric assessments for TH-contents in the CPu and the SN provided no evidence for dopaminergic deafferentiation, whereas a small significant increase of TH in the glomerular layer of the olfactory bulb and the adrenal marrow could be detected in PSI-treated rats. Nevertheless, stereological examination delivered no evidence for alterations in the volume of adrenal marrow or adrenal cortex. We also found no obvious differences in morphology and TH-contents of the carotid bodies.
Here, we could not replicate the findings of McNaught et al. (2004), our results are in line with a number of reports that also could not observe the effects described by McNaught et al. Because of several contraire reports, now there is a vital ongoing discussion whether proteasome inhibition is a breakthrough for animal models of Parkinson's disease or not.

# Influence of short-term deep brain stimulation of the subthalamic nucleus on Homer1 gene expression in rats with unilateral 6-hydroxydopamine lesion.

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Parkinson's disease is characterized by striatal dopamine deficit due to a progressive loss of nigrostriatal dopaminergic neurons leading to severe motor disturbances. The subthalamic nucleus (STN) which is driving the output activity of basal ganglia structures has been implied in the pathophysiology of PD. Therefore, it has become a target of deep brain stimulation (DBS). Although widely used clinically, little is known about the mechanisms at the molecular and cellular level. To elucidate those mechanisms, we investigated the effects of STN-DBS on gene expression levels in 6-hydroxydopamine (hemiparkinsonian) rats after short-term stimulation. We implanted microelectrodes into the STN and stimulated for 3 hours with pulses 60µs duration, 500µA intensity and a repetition frequency of 130Hz. We found a downregulation of *homer1* by 3h DBS in 6-hydroxydopamine lesioned rats in a microarray experiment. Here we show the regulation of *homer1* in the basal ganglia of the rat brain tissue of lesioned rats after 3h hours of DBS by *in situ* hybridization. *Homer1* encodes the proteins Homer1a, b and c which are responsible for localization and function of group I metabotropic glutamate receptors (mGluR). The downregulation of *homer1* may account for a DBS-induced decrease in the activity of output basal ganglia downstream from STN.

This work has been supported by a fellowship of the Landesgraduiertenförderung Mecklenburg-Vorpommern and a grant by the Federal Ministry of Education and Research (FKZ 01ZZ0108).

# Symposium

## S18: Compositionality: Neuronal Basis of Complex Behavior

***Theo Geisel and Moshe Abeles, Göttingen and Ramat Gan (Israel)***

### Slide

**S18-1** Opening remarks for Symposium 18
*Theo Geisel and Moshe Abeles, Göttingen and Ramat Gan (Israel)*

**S18-2** The compositional nature of drawing and neural coding
*Moshe Abeles, Ramat Gan (Israel)*

**S18-3** Syntactic rules governing the stringing of behavioral primitives
*Mina Teicher, Ramat Gan (Israel)*

**S18-4** Language Models based on Hebbian Cell Assemblies
*Thomas Wennekers, Plymouth (UK)*

**S18-5** On the compositionality of complex movements
*Tamar Flash, Moshe Abeles, Felix Polyakov, Rotem Drori and Eran Stark, Rehovot (Israel) and Ramat Gan (Israel)*

**S18-6** Spike-timing dependent plasticity in balanced random networks
*Markus Diesmann, Wako City, Saitama (J)*

**S18-7** Self-organization of behavioral primitives and rules for concatenating them
*J. Michael Herrmann and Theo Geisel, Göttingen*

### Poster

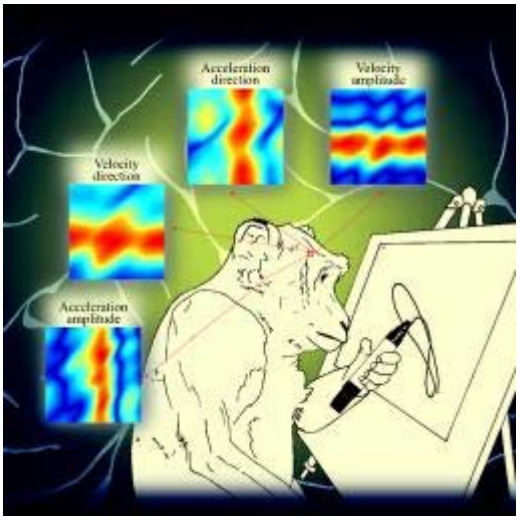
**TS18-1C** A composition machine for complex movements
*S. Schrader, A. Morrison and M. Diesmann, Freiburg i.Br. and Tokyo (J)*

**TS18-2C** Single-cell-resolution maps of the cerebral metabolism in the rodent CNS show regional differences in neuronal and astrocytic contribution to $K^+$ and energy metabolism.
*H. Lison, E. Budinger, H. Scheich and J. Goldschmidt, Magdeburg*

Introductory Remarks to Symposium 18

# Compositionality: Neuronal Basis of Complex Behavior

Theo Geisel and Moshe Abeles, Göttingen and Ramat Gan (Israel)




Compositionality may be manifested either by stringing components along time, such as in speech, or simultaneously, such as in understanding a visual scene. In motor behavior these two forms are found abundantly. Concatenating simpler strokes generates complex drawing motions. Picking up an object requires simultaneous coordination of three time evolving motions: the arm reaching, the hand orientation, and the finger shaping. Like in language, not all possible combinations are utilized. The rules, which stroke may be concatenated to which, constitute the syntax of action. Some speakers of the symposium take the point of view that there are neural mechanisms that are used to bind the elements of a composite movement to each other. These are referred to as the binding mechanisms. In voluntary movements, rather than planning and producing a limb motion anew for each individual movement, there is evidence to suggest that the system chooses from a limited repertoire of motor primitives. Several muscular co-activations (synergies) may be concatenated to generate simple units of movements. Several such strokes are concatenated to generate more complex shapes etc. While manipulating objects, the arm, the hand and the fingers are combined in a coordinated manner to perform a meaningful action.

In this symposium we present the current research on compositionality as a neuronal basis of complex behavior with contributions spanning the range of relevant disciplines. From the psychophysical level, via electrophysiology, to the level of network modeling and behavioral bio-robotics and prosthetics. The research on the motor system is contrastet with current research on the neurobiological basis of language. The aim of this symposium is to critically review the idea of compositionality as a universal principle on the basis of the latest findings in both fields of research. It is our hope that with this group of experts, the comparison of experimental results and neurobiological models of language and motor behavior will contribute to a clarification of concepts and the development of research strategies.

# The compositional nature of drawing and neural coding

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The higher levels of function show the property of "compositionality". We compose little strokes into letters, letters into words, words into sentences. At each level there are many more distinct items (the number of words is much higher then the number of letters), however much-much less then the number of possible combinations (The number of legitimate 3 character words is but a miniscule fraction of all possible 3 character combinations).
In spoken language, composition is carried out serially in time with possible partial overlap between components. The same may be true for voluntary movements of the arm and hand. Thus, studies of monkey arm motion may reveal a small set of basic movements which are concatenated into longer sequences (gestures). Here, too, the number of gestures which are actually performed, is expected to be much smaller then the total number of possible combinations of the basic movements.
This issue was studied in monkeys which were trained to generate drawing-like motion continuously and in which activity of motor and premotor single units were recorded through eight individually movable micro electrodes.
On the behavioral level, motions were parsed into little "strokelets" and the regularities of concatenating these were revealed by using information bottleneck. Starting with ~60 types of strokelets, we ended with ~10 distinct classes of motions with clear preference of transition among specific pairs of strokelets.
On the electrophysiological level, we first studied what is coded by activity of neurons in the motor cortices. By using partial correlation methods we were able to distinguish between the contributions of several movement features to the firing rates of the neurons. The 5 features studied were the position of the hand, the amplitude of the drawing-velocity vector, the direction of the drawing-velocity vector, the amplitude of the acceleration vector, and the direction of the acceleration vector. We found neurons whose rate change specifically with each of these 5 features. The number of neurons coding for mixtures of parameters were much less then expected by chance. In addition, the coding may change with behavioral state (drawing versus goal directed straight motion). This means that deciphering the cortical activity must use different decoding mechanisms in different states.
One simple mechanism to achieve such state-dependent decoding is by using time of spikes as a tag on the single neuron activity. This requires the ability to control the precise timing of firing an action potential. By analyzing the precision of spike timing in relation to the drawing shape we found that in most cases the spiking times had a precision of a few ms. In the best case it reached less then 0.5 ms. Thus, it is possible to view the state-dependent properties of any given neuron in different coding scheme as being due to participation in different cell assemblies in which the specificity is dictated by the exact timing relations among the firing of the participating neurons.

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# Syntactic rules governing the stringing of behavioral primitives

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I shall describe two experiments that were the ground for a statistical analysis that proved spike synchronization in behaving animals up to 3 miliseconds.

# Language Models based on Hebbian Cell Assemblies

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We demonstrate how associative neural networks as standard models for Hebbian cell assemblies can be extended to implement language processes in large-scale brain simulations [1]. To this end the classical auto- and hetero-associative paradigms of attractor nets and synfire chains (SFCs) are combined and complemented by "conditioned associations" as a third principle which allows for the implementation of complex graph-like transition structures between assemblies. We show example simulations of a multiple area network for object-naming, which categorises objects in a visual hierarchy and generates different specific syntactic motor sequences ("words") in response. The formation of cell assemblies due to ongoing plasticity in a multiple area network for word-learning is studied afterwards. Simulations show how assemblies can form by means of percolating activity across auditory and motor-related language areas, a process supported by rhythmic, synchronised propagating waves through the network.

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[1] Thomas Wennekers, Max Garagnani, and Friedemann Pulvermueller (2007)
Language Models based on Hebbian Cell Assemblies. Journal of Physiology
(Paris), in press.

# On the compositionality of complex movements

Tamar Flash[1], Moshe Abeles[2,3], Felix Polyakov[1], Rotem Drori[2] and Eran Stark[2]

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Different models for the description of complex arm movements have been developed. Some are based on the notion that the brain wishes to optimize an objective function, e.g. motion smoothness during the generation of point-to point and drawing movements, while others focus on the local characteristics of the movements: the relation of movement velocity to path curvature. I will discuss how these two approaches can be combined in order to investigate movement compositionality, the idea that more complex movements are constructed from elementary motivon primitives. I will also discuss a new approach to the analysis of arm trajectories based on the use of geometrical differential tools demonstrating how these tools can be applied to compositionality. Finally I will present recent analysis of the activities of neural recordings, lending support to our hypothesis concerning movement primitives which were based on differential geometrical analysis.

# Spike-timing dependent plasticity in balanced random networks

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The balanced random network model attracts considerable interest because it explains the irregular spiking activity at low rates and large membrane potential fluctuations exhibited by cortical neurons in vivo. Here, we investigate to what extent this model is also compatible with the experimentally observed phenomenon of spike-timing dependent plasticity (STDP). Confronted with the plethora of theoretical models for STDP available, we re-examine the experimental data. On this basis we propose a novel STDP update rule, with a multiplicative dependence on the synaptic weight for depression, and a power law dependence for potentiation. We show that this rule, when implemented in large (100,000 neurons) balanced networks of realistic connectivity and sparseness (10,000 synapses per neuron), is compatible with the asynchronous irregular activity regime. The resultant equilibrium weight distribution is unimodal with fluctuating individual weight trajectories, and does not exhibit development of structure. We investigate the robustness of our results with respect to the scaling of the depressing increments. We introduce synchronous stimulation to a group of neurons, and demonstrate that the decoupling of this group from the rest of the network is so severe that it cannot effectively control the spiking of other neurons, even those with the highest convergence from this group.

# Self-organization of behavioral primitives and rules for concatenating them

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We study the hypothesis that elementary movements are the result of a self-organized process which connects a generic controller and an adaptive internal model with the body and the environment. The self-organization is achieved by a tendency to reconcile two opposing processes, namely the prediction of the sensory effects of the movements and the causal coherency of the internal representation of sensory information. As a result behaviors are selected which minimize the complexity gap between model predictions and the actual behavioral feedback. In order to provide evidence for the hypothesis, we consider both robotic model systems as well as data from psychopysical experiments in humans. The schemes is further expanded by the adoption of self-supervised learning schemes of higher order that are based on the emerged low-level movement representations.

# A composition machine for complex movements

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Synfire chains that are mutually and weakly interconnected with excitatory synapses are capable of synchronizing and thus aggregating their traveling waves [1,2]. This process is considered to be a candidate neural mechanism for binding and compositionality, potentially underlying higher brain functions such as pattern recognition, memory retrieval, movement preparation and movement execution [4,5]. It has been shown that a layered structure of synfire chains can indeed provide the required building blocks [2]. Based on these principles, we present a prototypical but complete composition machine that composes complex movements out of movement primitives to generate scribbling trajectories. The system is realized by a spiking neuronal network model using the simulation tool NEST [3].

The movement trajectory is obtained by integrating the population vector of the directionally tuned motor neurons constituting the output of the network model. Depending on the spatio-temporal patterns applied to the synfire chains of the input layer, a sequence of movement primitives is activated which generates a movement trajectory. The length of a top level chain determines the duration of activation of the respective primitive. Autonomous activity in the lower layer generates a random sequence of primitives resulting in scribbling-like behavior. Thus, we have formulated a model in which we can test algorithms for the detection and categorization of primitives and conservation laws on the level of movement trajectories (behavior) as well as on the level of spikes (electrophysiological). Furthermore we can now derive predictions for the relationship between properties of the spiking activity (e.g. rate, synchrony) and properties of the movement trajectory (e.g. direction, acceleration).

**Figure.** Movement generation with synfire chains.
A) Various reaching task trajectories generated by the same network of synfire chains. The precise shape is determined by the relative timing of the initial stimuli activating the movement primitives.
B) Scribbling-like trajectory generated by the same network as in A operating autonomously.

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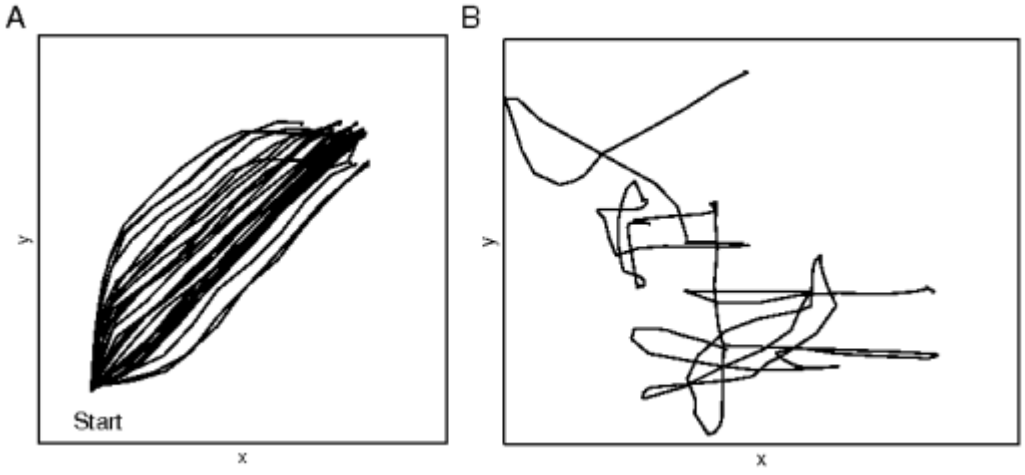

# Single-cell-resolution maps of the cerebral metabolism in the rodent CNS show regional differences in neuronal and astrocytic contribution to $K^+$ and energy metabolism.

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Auditory Learning and Speech, Leibniz-Institute for Neurobiology, Brenneckestr. 9, Magdeburg, Germany

In awake humans and animals in the resting state the cerebral metabolic rates of glucose and oxygen differ in different brain regions. The degree to which energy consuming processes in excitatory neurons, inhibitory neurons or astrocytes, and in different subcellular compartments such as axons or dendrites contribute to these regional differences in brain energy metabolism are largely unknown since no methods existed for mapping, cell-by-cell, oxygen- or glucose-consumption in the brain.

In order to gain insight into the patterns of brain energy metabolism and neuronal activity in the resting state at the single-cell level we here made use of a recently developed technique, thallium autometallography, that makes it possible to non-radioactively map patterns of potassium ($K^+$) uptake in the brain of experimental animals. We injected awake behaving unstimulated rodents with the $K^+$-probe thallium ($Tl^+$) and mapped the distribution of the tracer in the central nervous system.

So produced images are highly complex and show distinct differences in the distribution of the tracer at the regional, cellular and subcellular level throughout the CNS. In many brain regions, e.g. cerebral cortex and brainstem, the tracer is found predominantly in the somatodendritic compartments of neurons. Since neuronal $K^+$-uptake is mediated to a large degree by the $Na^+/K^+$-ATPase this distribution argues for predominant postsynaptic energy consumption. In cerebral cortex also exist regional differences with respect to neuronal uptake patterns in different cortical layers and cell types. In primary sensory areas for example uptake seems highest in inhibitory interneurons in layer IV. In the Cerebellum inhibitory interneurons also have a high $Tl^+$ uptake, but uptake in the GABAergic Purkinje cells is very low. In other regions, e.g. certain thalamic nuclei, neuronal $Tl^+$ uptake is generally low despite high glucose consumption, and astrocytic uptake is much more pronounced.

We conclude from this highly complex distribution that, in the mammalian CNS, no general rules exist with respect to the metabolic demands of neuronal vs. astrocytic processing or of excitation vs. inhibition. Instead, region-specific intricate networks seem to exist, in which the metabolic demands of the constituent elements vary in different ways depending on the different modes of neuronal processing

# Symposium

## S19: Spatial Cognition: From Rodents to Humans

***Hanspeter A. Mallot and Johannes Thiele, Tübingen***

### Slide

**S19-1** Opening remarks for Symposium 19
*Hanspeter A. Mallot and Johannes Thiele, Tübingen*

**S19-2** Reproducing self-motion from memory
*Stefan Glasauer, Erich Schneider, Alexandra Stein, Anna Günther, Yuri P. Ivanenko and Thomas Brandt, München and Rome (I)*

**S19-3** What do hippocampal place cells code?
*Christian Hölscher, Coleraine (UK)*

**S19-4** Representation of objects and landmarks in the human brain
*Gabriele Janzen, AH Nijmegen (NL)*

**S19-5** Rat navigation and wayfinding in virtual environments
*Hanspeter A. Mallot, Alexander Schnee, Johannes Thiele and Hansjürgen Dahmen, Tübingen*

**S19-6** Cognitive strategies and mechanisms in human path planning
*Jan Malte Wiener, Paris (F)*

**S19-7** Update your environment: Characterizing the neural systems mediating path integration and spatial updating in humans
*Thomas Wolbers, Jan Wiener, Hanspeter Mallot, Jack Loomis, Mary Hegarty and Christian Büchel, Santa Barbara (USA), Paris (F), Tübingen and Hamburg*

### Poster

**TS19-1C** Unsupervised Learning of Place Cells and Head Direction Cells with Slow Feature Analysis
*M. Franzius, H. Sprekeler and L. Wiskott, Berlin*

**TS19-2C** Hippocampal Activation in the Zebra Finch During Learning and Recall of a Spatial Memory Task.
*U. Mayer, Y. Hardes, L. Hertel, S. Watanabe and HJ. Bischof, Bielefeld and Tokyo (J)*

**TS19-3C** Hippocampal ca3 subfield cell degeneration induces spatial cognition dysfunction in kainic acid model of epilepsy;correlation of anatomy and behaviour.
*M. Sajad, N. Srivastava, K. Seth and AK. Agrawal, Lucknow (India)*

**TS19-4C** Coding of interaural time sensitivity of concurrent sounds
*I. Siveke, C. Leibold and B. Grothe, Muenchen and Berlin*

**TS19-5C** The effect of experimental neoplastic disease in rats on motor and spatial behavior.
*M. Szulc, M. Wieczorek, M. Medon, L. Luczak, A. Rybarczyk, M. Gorka, S. Michalak, W. Ambrosius, K. Osztynowicz and W. Kozubski, Poznan (PL)*

**TS19-6C** Psychophysics and Electrophysiology in Virtual Environments
*HA. Mallot, A. Schnee, H. Dahmen, A. Fenton, E. Kelemen, HY. Kao and C. Hölscher, Tübingen, Brooklyn (USA) and Coleraine NI (UK)*

**TS19-7C** Do rats use optical flow for motion control ?

*J. Thiele, Tübingen*

Introductory Remarks to Symposium 19

# Spatial Cognition: From Rodents to Humans

Hanspeter A. Mallot and Johannes Thiele, Tübingen

Spatial cognitive performance includes a wide variety of tasks that require memory and processing structures of different complexity. Examples include path integration (use egomotion information to return to a starting place), place recognition (landmarks, or local position information), and path planning (place adjacencies, cognitive map, regions). In recent years, the breakdown of spatial cognition into the mechanisms underlying these different tasks has paved the way for a better understanding of similarities and differences in animal and human spatial behaviour and spatial cognition. The symposium will give an overview of different mechanisms in spatial cognition and their implementation in different species.

The perception of egomotion by visual, vestibular, or proprioceptive modalities and its integration into a position estimate are among the most basic sources of information in spatial cognition. In insects, the underlying representation is thought to be the “homing vector”, i.e. a two-component vector representing distance and direction to the home. In humans, egomotion information can also be used for returns after excursions (triangle completion task), or for the reproduction of a distance travelled previously. The assumed mechanisms for path integration in insects and humans differ in two respects: first, while update is assumed to be continuous in insects, it is thought to happen at discrete times in humans. Second, velocity profiles are also remembered in humans. In the symposium, we will discuss insect path integration together with evidence from human metric navigation performance.

Place recognition has to be based on sensory, often visual information that can be obtained when looking from a particular place. This notion is much more general than the familiar idea of a landmark, where places are recognized from well defined, namable objects. Indeed, fMRI studies have recently revealed a difference between object recognition in general, and landmark definition, where landmarks haven been defined by there role in marking a decision place along a route. Other problems in place recognition include the question which cues are actually employed in recognizing a place and whether places are represented in the brain as purely spatial items or as more general “situations” that also include values for various behaviours. The latter issue will be discussed in terms of hippocampal place fields of the rat.

Wayfinding and planning of multistep routes in cluttered environments requires an explicit or declarative type of spatial memory that, following the tradition of Tolman and O’Keefe & Nadel is usually called a cognitive map. Surprisingly, clear evidence of route navigation and route planning in animals is rare. Virtual reality has recently been used to investigate rat navigation performance in large scale environments. The simplest version of a cognitive map can be looked at as a network of place representations and motor programs effecting travel from one place to an adjacent one. The representation of additional types of knowledge in more advanced cognitive maps remains an open question. Recent experiments with route planning in humans suggest that cognitive maps are organized in a hierarchical way, representing not only places but also regions at a higher, or coarser, level.

# Reproducing self-motion from memory

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Navigation involves various skills, including updating of one's position and orientation during self-motion. If positional cues such as landmarks are not available, navigation is based on information about self-velocity, which is transformed to position or orientation by a process called path integration. One of the most basic navigation tasks, homing after an outbound travel, can be solved by reproducing the outbound travel in the opposite direction. Experiments on reproducing imposed self-motion without positional cues showed that not only final distance or angle of motion, but also the temporal profile are reproduced. On the basis of this observation, it has been suggested that memorizing self-velocity is sufficient for self-motion reproduction, thus eliminating the need for path integration. Reproduction errors during this task have been attributed to sensory inputs, inaccurate memorization of the motion variable, or motor errors. However, another possible source of error has so far been neglected. The internal time base for path integration or movement memorization may be distorted, and thus not reflect physical time. Since additional cognitive load has previously been shown to affect subjective estimation of duration, we used a dual task paradigm during either the stimulation or reproduction phase of different self-motion reproduction tasks. The cognitive load changed the distance of reproduced self-motion by about 25% depending on whether the mental task was performed while experiencing or reproducing the motion. While imposed velocity was reproduced accurately in all conditions, reproduced movement duration was affected in the same way as distance. This result implies that for the perception of distance travelled, perceptual space and time are closely interrelated. Since, so far, our results were consistent with memorization of either self-velocity or self-displacement, the latter being derived by path integration, we performed two additional experiments testing whether path integration was also affected by the dual-task. In the goal-directed locomotor task, blindfolded subjects had to walk towards a previously seen target. In the self-rotation targeting task, subjects pressed a button upon reaching a desired angular displacement. In both experiments, produced displacement and movement duration increased when performing a dual task. Thus, not only reproduction of self-motion, but also path integration is systematically affected by dual tasks. Our findings are consistent with shared processing of temporal and spatial information. Comparison of recent findings on the neural basis of path integration in rodents with hypotheses on interval timing suggest a close relation of both mechanisms. Thus, a possible reason for the similarity of the effect of dual tasks on temporal and spatial perception may be the equivalence of neural mechanisms.

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# What do hippocampal place cells code?

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Single cell recording in the hippocampus of freely moving animals has been analysed for several decades. One prominent feature of their firing properties is that they fire when the animal is in a particular location within the navigational space. Because of this, they have been named 'place cells' and the concept had been proposed that they form part of a 'cognitive map' located within the hippocampus (O'Keefe and Dostrovsky, 1971).
Since then, a lot of information has been gathered concerning the type of information that is encoded by these hippocampal neurons. It was found that apart from spatial information, directional information, temporal, and procedural information is encoded (Hölscher et al., 2004; Gengler et al., 2005). While these information qualities are found in individual neurons, networks of neurons that encode different qualities evolve that can associate multi-dimensional aspects of the spatial environment that the animal navigates in (Leutgeb et al., 2005). Additionally, sequences of events are encoded, and specific networks are called up at their respective position in time and space when the animals navigates a maze (Louie and Wilson, 2001). Furthermore, procedural information is found in a subset of cells that can be used for path integration in environments that do not allow visual navigation (Hölscher et al., 2004).
Recordings in areas of the temporal cortex revealed that most of the spatial information seen in place cells is already available in areas that project to the hippocampus, showing that 'place cells' are not constructed in the hippocampus (Frank et al., 2000). Further studies showed that the entorhinal cortex contains cell populations that are active at discrete areas of the environment in a highly regular and evenly spaced fashion (Hafting et al., 2005). These 'grid cells' contain valuable information about spatial metrics that is fed to the hippocampal formation and can underlie path integration computations.
Anatomical studies show that the hippocampal formation receives object information from temporal visual areas ('what') as well as spatial information from a separate information pathway ('where') (Mishkin et al., 1983). These two information categories are associated to larger neuronal assemblies that contain spatial ('where'), temporal ('when'), procedural ('how'), and object identity ('what') information (Hölscher, 2003).
The picture that emerges from this is that the hippocampus is not simply a 'cognitive map' which is read by the animal during navigation, but an extended association system that is used to combine information about discrete objects and events in time and space, thereby supporting the formation of sequential 'episodic' memories.

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# Representation of objects and landmarks in the human brain

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Simply perceiving our spatial environment and moving in a certain direction is not enough to successfully navigate through space. It is necessary to know which route to take to reach the desired destination. Therefore, crucial locations need to be stored in memory. Objects along a route can serve as such crucial landmarks when they are placed at locations relevant for successful wayfinding, thus, intersections in environments where a decision about the further route has to be taken (decision points). In recent functional magnetic resonance imaging (fMRI) studies, we have obtained evidence that the parahippocampal gyrus selectively responds to the navigational relevance of landmarks (Janzen & van Turennout, 2004; Janzen, et al., 2006). The parahippocampal gyrus has been implicated in the encoding of visual scenes and in object-place associations (e.g. Maguire, et al., 1998). The neural marking of navigationally relevant objects observed in bilateral parahippocampal gyrus is independent of attentional processes suggesting automatic retrieval of the navigational information. Relevant objects were represented in the parahippocampal gyrus immediately after learning a route through a maze. Moreover, the effect became even stronger after a delay of one day, suggesting consolidation of navigational relevant information.

It has been shown that the medial temporal lobe, including the hippocampus and the parahippocampal gyrus, is crucially involved in memory consolidation (e.g. Walker, 2005). We investigated memory consolidation of navigationally relevant landmarks in the medial temporal lobe after route learning. We observed an effect of memory consolidation in the hippocampus that interacted with navigational ability. Increased activity in the hippocampus for remote objects was observed in good navigators only (see Epstein, et al. 2005). The results provide evidence for a contribution of memory consolidation to successful navigation.

The observed automatic changes in the neural representation of objects show rapid learning after only one exposure to a spatial maze and remain unchanged with additional maze practice. The automatic and long-lasting storage of navigationally relevant objects can provide a neural mechanism underlying successful wayfinding and navigation.

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# Rat navigation and wayfinding in virtual environments

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Rat spatial behavior in large-scale environments with controlled visual stimulation has been studied in a virtual reality setup (Hölscher et al., J. Expt. Biol. 2005). The setup consists of an air-cushioned running ball and a panoramic projection unit. The rat is fixated on top of the ball, touching it with its paws. Translatory running movements are transformed into ball rotations about horizontal axes which are measured by an optical system and transferred to the graphics computer. The rat is free to turn on the spot, while ball rotations about the vertical axis are blocked. Translation signals are used to control a virtual reality graphics system generating panoramic visual views of virtual environments. The panoramas are projected from the inside into a toroidal screen, using suitable mirror systems. The rat received reward with sugar solution delivered from a brass tube in front of the rat's mouth.

Initial experiments used repetitive environments composed of a ground plane and cylindrical objects hanging from a ceiling. Thus, the rat would not experience cue conflicts from visually running into obstacles without tactile feedback. Reward was given whenever the rat stepped under one cylinder. Results show that rats can be trained to approach the virtual cylinders. Running distances in well trained rats during a session of 10 minutes are in the order of hundreds of meters.

In recent studies, these experiments have been extended in three directions. Spatial memory has been tested in a virtual watermaze paradigm using a square room with walls covered by stripe pattern of four different orientations. The rat was released in the center with random orientation and trained to receive a reward under a cylinder presented in one corner of the room. During training, the cylinder was gradually reduced in size until it completely vanished. In the test phase, rats searched for non-existing reward in the correct quadrant of the room. This result indicates that spatial learning takes place in virtual environments.

Visual object discrimination was studied using a circular arrangement of 12 cylinders, covered with dark or bright surfaces in an alternating sequence. Rats were released in the center of the ring and rewarded either under the dark or he bright cylinders. We found a spontaneous preference for bright cylinders but training to dark cylinders was possible, Psychometric functions for brightness discrimination have been measured by gradually reducing the difference between bright and dark cylinders (see Schnee et al., this meeting).

Visual course stabilization by goal tracking and optic flow was tested in a virtual environment with added optic flow. While approaching a rewarded cylinder, rats were continuously displaced sideways. The only way to detect this displacement in the virtual environment is optic flow. If rats use optic flow information, displacement can be compensated and straight paths should result. If optic flow is not used, curved paths are to be expected (cf. Warren et al, Nature Neuroscience 2001). We found curved and straight approach paths in different environments, but no clear correlation with the type and visual richness of the environment (Thiele et al., this meeting).

# Cognitive strategies and mechanisms in human path planning

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For large numbers of targets, path planning can be a very complex and computationally expensive task. Human navigators, however, usually solve path planning tasks fastly and efficiently. Here a series of experiments is presented that studied human path planning performance as well as the cognitive strategies and processes involved.

25 places were arranged on a regular grid in a large room. Each place was marked by a unique colored symbol. Subjects were repeatedly asked to solve traveling salesman problems (TSP), i.e. to find the shortest closed loop connecting a given start place with a number of target places. For each trial, subjects were given a so-called 'shopping list' depicting the symbol of the start place and the symbols of the target places. While the exact TSP is computationally hard, approximate solutions can be found by simple strategies such as the nearest neighbor strategy (NN) which has been repeatedly reported to be involved in animal and human path planning behavior. One experiment tested whether humans employed the NN strategy when solving TSPs. Results showed that subjects outperformed the NN strategy in cases in which the NN algorithm did not predict the optimal solution, demonstrating that the NN strategy is not sufficient to explain human path planning behavior. As a second possible path planning strategy a region-based approach was tested in a second experiment. When optimal paths required more region transitions than other, sub-optimal paths, subjects preferred these sub-optimal paths. This result suggests that subjects first planned a coarse path on the region level and then refined this path plan during navigation. Such a hierarchical planning scheme allows for the reduction of both, computational effort and memory load during path planning while still resulting in reasonably short paths.

To specifically test for the relevance of spatial working memory (SWM) and spatial long-term memory (LTM) for path planning, the number of taget places (ranging between 4 and 9 targets) as well as the mode of presenting targets was varied in further experiments. In one experiment the target places were directly marked in the environment such that no memory was required (no memory condition); in a second experiment, the symbols of the target places of the TSPs were depicted on so-called 'shopping lists', which required to first locate the target symbols in the environment and to generate a spatial working memory of the their positions before planning a path (SWM condition); in a third experiment, additionally the symbols of the 25 places were hidden during path planning and navigation. To solve the TSPs, subjects therefore had to recall the exact positions of the symbols, learned in a initial training phase, from long-term memory (SWM & LTM condition).

As expected, results showed that subjects' path planning performance, i.e. the quality of the chosen paths, systematically decreased with increasing TSP size. Furthermore, performance between the latter three experiments differed systematically. Performance was best in the 'no memory' condition and worst in the 'SWM & LTM' condition. This result suggests the usage of different path planning strategies according to the specific memory demands.

# Update your environment: Characterizing the neural systems mediating path integration and spatial updating in humans

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Path integration, the ability to sense self-motion for keeping track of changes in orientation and position, and spatial updating, the constant updating of the positions of surrounding objects as we move through an environment, constitute fundamental mechanisms of spatial navigation and keystones for the development of cognitive maps. Contrary to abundant findings in rodents, the neural foundations are not well understood in humans. Here we show that human participants can reliably extract self-motion information from optic flow in a virtual environment. Functional magnetic resonance imaging revealed visual path integration to rely upon the dynamic interplay of self-motion processing in human MST, higher-level spatial processes in the hippocampus and spatial working memory in medial prefrontal cortex. The additional requirement to compute changing object coordinates during spatial updating evoked context-dependent contributions of intraparietal and inferior temporal areas. Most importantly, effective connectivity analysis demonstrated that behavioural performance is directly related to degree of coordination between the network constituents. These results are in accordance with animal models and will be discussed in the context of an emerging neuroanatomical model of navigational learning.

# Unsupervised Learning of Place Cells and Head Direction Cells with Slow Feature Analysis

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We present a model for the self-organized formation of hippocampal place cells and limbic head direction cells based on unsupervised learning on natural visual stimuli. The model is based on a hierarchy of Slow Feature Analysis (SFA) modules, which were recently shown to be a good model for complex cells in the early visual system. The system extracts a distributed representation of position and orientation, which is transcoded into a localized place field or head direction representation, respectively, by sparse coding (ICA). We introduce a mathematical framework for determining the solutions of SFA, which accurately predicts the distributed representation of computer simulations. The model's output characteristic of orientation-independent or orientation-dependent place cell-type or position-independent head direction cell-type solely depends on the animal's movement pattern, which explains experimental findings about place cell head direction selectivity depending on an animal's behavioral task.

# Hippocampal Activation in the Zebra Finch During Learning and Recall of a Spatial Memory Task.

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The avian hippocampus has been shown to be involved in spatial memory tasks like food hoarding and navigation. We have recently demonstrated that this is not a speciality of food hoarding or migrating birds. The zebra finch, a nonmigrating Australian songbird, is able to solve a spatial memory task where the bird has to find hidden food available at only one of four identical food trays in an open arena. In the course of learning the apatial task, the hippocampus shows high expression of the immediate early gene ZENK, indicating high activation of this area. Controls performing the same experiment but without learning (all four feeders contained food) did not show hippocampal activation.

In the present experiment we wanted to know whether hippocampal activation can also be observed if the learned task is recalled. For this purpose, we compared hippocampal ZENK activation in birds during the learning stage with others having well reached their maximal performance.

As in the previous experiment, hippocampal ZENK expression was quite patchy instead of homogeneous. Thus,exact cell density measurements were not appropriate. Instead, we scored the overall number of neurons within the different hippocampal subdivisions on a scale from zero to four for each of the 5 birds of each group.

The results unequivocally show that the avian hippocampus is also active during recall of a learned spatial task. However, the activation is in all areas examined lower than in the learning animals. In contrast, ZENK expression within the septum which is in general coactivated with hippocampus, is lower in the learner group. Strong differences in the amount of activation between the hippocampal subareas indicate varying contributions of these subareas to both, learning and recall.

# Hippocampal ca3 subfield cell degeneration induces spatial cognition dysfunction in kainic acid model of epilepsy;correlation of anatomy and behaviour.

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The inter and intraction of the two hippocampal sides have been associated with the memory and learning abilities. In case damage persists to any part of the hippocampus, memory and learning is impaired, although to a different extent and types. These reports came after the famous HM case when lobectomy was preferred as a tool for untraceable epilepsy. Later on many experimental models were designed in effect to cause type relationship. We by making use of one these models tried to define the relationship between the ipsilateral and contralateral side of hippocampus. The assessment of the functional relationship was done by the neurobehavioral, neurochemical and Immunohistochemical indices. The results were quite like the literature and confirmed the effect of the ipsilateral lesioning on the contralateral non lesioned side and also bilateral groups showed significant spatial memory loss in Y Maze for reverse learning tasks. Various antioxidant enzymes showed rise in the contralateral side as compared to lesioned side. Cholinergic receptor binding assay showed significant decrease in receptor binding of cholinergic neurons in lesioned side as compared to the non lesioned side and bilateral rats showed great receptor binding loss. Immunohistochemical index showed decreased ChAT Immunoreactivity in the lesioned side of unilaterally lesioned animals as compared to sham operated group. In case of bilaterally lesioned group the ChAT Immunoreactivity was found to be the minimal of all the groups.

# Coding of interaural time sensitivity of concurrent sounds

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In a natural auditory environment we are continuously exposed to multiple concurrent sounds originating from different directions. To extract information from this mixture of sounds our auditory system needs to detect, localize, and separate the different sources. The ability to detect and separate a single target has been studied in various psychoacoustic, physiological, and theoretical studies. Furthermore it has been shown that the identification of different sounds depends on the locations of the concurrent sounds. However, data focusing on the localization of concurrent sounds are rare with most of the studies investigating sound localization using only one single acoustic stimulus at a time. In this study we investigated the neuronal mechanisms underlying the localization of concurrent sound sources by measuring the sensitivity of low frequency neurons to interaural time differences (ITDs) in the presence of a concurrent signal.

We recorded single cell responses from ITD sensitive neurons in the dorsal nucleus of the lateral lemniscus (DNLL). This nucleus receives direct inputs from the superior olivary complex, the initial site of ITD processing. We found that ITD sensitivity of the two different signals is strongly influenced by each other. In particular, adding a pure tone with an unfavorable ITD to the masking signal led to a suppression of the response that would be evoked by the masker alone.

This finding contradicts the hypothesis that neuronal activity evoked by concurrent stimuli is a linear superposition of the neuronal responses evoked by the two sources independently. Furthermore our results indicate that the troughs of the pure tone ITD tuning curves might be generated by inhibitory mechanisms - a hypothesis that is corroborated by a model of ITD processing in the medial superior olive with unilateral inhibition.

By modeling an array of ITD detector neurons with a broad range of characteristic frequencies, we find that the suppression of the neuronal response by a concurrent stimulus leads to a distinct separation of active neuronal populations along the tonotopic gradient. This is likely to be achieved by inhibition at the level of the ITD detector itself. We, therefore, hypothesize that ITD sensitive neurons take a prominent role in localization as well as in separation of concurrent sounds.

# The effect of experimental neoplastic disease in rats on motor and spatial behavior.

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Mental status alterations and cognitive impairment are observed in non-central nervous cancers. It remains unclear if they result from remote effects of malignancy, paraneoplasia or adjuvant treatment. Limbic encephalitis is a paraneoplastic syndrome consisting of severe memory deficits, depression, irritability, seizures, and dementia. Cognitive impairment in patients with paraneoplastic cerebellar degeneration was suggested as related to the loss of cerebellar efferents to thalamus and forebrain structures causing a reverse cerebellar diaschisis.

The aim of the study was to test behavior of rats with experimental neoplastic disease and comparison breast cancer and Morris hepatoma bearing animals.

Materials and methods: Female Wistar rats inoculated with transplantable breast cancer and male Buffalo rats with Morris hepatoma 5123 were used for experiments. The observations were carried-on during first and second week in breast cancer bearing rats and one, two and three weeks after Morris hepatoma transplantation. The animals were tested in open field, T-maze and elevated plus maze and video-taped. The authors used own analytic program based on two-step procedure based on the image processing algorithms. In the first step the differential image between the current capture and the so-called "background" image is created. The result of this step is the isolated "raw" rat's image. In the second step the rat's center is calculated with the help of the modified weighted average algorithm. The auxiliary image enhancing techniques are also applied with the median and morphological based filters being the most important ones. The system is able to determine the movement path of the rat, its speed, acceleration and several other parameters, i.e. the entrance-abandonment times of to the selected region, time spend in the selected region, etc. It is also able to analyze the movie "on-line" - directly from the digital camera equipped with the IEEE1394 interface or "off-line" - previously recorded in the AVI format. The system is highly configurable.

Results: We have found reduced motor activity, limited exploratory behavior in open field test, increasing transfer latency in elevated plus - maze and changes in side preferences as well as alteration in T-maze. The behavior disturbances increased with the progression of breast cancer and Morris hepatoma 5123. With the growth of experimental tumors we observed that rats preferred to spend longer time in particular sectors of open field chamber or mazes used. In our previous study we have found up-regulation of acethylcholinesterase and butyrylcholinesterase in brains of rats with breast cancer and Morris hepatoma which may have effect on behavior.

Conclusion: The behavior impairment of tumor bearing rats depend on the stage of experimental neoplastic disease and may be related to up-regulation of acethylcholinesterase and butyrylcholinesterase.

# Psychophysics and Electrophysiology in Virtual Environments

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By using a newly developed virtual reality setup a variety of experiments have been performed to demonstrate the applicability of this method (for a detailed description of the setup see Hölscher et al., J. Expt. Biol. 2005). Preceding experiments have shown that the animals are able to interact with the virtual environments that where presented to them (see also Hölscher et al., J. Expt. Biol. 2005).

The more recent experiments were designed to demonstrate that the animals can also perform spatial tasks within a virtual environment. On a purely behavioral basis an experiment was designed that bases on the paradigm of the Morris-Water-maze experiments. In order to adopt this task in V.R., a 20 by 20 m squared environment was created with a rewarded area in the center of one quadrant. The walls of this arena where textured with different patterns to make them distinguishable. By starting from the center of the environment, the animals learned to approach a visually marked rewarding area reliably. The diameter of this visual cue was then stepwise reduced until it was finally removed. By this time the animals had to orient themselves at the distinct landmarks, e.g. walls. In a final testing trial the reward area was also removed. The data show that the animals predominantly searched in the formerly rewarded quadrant.

Another approach to investigate above mentioned question is realized in a pilot study which revealed the applicability of electrophysiological cell recordings in combination with our virtual reality set-up. To ensure that the animals repeatedly visit the same position in the large environment that was used, which is crucial to examine the correlations between position and firing, the animals were trained to run on a ring-shaped road. This road had a diameter of 5 m and was placed in the center of a 20 m by 20 m squared environment. In a small number of animals typical hippocampal place cells could be identified, responding to certain places in the virtual environment.

A second branch of current experiments was designed to reveal the necessary physical properties of a virtual environment to become perceived by the animals. In this case standard discrimination tasks being performed, in which two targets with different physical properties had to be discriminated. First data were evaluated regarding the required brightness and contrast values to be used for the design of further experiments.

In summary this results make clear that virtual reality technique gives us a potent tool at hand to further investigate spatial behavior in rats.

# Do rats use optical flow for motion control ?

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Animals in motion may control their movements based on different visual stimuli. Under common conditions a subject's egocentric relation towards a visible goal and the information about its selfmotion based on optical flow are concordant, which makes it impossible to distinguish how they contribute to navigational control. Recent studies with human subjects used virtual environments where the visual information was manipulated. For example, Warren et al. [1] could show that humans in fact do use the optical flow information for navigational control. I used a virtual reality setup designed for rodents [2] to test if this result also applies to rats. The setup consists of a running ball surrounded by a 360° video screen. In this virtual setup, the subjects first learned to navigate towards visual goals. In experimental trials the rats' movements in the virtual world were shifted by a fixed angle relative to its real-world movements, resulting in an optical flow field with a focus of expansion (FOE) that was shifted from their moving direction. Depending on how visual information is used in course control, different trajectories for the approach to the goal can be predicted. Straight approach lines can only be achieved using optic flow information whereas orientation based on the egocentric relation to the goal alone will effect a curved line. In different experimental conditions, I varied the amount of optic flow information available to the navigating rat. These variations had a clear effect on the rats' trajectories, indicating that optical flow information has an impact on the rats' motion control. However, further experiments are required to ascertain how the rats perceive and process the optical flow information.

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# Symposium

## S20: The Drosophila NMJ: Unravelling Principal Mechanisms of Synapse Formation, Function and Plasticity

*Andreas Prokop and Stephan S. Sigrist, Manchester (UK) and Göttingen*

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### Poster

**TS20-1C** The SOC-1 protein modulates levamisole and $GABA_A$ receptor functions at the NMJ in *C. elegans*
*T. Schedletzky, J. Liewald and A. Gottschalk, Frankfurt*

**TS20-2C** Quest for the role of IRM-proteins in neuromuscular junctions of *Drosophila melanogaster*
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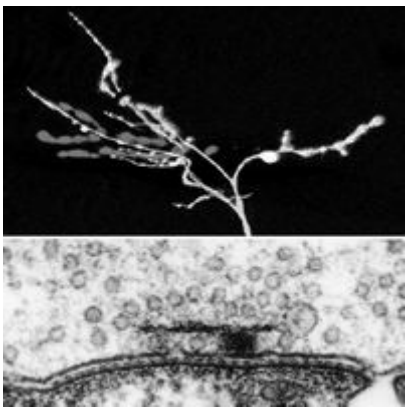
**TS20-3C** *Drosophila* synapses viewed at the level of protein complexes: recent advances using EM tomography
*B. Greiner, N. Delaney, N. Butcher, RM. Marshall and IA. Meinertzhagen, Halifax (Canada) and Stanford (USA)*

Introductory Remarks to Symposium 20

# The Drosophila NMJ: Unravelling Principal Mechanisms of Synapse Formation, Function and Plasticity

Andreas Prokop and Stephan S. Sigrist, Manchester (UK) and Göttingen



More than 3 decades ago, the neuromuscular junction (NMJ) of *Drosophila* has been introduced as a model system for neurogenetic research, and has since been used most successfully to investigate genes and mechanisms underlying synaptic development, function and plasticity, in several instances pioneering new areas of research. Mutant phenotypes of far more than 100 genes have since been documented for Drosophila NMJs, a number hardly achieved by any other synaptic model. Many of such insights have been and will be translated into vertebrate research, adding significantly to our general knowledge about synapses. The fact that the fly NMJ is glutamatergic, with glutamate receptors closely homologous to those of mammalian central synapses, further supports this statement.

The combination of two principal factors makes the *Drosophila* NMJ so efficient for neurogenetic research: First, the high resolution at which the *Drosophila* neuromuscular system has been structurally and functionally described in situ (i.e. within the whole organism), provides efficient and sensitive read-outs for genetic studies, including *in vivo* visualisation of protein dynamics, physiological and ultrastructural analyses. Second, capitalising on powerful genetics and tools available for *Drosophila*, gene functions can be uncovered in an unbiased way via high-throughput mutational screens. Subsequently, such gene functions can be investigated further in effective manners.

This symposium will provide an overview over the *Drosophila* NMJ as a neurogenetic model system. The individual presentations will inform about examples of ongoing *Drosophila* NMJ research on mechanisms underlying synaptogenesis, synapse structure, activity-dependent plasticity, and synaptic transmission.

# BRUCHPILOT PROTEIN IS NEEDED FOR $CA^{2+}$-CHANNEL CLUSTERING AND ACTIVE ZONE FORMATION TO ALLOW EFFICIENT SYNAPTIC TRANSMITTER RELEASE

Stephan J. Sigrist and Manfred Heckmann

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Uncovering how changes in presynaptic transmitter release are controlled in a synapse specific manner is important for advancing our understanding of circuit function. Presynaptic active zones are characterised by clustered Ca2+-channels and docked transmitter-filled vesicles, the spacing between which is critical for the transmission characteristics of a given synapse. Moreover, active zone membranes are electron dense in appearance and are associated with dense projections. However, the correlation between active zone differentiation, clustering of Ca2+-channels and docking of release ready vesicles remains largely unknown.
We recently identified Bruchpilot (Brp), a large coiled coil domain protein in parts homologous to mammalian CAST/ERC/ELKS proteins, as an active zone component of Drosophila synapses. We recently showed that Brp is essential for active zone differentiation and proper Ca2+-channel clustering. While vesicle docking and synapse formation per se appeared largely unaffected at neuromuscular junctions of brp mutant larvae, presynaptic dense projections were completely eliminated, membrane organisation of the active zones was defective and evoked vesicle release was severely reduced and delayed. Furthermore, marked facilitation following paired pulse stimulation and increased EGTA sensitivity of release indicated defective Ca2+-channel-vesicle spacing. Consistently, the N-type Ca2+-channel subunit Cacophony was diffusely distributed in brp mutants. Thus, active zones might be specialized structures evolved to establish high vesicle release probability via the clustering of Ca2+-channels.
We will present new data on the active zone function of BRP.

# Sphingolipid regulation of synapse structure and function

Sean T. Sweeney and Laura Briggs

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Sphingolipids have been implicated in many aspects of synapse structure, function and degeneration. Their precise role in these processes however, has yet to be determined. Palmitoyl-serine transferase is the first enzyme step for synthesis of sphingolipids. We have initiated a study of mutations in the two palmitoyl-serine transferase enzyme subunits in Drosophila, sptI and lace (sptII). Previous studies have demonstrated that mutations in lace have severely reduced sphingolipid content (Herr et al., 2003 Development 130, 2443-2453). Examination of the larval synapses of lace mutants show many aberrations in normal synapse structure. Although synapse length is normal, the number of boutons per synapse is reduced to 40% of the wild type number. Boutons in the lace mutant are also larger with a high incidence of malformed terminal boutons with a 'spur-like' morphology. The distribution of synaptic adhesion molecules on the neuronal plasma membrane of lace mutant synapses is also aberrant with observed 'clumps' of adhesion molecules in contrast to the more even distribution observed in wild type synapses. Functional studies of the synaptic lace mutant phenotype are ongoing and will be described. In humans, dominant mutations have been described in sptII that give rise to Hereditary Sensory Neuropathy type 1. We are currently investigating lace and sptII mutations in Drosophila for similar sensory structural and functional defects.

# ORGANISATION AND DYNAMICS OF MAGUK-BASED PROTEIN COMPLEXES AT *DROSOPHILA* LARVAL NEUROMUSCULAR JUNCTIONS.

Ulrich Thomas[1], André Bachmann[2], Oliver Kobler[1], Carolin Wichmann[3], Robert Kittel[3], Stephan S. Sigrist[3], Jimena Sierralta[4], Elisabeth Knust[2] and Eckart D. Gundelfinger[1]

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[3] European Neuroscience Institute, Göttingen

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Dlg is a prototypic membrane-associated guanylate kinase (MAGUK), enriched at glutamatergic neuromuscular junctions (NMJs) of Drosophila larvae. Here it serves as a scaffolding molecule to ensure proper localization of Shaker-type potassium channels, the cell adhesion molecule FasII and of other scaffolding proteins such as Scribble, Metro and DLin-7. Analysis of isoform-specific mutants and DNA-based siRNA expression allows us to conclude that two major subclasses of Dlg, Dlg-A and Dlg-S97, are expressed at NMJs, suggesting the co-existence of separate Dlg-based complexes. The postsynaptic recruitment of DLin-7 and Metro largely depends on Dlg-S97, with Metro, an MPP-like MAGUK, providing a physical link between Dlg-S97 and DLin-7. Interestingly, genetic analyses revealed that the epistatic relationship between all three proteins is bidirectional. Metro is undetectable in DLin-7 mutants and preliminary results suggest that DLin-7 protects Metro form degradation. Moreover, postsynaptic Dlg-S97 is reduced in both metro and DLin-7 mutant larvae. Basal synaptic function appears to be independent of Metro. Loss of Metro, however, is associated with a reduction in bouton number and with abnormal shape of boutons, a phenotype reminiscent to that observed in dlg mutants. Initial EM-analyses point to discrete ultrastructural defects including expanded active zones and a disturbed alignment of the pre- and postsynaptic membrane. We thus conclude that Dlg-S97, Metro and DLin-7 interact in an interdependent manner to form a scaffolding complex required for proper NMJ structure and plasticity. Using a life imaging set-up we have recently started to analyse the dynamics of Dlg and its binding partners at NMJs under varying conditions. First data sets imply, that different isoforms of Dlg display considerable differences in their local motility, suggesting that isoform diversity of MAGUKs may serve as a means to tare stable versus dynamic building blocks at synaptic junctions.

# Fos, Jun and the transcriptional regulation of neuronal plasticity

Mani Ramaswami

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(and University of Arizona, Tucson, AZ 85721, USA)

Persistent increases in synaptic weight believed to underlie long-term memory formation require new gene expression. My laboratory has recently studied: a) the hierarchy of transcription factors in control of long-term plasticity at Drosophila motor synapses; b) the underlying cellular mechanisms; and c) the relevance of these mechanisms to experience-induced behavioral plasticity.

In Drosophila motor neurons, activation of AP-1, a heterodimer of the immediate early transcription factors Fos and Jun, induces cAMP and CREB dependent forms of long-term facilitation (LTF). Contrary to expectations from current models of long-term plasticity, AP-1 mediated LTF occurs without any increase in the number of release sites: thus, transmitter release is potentiated at individual active zones. Short-term potentiation (PTP) of transmitter release which occurs in control synapses after brief 10Hz tetani is not shown at synapses that have undergone LTF. Data will be presented to show that LTF is accompanied by an increased size of an actively cycling synaptic-vesicle pool at the expense of the reserve pool. Interpreted in the context of previous work, our data show: a) that transient reserve pool mobilization underlies post-tetanic potentiation and b) the sustained reserve-pool mobilization mediates long-term facilitation. Most importantly, these data show that synapse growth is not an absolute requirement for transcription-dependent long-term plasticity. Parallel studies of AP-1 function in long-term habituation, a form of olfactory memory, will show that Fos and Jun have essential functions in this form of plasticity. Preliminary experiments to identify olfactory circuit elements in which Fos and Jun mediate this form of behavioral change will also be presented.

Key contributers:
Susy M Kim, Dr. Angel Pimentel, Avni Gandhi, Dr. Vimlesh Kumar, Prof Subhabrata Sanyal; Prof Veronica Rodrigues.

# Mechanisms of experience-dependent potentiation of glutamatergic synapses

Christoph M. Schuster and Jörn Steinert

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We have recently developed an experimental strategy to systematically analyze the consequences of natural behavior on the function and structure of glutamatergic synapses. This strategy is based on the continuous monitoring of crawling activities of Drosophila larvae and the subsequent analysis of synaptic properties at NMJs of body wall muscles. We found that the glutamatergic synapses of larval NMJs can undergo robust potentiation of their transmission strength strictly depending on the before experienced crawling activities. We further found that the first 120 min of experience-dependent synaptic potentiation are mediated by a hierarchy of subsequent mechanisms that allowed us to define three distinct phases of experience-dependent potentiation so far. Here we will focus on the mechanisms underlying phase-II of experience-dependent potentiation, which are induced in larvae following more than 90 min of continuous high crawling activities.
We provide evidence that retrograde NO/sGC/cGMP/PKG-signaling is sufficient and necessary to induce and maintain phase-II of experience-dependent synaptic potentiation. We further show that this potentiation is mediated by presynaptic N-methyl-D-aspartate receptors (DNMDARs) that are activated by PKG-mediated phosphorylation of DNMDAR1T886. These results demonstrate that retrograde NO/sGC/cGMP/PKG-signaling activates presynaptic DNMDARs in vivo to facilitate presynaptic vesicle release in an experience-dependent manner.

# Unsaturated Fatty Acid Regulation of Synaptic Transmission

Peter Robin Hiesinger

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The link between the intake and metabolism of specific lipids and the molecular mechanism of synaptic transmission presents us mostly with gaps in our knowledge, depsite its possible importance for the function of neurons in health and disease. Here we report the discovery of mutations in a novel lipid modifying enzyme that cause specific synaptic transmission defects. Using the fly as a model organism we have identified synoil, a fatty acid desaturase that produces palmitoleic acid in neurons. Our data indicate genetic and biochemical links of synoil with the core membrane fusion protein Syntaxin, suggesting a direct action in synaptic vesicle exocytosis. The discovery of synoil in a genetic model organism provides a novel genetic inroad into the notoriously difficult study of specific lipid functions at synapses in vivo.

# The SOC-1 protein modulates levamisole and $GABA_A$ receptor functions at the NMJ in *C. elegans*

Thorsten Schedletzky, Jana Liewald and Alexander Gottschalk

Institute of Biochemistry, Johann Wolfgang Goethe-University, Max-von-Laue-Straße 9, Frankfurt, Germany

We have previously identified the SOC-1 protein, a receptor-tyrosine kinase signaling protein, associated with the levamisole receptor, one of two nicotinic acetylcholine receptors (nAChRs) acting at the neuromuscular junction (NMJ) of Caenorhabditis elegans, using a tandem affinity purification (TAP) approach, mass spectromety and RNAi-based functional screening (Gottschalk *et al.*, 2005).

*soc-1(n1789)* mutants are resistant to nicotine, levamisole and the choline esterase inhibitor aldicarb in paralysis assays. Loss of SOC-1 causes reduced synaptic levamisole and $GABA_A$ receptor levels and leads to muscimol resistance in liquid-thrashing assays. We could show that the muscle specific expression of SOC-1::GFP fusion protein rescues this phenotypes. The SOC-1 protein localizes to membranous structures and forms clusters along the nerve cord. To investigate whether SOC-1 colocalizes with levemisole and/or $GABA_A$ receptors at postsynaptic sites, we used an antibody-injection assay.

To identify novel regulators of levamisole and/or $GABA_A$ receptor clustering at the NMJ, we used the expression of TAP-tagged SOC-1 protein in muscle cells in *soc-1(n1789)* mutants followed by tandem affinity purification. In a second approach, we are currently screening for receptor tyrosine kinases in C. elegans causing a levamisole or muscimol resistant phenotype, to identify novel upstream regulators of SOC-1, that may also affect nAChR and/or $GABA_A$ receptor clustering.

Currently, we are analyzing the *soc-1(n1789)* mutant electrophysiogically for effects on the functional properties of levamisole and $GABA_A$ receptors using whole-cell recordings from body wall muscle cells in dissected worms. Prelimanary data indicate significantly reduced currents mediated by the cholinergic agonist levamisole in the *soc-1(n1789)* mutant. The further electrophysiological evaluation is in progress and results will be presented at the meeting.

Reference: Gottschalk A, Almedom R, Schedletzky T, Anderson SD, Yates JR, and Schafer WR (2005) *EMBO J* 24: 2566-2578

# Quest for the role of IRM-proteins in neuromuscular junctions of *Drosophila melanogaster*

Kokil Chaudhary, Sebastian Schiller and Karl-Friedrich Fischbach

Institute of Biology III, Albert-Ludwigs University of Freiburg, Schaenzlestr.1, Freiburg, Germany

IrreC-roughest (Rst), Kin-of-Irre (Kirre) and their ligands Sticks-and-Stones (Sns) and Hibris (Hbs) are members of the immunoglobulin superfamily and these together constitute the "Irre Cell Recognition Module (IRM)". These proteins play key roles at several developmental stages in *D. melanogaster*.

We have shown that their common theme of action is to mediate recognition between two different cell types. Previous work of our lab has demonstrated the essential requirement of either Rst or Kirre protein during the fusion of muscle founder cells and fusion competent myoblasts. Here we present our data showing their localisation in motorneurons and muscles and illustrate our progress in elucidating their function at the neuromuscular junction.

# *Drosophila* synapses viewed at the level of protein complexes: recent advances using EM tomography

Birgit Greiner[1], Nicole Delaney[1], Nancy Butcher[1], Robert M. Marshall[2] and Ian A. Meinertzhagen[1]

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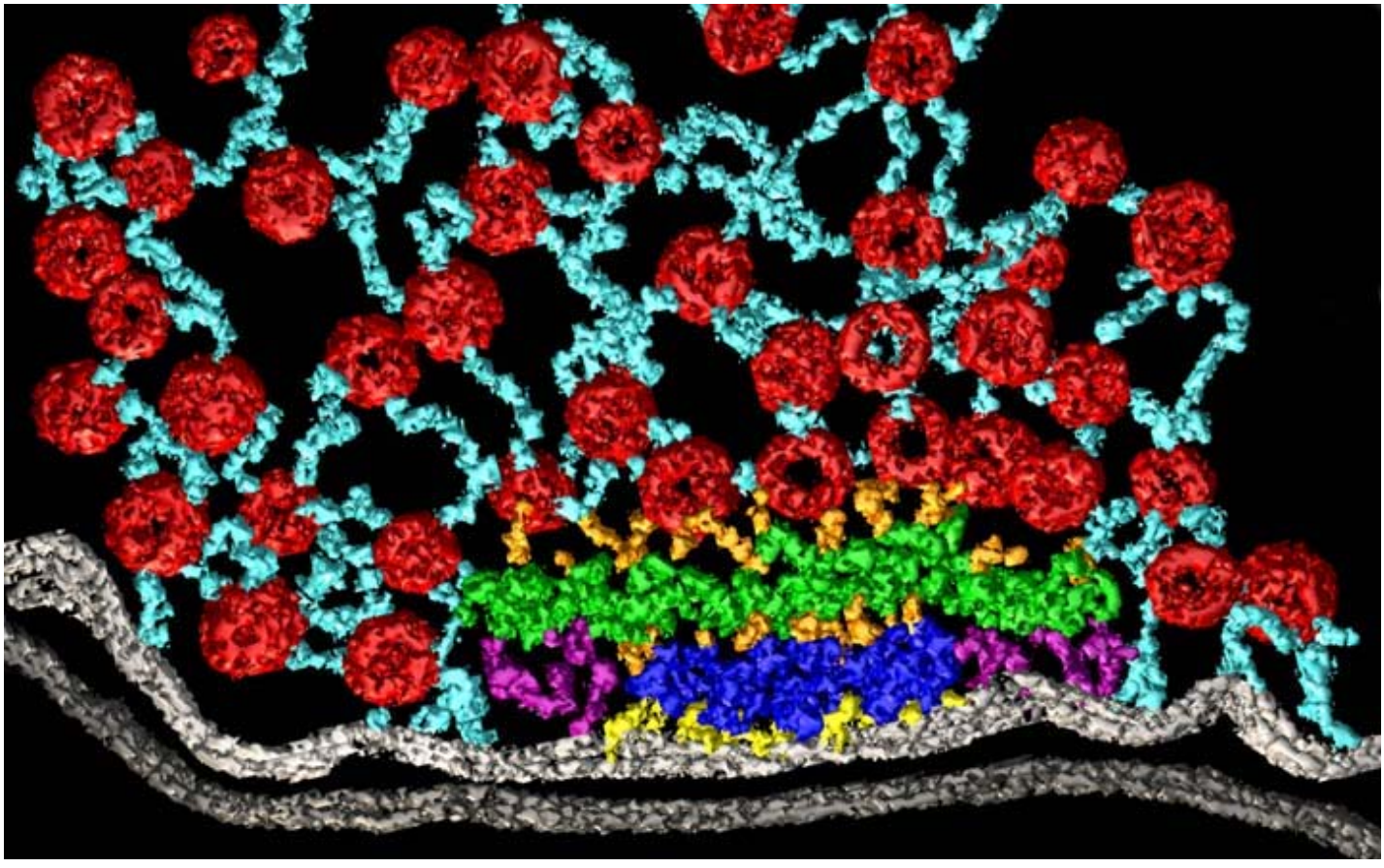
Genes programme the physiology of synapses by coding for the synaptic proteins that regulate synaptic function. Many such proteins, such as those for exocytosis, act as macromolecular complexes. Conventional electron microscopy (EM) of thin sections is unable to visualise the organisation of such complexes, however, because these overlap each other within the depth of an ultrathin section and cannot be resolved. Recent developments in EM tomography solve the problem of overlap by means of wide-angle image tilt series, to yield depth information. After tomography, 3-D information can then be carved out manually from the merged image block using advanced software tools (EM3D), according to published methods (Harlow et al., 2001, *Nature*).

Using these methods we have reconstructed the T-bar ribbons and other synaptic organelles at the release sites of two types of *Drosophila* wild-type synapse, the neuromuscular junction (*Figure*) and photoreceptor tetrad synapses of the fly's lamina. Some of our major findings include resolution of: (1) a clear regular network of filaments (light blue) that connects the vesicles (red) surrounding the platform of the T-bar ribbon (green); (2) the platform and the T-bar pedestal (dark blue), which are vertically striated; and (3) distinct proteins (purple) that reach from the platform to the presynaptic membrane (white). These reconstructions provide a basis against which we will compare structural perturbations in synapses of flies mutant for the genes coding for synaptic proteins (e.g. *endo, bruchpilot*).



# Symposium

## S21: Glia Development: Molecular Control of Specification, Migration, Differentiation and Myelination of Oligodendrocytes and SchwannCells

*Michael Wegner, Erlangen*

### Slide

**S21-1** Oligodendrocyte precursor cells (OPCs): an historical perspective
*Martin Raff, London (UK)*

**S21-2** Multiple oligodendrocyte origins
*WD Richardson, N Kessaris, M Fogarty, P Iannarelli and K Young, London (UK)*

**S21-3** Transcriptional control of oligodendrocyte development by Sox proteins
*Michael Wegner, Erlangen*

**S21-4** Differential Control of OPC Differentiation by Tenascins and Chondroitin Sulfates
*Alexander von Holst, Bochum*

**S21-5** The role of extracellular matrix in the regulation of oligodendrocyte development and myelination
*Charles ffrench-Constant, Joana Camara and Jan Wang, Cambridge (UK)*

**S21-6** Axonal Control of Myelination
*Klaus-Armin Nave, Göttingen*

### Poster

**TS21-1C** Control of Peripheral Nerve Myelination by the β-Secretase BACE1
*AN. Garratt, M. Willem, P. Saftig, B. De Strooper, C. Birchmeier and C. Haass, Berlin, Munich, Kiel and Leuven (B)*

**TS21-2C** CHONDROITIN SULPHATE GLYCOSAMINOGLYCANS ARE REQUIRED FOR THE DEVELOPMENT OF TELENCEPHALIC NEURAL STEM/ PROGENITOR CELLS
*S. Sirko, A. von Holst, A. Wizenmann, M. Götz and A. Faissner, Bochum and Neuherberg/Munich*

**TS21-3C** PPARdelta agonist promotes differentiation of oligodendrocytes from oligospheres
*AI. Boullerne, P. Polak, S. Vujicic, A. Othman and DL. Feinstein, Chicago, Illinois (USA)*

**TS21-4C** Identification of an intrinsic inhibitor of myelinating Schwann cell differentiation
*A. Heinen, T. Zimmermann and P. Küry, Duesseldorf*

**TS21-5C** Distinct function of neuregulin-1 in myelination of the peripheral and central nervous system
*BG. Brinkmann, MW. Sereda, A. Agarwal, M. Schwab, A. Garrat, C. Birchmeier and KA. Nave, Göttingen and Berlin*

**TS21-6C** PRENATAL GLUCOCORTICOID TREATMENT REDUCES MYELIN BASIC PROTEIN IN THE FETAL SHEEP BRAIN
*I. Antonow-Schlorke, A. Helgert, T. Müller, H. Schubert, PW. Nathanielsz, OW. Witte and M. Schwab, JENA, Jena and San Antonio, Texas (USA)*

**TS21-7C** Lineage analysis of Olig2-positive cells in the adult brain after injury
*L. Dimou, A. Buffo and M. Götz, Munich*

Introductory Remarks to Symposium 21

# Glia Development: Molecular Control of Specification, Migration, Differentiation and Myelination...

Michael Wegner, Erlangen

Oligodendrocytes are the myelin forming cells of the CNS that are crucial for proper neuronal information propagation. Although oligodendrocytes belong to one of the most studied mammalian cells types, we still need a better understanding of how and where they are generated during development and, which cues are essential to generate functional myelinating cells of the nervous system. The symposium intends to summarize scientific efforts and achievements by bringing together researchers that will present their cell biological and molecular insights into the development of oligodendrocytes. The symposium will be opened by Martin Raff, who gives an (historical) overview on oligodendrocyte precursor cells (OPCs), summarizing 30 years of work, which will be the frame for the audience and for the following presentations. Bill Richardson will report on their findings that additional oligodendroglial lineages exist, which not only derive from different sources along the neuraxis but also at different time points. This raises the question which transcription factors control the generation of oligodendrocytes and Michael Wegner will provide evidence for the importance of Sox family proteins. However, the oligodendrocyte progenitor cells are located in an environment rich of extracellular matrix (ECM) molecules, and Alexander von Holst will report how tenascin family proteins and chondroitin sulfates influence their differentiation. Many ECM components signal via integrin receptors and Charles ffrench-Constant will present data on the importance of integrin-mediated signaling cascades for the maturation of OPCs to a myelin forming oligodendrocyte. In order to become such a specified glial cell the OPCs as well as Schwann cells, the myelinating glia cells ot the peripheral nervous system, depend on signals derived from the axon. Klaus-Achim Nave will elaborate on this intricate neuron-glia interaction including how neuregulins contribute to the control of myelination.

All these facettes of oligodendrocyte development need to be understood in detail, if we ever seriously want to cure diseases that are caused by a failure or loss of this fascinating glial cell.

# Oligodendrocyte precursor cells (OPCs): an historical perspective

Martin Raff

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I will review how my colleagues and I stumbled upon oligodendrocyte precursor cells (OPCs) in cultures of rat optic nerve cells and how we used these cells as a model for studying developmental timing, cell survival control, cell number control, intracellular developmental programmes, and cell plasticity. Finally, I will consider the mystery of adult OPCs and the knotty problem of type-2 astrocytes.

# Multiple oligodendrocyte origins

WD Richardson, N Kessaris, M Fogarty, P Iannarelli and K Young

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Oligodendrocyte precursors (OLPs) are generated from neuroepithelial stem cells in the ventricular zones (VZ) of the embryonic spinal cord and brain. In the cord, most OLPs (80%) come from the ventral progenitor domain pMN, which also generates motor neurons. The remaining 20% come from more dorsal domains dP3-dP5. Ventrally- and dorsally-derived oligodendrocytes (OLs) are differentially distributed in the cord, so that dorsally-derived OLs contribute predominantly to dorsal axon tracts. In the forebrain, there are also ventrally- and dorsally-derived populations. OLPs arise first in the VZ of the medial ganglionic eminence (MGE), then the lateral ganglionic eminence (LGE) and finally the cerebral cortex, in a "Mexican wave" of production from ventral to dorsal. As in the spinal cord, the different populations are differentially distributed; the ventrally-derived (MGE and LGE) OLPs migrate all through the forebrain including the cerebral cortex, while the dorsally-derived (cortical) OLPs remain within the cortex. Are they all functionally equivalent? We addressed this by killing one or other OLP population in transgenic mice by targeted expression of Diphtheria toxin (DT). When the cortex-derived population was eliminated in (*Emx1-Cre/ Sox10-DT*) double-transgenic mice, neighbouring OLP populations expanded to make up the loss. Even when we killed all telencephalic OLPs in (*Emx1-Cre/ Gsh2-Cre/ Nkx2.1-Cre/ Sox10-DT*) quadruple-transgenics, there was an approximately one-week delay in the onset of myelination but the mice recovered, bred and lived a normal lifespan. It appears that OLPs from the diencephalon migrate forward into the telencephalon to replace the missing cells. It is likely, therefore, that OLPs in the forebrain are functionally equivalent, regardless of their site of origin.

OLPs and OLs derived from the MGE (*Nkx2.1*-expressing territory) are eliminated during postnatal life and are almost undetectable in the adult, even in ventral regions close to their original source. Consistent with this we found that the postnatal forebrain SVZ, known to be a source of new oligodendrocytes in the adult, contains stem cells descended from the embryonic LGE and cortex (*Gsh2*- and *Emx1*-expressing territories) but no MGE-derived stem cells. Therefore, if there is natural turnover of OLs during postnatal life, we expect the MGE-derived OLs to be progressively lost and replaced by their more dorsally-derived counterparts.

We are fate-mapping the LGE-and cortex-derived SVZ stem cells to see if they generate different classes of neurons or glia in the adult. We are also fate-mapping adult OLPs using *Pdgfra-CreER*$^{T2}$ mice, in which Cre can be activated on demand by tamoxifen administration. By activating Cre and reporter gene expression in OLPs at different postnatal ages and characterizing the cells that become labelled post-tamoxifen, we have shown that *Pdgfra*-expressing cells divide and give rise to new myelinating OLs throughout adult life. We also found small numbers of new labelled neurons produced in the ventral forebrain but no astrocytes. Thus, *Pdgfra+* OLPs mainly generate oligodendrocytes in the adult. It is possible that they also generate some ventral neurons but this needs to be confirmed.

# Transcriptional control of oligodendrocyte development by Sox proteins

Michael Wegner

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The myelin forming oligodendrocytes are an excellent model to study transcriptional regulation of specification events, lineage progression and terminal differentiation in the central nervous system. Their development is strongly influenced by transcription factors from the Sox protein family, especially SoxE proteins. Sox9 is, for instance, essential for specification of oligodendrocyte precursors, whereas Sox10 is required for terminal differentiation and myelin gene expression. Interestingly, cells of the oligodendrocyte lineage also express the SoxD proteins Sox5 and Sox6 during their development. SoxD transcription factors repress specification and terminal differentiation and influence migration patterns. As a consequence, oligodendrocyte precursors and terminally differentiating oligodendrocytes appear precociously in spinal cords deficient for both Sox5 and Sox6. SoxD and SoxE proteins thus have opposite functions. Both genetic as well as molecular evidence suggests that SoxD proteins directly interfere with the function of SoxE proteins. A complex regulatory network between different groups of Sox proteins is thus essential for proper progression of oligodendrocyte development.

# Differential Control of OPC Differentiation by Tenascins and Chondroitin Sulfates

Alexander von Holst

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Oligodendrocyte precursor cells (OPCs) are present in large numbers in both the developing as well as in the adult brain. OPCs are specified to become the myelin-forming cells of the central nervous system (CNS). However, it remains incompletely understood which extracellular cues drive an OPC to lose its precursor state and, subsequently, to modulate their differentiation and myelination programmes.

Among the large variety of ECM constituents found in the developing brain, we concentrate on the ECM-glycoproteins Tenascin C (Tnc), Tenascin R (Tnr) and a defined class of carbohydrates, the chondroitin sulfate glycosaminoglycans (CS-GAGs). Tnc, Tnr and CS-GAGs are dynamically expressed during CNS development including the germinal layers where OPC are born as well as in the environment of their migration pathways.

We report here that these ECM components are potent regulators of oligodendrocyte development and that they act in opposite manners at different stages of their lineage progression. Tnc has been shown to be a major component of the inhibitory properties of astrocytic ECM whereas Tnr, which is mostly expressed by OPCs themselves, promotes their differentiation. Furthermore, we could show that the enzymatic removal of CS-chains leads to increased process outgrowth and membrane formation in OPC cultures, which indicates differentiation towards mature oligodendrocytes.

Our results provide insights towards a better understanding of OPC behaviour in a complex environment, which could be useful also for understanding pathological situations such as the loss of oligodendrocytes in multiple sclerosis

# The role of extracellular matrix in the regulation of oligodendrocyte development and myelination

Charles ffrench-Constant, Joana Camara and Jan Wang

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Cell culture experiments examining growth factor signalling in oligodendrocytes show significant amplification by integrins, suggesting that this family of extracellular matrix receptors plays an important role in myelination. In keeping with this, expression of the laminin alpha2 chain has been reported on axons at the time of myelination, and abnormalities of CNS myelination have been described in mice with mutant laminin alpha2 (dy/dy mice). Laminins are recognised by the alpha6beta1 integrin, and mice lacking alpha6 show increased apoptosis of newly-formed oligodendrocytes. I will describe experiments using expression of dominant negative integrins in transgenic mice to examine the role of this receptor in vivo. The results suggest that beta1 integrin is dispensible for myelination in the CNS, as do studies using cre/lox technology to remove beta 1 integrin from myelinating oligodendrocytes. Together, these studies suggest a model in which integrins are required for the early stages of oligodendrocyte development, while other laminin receptors play a major role in myelination. We are exploring this hypothesis using myelinating co-cultures, and I will describe the progress of this work.

# Control of Peripheral Nerve Myelination by the β-Secretase BACE1

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Although BACE1 (beta-site amyloid precursor proteincleaving enzyme 1) is essential for the generation of amyloid β-peptide in Alzheimer's disease, its physiological function is unclear. Here we found that very high levels of BACE1 were expressed at time points when peripheral nerves become myelinated. Deficiency of BACE1 resulted in the accumulation of unprocessed neuregulin 1 (NRG1), an axonally expressed factor required for glial cell development and myelination. BACE1−/− mice displayed hypomyelination of peripheral nerves and aberrant axonal segregation of small diameter afferent fibers very similar to mice with mutations in type III NRG1, or Schwann cell-specific ErbB2 knockouts. Thus, BACE1 is required for myelination and correct bundling of axons by Schwann cells most likely via processing of type III NRG1.

# CHONDROITIN SULPHATE GLYCOSAMINOGLYCANS ARE REQUIRED FOR THE DEVELOPMENT OF TELENCEPHALIC NEURAL STEM/ PROGENITOR CELLS

Swetlana Sirko[1], Alexander von Holst[1], Andrea Wizenmann[2], Magdalena Götz[2] and Andreas Faissner[1]

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The germinal regions of the embryonic cerebral cortex contain stem cells whose development is driven by the combination of intrinsic programmes and extracellular signals from the ECM. The chondroitin sulphate proteoglycans (CSPGs) as ECM component are present in the ventricular and subventricular zones of the developing forebrain. The DSD-1-PG/Phosphacan is a CSPG and represents a splice variant of the receptor protein tyrosine phosphatase (RPTP)-beta gene. It is thought to be involved in the regulation of many developmental events. The DSD-1 epitope is a defined but complex cell surface-associated chondroitin sulphate-glycosaminoglycan (CS-GAG) motif recognized by the monoclonal antibody 473HD. A developmental immunohistochemical analysis of 473HD expression during corticogenesis was performed. In the neurogenic phase a prominent expression of DSD-1-PG/RPTP-beta on nestin-positive cells in the ventricular zone, with radial processes extending towards the pial surface was detected. After the neurogenic period, the expression of the 473HD-epitope shifted to wards, the secondary germinal area, the subventricular zone. When the number of 473HD-positive cells was determined on freshly isolated cortical tissue, we observed that they persisted throughout neurogenesis and decreased during gliogenesis. We have isolated 473HD-positive cells by immunopanning and characterized them as actively cycling, BLBP-positive neurogenic radial glia during cortical development. When the 473HD-positive cells were cultivated under neurosphere-forming conditions an increase in the number of neurospheres compared to the non-selected cell population was observed at all stages examined. This describes a significant aspect of the biology of embryonic neural stem cells - a subset of early multipotent neural progenitor/stem cells express DSD-1-PG/RPTPβ, which aids their identification and enrichment. To address the functional importance of CS-GAGs for development and differentiation of telencephalic neural stem/progenitor cells in vitro and in vivo we used GAG-lyases for deglycanation of CSPG and investigated the functional consequences of CS-GAG removal on neural stem/progenitor cell behaviour using several cell biological assays. Our results show that removal of CS-GAGs from neural stem/progenitor cell-surface caused: (1) reduction in proliferation of these cells and their capacity to generate neurospheres in presence of mitogen factors EGF and bFGF and (2) changes of the composition of the precursor cell pool by a shift from neurogenic radial glia towards gliogenic radial glia after treatment. These findings revealed an important role of CS-GAGs for proliferation of neural stem/progenitor cells and suggest that this specific class of carbohydrates has a pro-neurogenic as well as an anti-gliogenic role during forebrain development. (Supported by BMBF and SPP 1172)

# PPARdelta agonist promotes differentiation of oligodendrocytes from oligospheres

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We recently showed that agonists of Peroxisome Proliferator Activated Receptor delta (PPARdelta) ameliorate EAE. To determine the effects of PPARdelta agonists on oligodendrocyte maturation and proliferation, we established a method to isolate oligospheres from E12-13 mouse embryos (with assistance from W. Macklin and C. Pedraza). Cells immediately started to migrate out of the spheres upon plating onto poly-lysine and after 2 days had the bipolar morphology of progenitors expressing A2B5, with 12% GFAP+ astrocytes. Cells in clusters (spheres) remained dividing early progenitors expressing Notch-1 and Nestin. After 2 days, cells were rinsed to remove growth factors, and fresh media with Thyroid hormone T3 was added to slow down proliferation and promote differentiation. After 7 days the cells stopped expressing A2B5, Notch-1 and Nestin, and expressed O4 and O1 with greater staining intensity on a network of processes. We then supplemented the media containing T3 with GW0742, a selective PPARdelta agonist; Pioglitazone, a selective PPARgamma agonist; or with the equivalent amount of DMSO vehicle (viability control). Clear differences were observed after 7 days; in Pioglitazone treated cells, there were a larger number of astrocytes; in the GW0742 treated cells, there were longer processes in a greater number of cells. Immunocytochemistry for MBP, O4, or PLP showed that the GW0742 treated cells had much greater arborization than vehicle or Pioglitazone treated cells. Staining for PPARdelta after 16 days showed it was strongly expressed in cell clusters in contrast to cells that had migrated out of the spheres, with O1 expressed on all cells. This suggests that PPARdelta may be required only at an early stage of bipotential glial progenitors, and therefore its expression is restricted within the spheres. We examined the expression of PPARs in HOG cells, a model for human oligodendrocyte progenitors. HOG cells were cultured in low-serum medium with T3 similar to the medium used for mouse oligospheres to induce differentiation. Under these conditions, HOG cells expressed mRNA for the myelin proteins MAG, CNP, MBP, MOG, and for the transcription factors SOX10, NKX2-2 and Olig-1, known to be involved in oligodendrocyte maturation. HOG cells expressed both PPARdelta and gamma mRNA. In summary, under our culture conditions, the PPARdelta agonist GW0742 significantly promoted the number of mature oligodendrocytes differentiating from mouse oligospheres as well as the size of their arborization. In contrast, incubation with the PPARgamma agonist Pioglitazone did not show increased maturation compared to vehicle treated controls, despite a probable presence in the oligospheres of PPARdelta and PPARgamma, similarly as in HOG cells. These results suggest that treatment of demyelinating disease with PPARdelta agonists, in addition to attenuating clinical signs, may also promote oligodendrocyte maturation and remyelination. Whether these agonists induce expression of transcription factors SOX10, NKX2-2 and Olig-1 in oligospheres will be determined. This work was supported in part by a grant from the NMSS (DLF).

# Identification of an intrinsic inhibitor of myelinating Schwann cell differentiation

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The role of the vertebrate nervous system is the propagation of electrical signals. In order to accelerate signal propagation and to insulate axons from the environment, large diameter axons are surrounded and enwrapped by a myelin sheath generated by oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). During the postnatal development, most Schwann cells segregate a large diameter axon and establish a one-to-one cellular relationship in order to ensheath this axon. Intrinsic as well as extrinsic signals are likely to be responsible for the successful establishment of interactions between Schwann cells and axons and for the execution of glial and neural differentiation programs. During myelinating Schwann cell differentiation a number of cellular processes must be tightly regulated and executed. This includes cell cycle control and exit, cellular growth as well as radial migration around a segregated axon. Finally, Schwann cells express myelin genes and expose myelin proteins in order to achieve electrical insulation. We could recently define a gene network which implicates known as well as new genes regarding Schwann cell differentiation. In order to assess if these genes are functionally involved in Schwann cell differentiation, we suppressed their expression by means of long-term RNA interference (RNAi) in cell culture. Among others we also suppressed a kinase inhibitor gene in primary rat Schwann cells. While this gene is known to interfere with the cell cycle, we observed that it is downregulated during the postnatal peripheral nerve development implying that cell cycle control is not its primary function in this context. Although cultured Schwann cells do not differentiate, long term gene suppression leads to cell cycle exit, induction of massive cellular growth accompanied by enhanced actin filament assembly and the formation of filopodia-like processes. Quantitative RT-PCR, Western Blot analysis and immunocytochemistry revealed that these suppressed Schwann cells express myelin genes and produce myelin proteins which are subsequently exposed on their surface. Taking together, these results demonstrate that we have identified a novel intrinsic inhibitor of myelinating Schwann cell differentiation.

# Distinct function of neuregulin-1 in myelination of the peripheral and central nervous system

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The neuregulins comprise a family of secreted or transmembrane proteins involved in distinct processes of nervous system development. The neuronal growth factor neuregulin-1 (NRG-1) includes over 15 isoforms that share the epidermal growth factor (EGF)-like domain and can be subdivided by their different amino-termini. Previously, we have shown that neuronal NRG-1 type III signals information about the axon size to Schwann cells in the peripheral nervous system (PNS). A reduction of 50% of the NRG-1 type III gene dosage leads to hypomyelination and neuronal overexpression of NRG-1 type III causes hypermyelination (Michailov et al 2004 Science).

Here, we investigate whether NRG-1 also regulates central nervous system (CNS) myelination. We therefore examined mutant mice expressing reduced levels of NRG1, and transgenic mice overexpressing specific isoforms of NRG-1. In mice heterozygous for NRG-1, myelin thickness in the corpus callosum was unaltered. Moreover, also in mice with conditional inactivation of the NRG1 gene in postmitotic pyramidal neurons, myelin thickness in the corpus callosum appeared unaltered. However, in mice with neuronal overexpression of either NRG-1 type III or type I, we observed hypermyelination in the corpus callosum, as well as in the cortical grey matter. This reveals that in contrast to the situation in the PNS, neuronal NRG-1 is not necessary for CNS myelination. Overexpression of NRG-1, however, is sufficient to induce hypermyelination in both systems. This suggests that oligodendrocytes may respond to additional myelination signals.

# PRENATAL GLUCOCORTICOID TREATMENT REDUCES MYELIN BASIC PROTEIN IN THE FETAL SHEEP BRAIN

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The synthetic glucocorticoid betamethasone is routinely used in perinatal medicine to accelerate fetal lung maturation in immaturely born babies. There are controversial experimental data on the effects of glucocorticoids on myelination. The aim of the study was to show the effect of betamethasone at the dose used clinically on the expression of myelin basic protein (MBP) during cerebral myelination in fetal sheep.

One course of betamethasone treatment (2x 110 µg/ kg maternal body weight 24 apart) was administered to the ewe i.m. at 0.5 (n=6), 0.6 (n=6), 0.7 (n=3) and 0.9 (n=6) of gestation (1= term, i.e. 150 days in sheep), respectively. Control ewes received an equal volume of saline. In order to show acute glucocorticoid effects, fetal brains were harvested 24h after the second betamethasone injection. For chronic effects, ewes received one course of betamethasone at 0.73 (n=5) of gestation or two courses of betamethasone at 0.7 and 0.75 (n=5) of gestation, and fetuses were allowed to survive until 0.9 of gestation. Fetal brains were fixed in paraformaldehyde and embedded in paraffin. The immunohistochemical distribution of MBP expressed by mature oligodendrocytes was estimated by light microscopy and assessed by the use of an image analysis system in the deep white matter of the fetal forebrain.

MBP immunoreactivity was detected firstly at 0.6 of gestation in the deep white matter of the fetal forebrain in sheep, and increased continuously during gestation. Maternal administration of betamethasone acutely reduces MBP immunoreactivity by 35.7 % ($p<0.05$) and 23.6 % ($p<0.01$) in the fetal brain at 0.6 and 0.7 of gestation, respectively. This effect was reversed 20 days after a single but not a double course of betamethasone treatment (decrease by 42.8 %, $p<0.01$). In contrast to the effects at 0.6 and 0.7 of gestation, betamethasone did not affect MBP immunoreactivity acutely at 0.9 of gestation.

The results indicate a time window of MBP susceptibility for glucocorticoids during early myelination. Disturbances of MBP seem to be reversible but might sustain after repeated glucocorticoid treatment at the dose used clinically.

# Lineage analysis of Olig2-positive cells in the adult brain after injury

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Adult neural progenitors proliferate in vivo in response to various insults such as ischemia, trauma and neurodegeneration. However, their regenerative capacity is very limited and only a few or even no neurons can get generated without any extrinsic manipulation. Astrocytes and glia progenitors have the potential to generate neurons in vitro and in vivo but fail to do so upon injury, implying the presence of potent inhibitory cues for neurogenesis after brain injury in vivo. We recently identified the transcription factor Olig2 as one such inhibitor (Buffo et al., 2005). Interestingly enough, while no markers for neurogenesis or neurogenic fate determinants could be detected after acute brain injuries, the number of Olig2 positive cells strongly increases in diverse injury models (Buffo et al., 2005). Interestingly, the composition of Olig2-positive cells increasing in response to injury was notably distinct in acute and chronic injury models (Buffo et al., 2005). The transcription factor Olig2 is expressed in bi-potent precursors during development and in adult neurogenesis, but has to be down-regulated to allow neurogenesis. Indeed, upon suppression of Olig2 after stab wound injury in the adult mouse cortex a small but significant number of neuroblasts was generated, suggesting a role of Olig2 in suppressing neurogenesis, promoting gliogenesis and in maintaining an un/dedifferianted status of the expressing cells.
In order to investigate the fate of Olig2 positive cells after different types of injury, we took advantage of the inducible Olig2-CreERT2 mice crossed with reporter animals, where we are able to map the Olig2-expressing cells at several time points after the injury. These fate mapping experiments not only allow to monitor the progeny of this important precursor population, but also to delete specific fate determinants that may restrict these cells in a glial lineage.

Buffo et al. (2005)PNAS 102(50): 18183-18188

# Symposium

## S22: Real-Time Voltage-Sensitive Dye Imaging of Cortical Network Activities across Sensory Modalities

***Dirk Jancke and Hartwig Spors, Bochum and Heidelberg***

### Slide

**S22-1** Opening remarks for Symposium 22
*Dirk Jancke and Hartwig Spors, Bochum and Heidelberg*

**S22-2** Voltage sensitive dye imaging in somatosensory cortex: response characteristics, spread and plasticity
*Damian Haydon Wallace, Heidelberg*

**S22-3** Population dynamics in cat early visual cortex evoked by local motion
*Dirk Jancke, Frédéric Chavane and Amiram Grinvald, Bochum and Rehovot (Israel)*

**S22-4** Input-output transformation in the visuo-oculomotor loop: Comparison of real-time optical imaging recordings in V1 to ocular following responses.
*Frederic Chavane, Alexandre Reynaud, Arjan Boonman, Frédéric Barthélémy and Guillaume Masson, Marseille (F)*

**S22-5** Dynamics of odor representations.
*Hartwig Spors, Heidelberg*

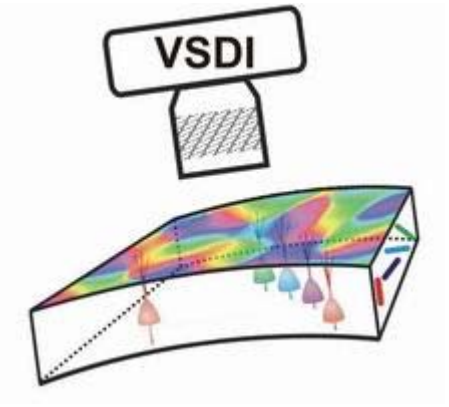
**S22-6** Optimal Decoding of Correlated Neural Population Responses in the Primate Visual Cortex
*Eyal Seidemann, Austin (USA)*

**S22-7** THE DYNAMICS OF EVOKED AND ONGOING ACTIVITY IN THE BEHAVING MONKEY
*Amiram Grinvald and David B. Omer, Rehovot (Israel)*

Introductory Remarks to Symposium 22

# Real-Time Voltage-Sensitive Dye Imaging of Cortical Network Activities across Sensory Modalities

Dirk Jancke and Hartwig Spors, Bochum and Heidelberg




Information processing in the mammalian brain is distributed across cortical areas and occurs in milliseconds. In order to determine how the outside world is represented internally and how sensory representations evolve and interact over short time scales it is crucial to measure neuronal electrical activity simultaneously across populations with both high spatial and high temporal resolution. Voltage sensitive dyes (VSD) and new camera systems allow for these challenging measurements. VSD Imaging permits sensitive monitoring of the changes in membrane potentials, both at sub- and supra-threshold level, over large areas of the cortex. Interactions via long-range horizontal cortical connections can be observed at millisecond temporal resolution and intra-cortical processing can be directly related to cortical anatomy. For example, VSD Imaging revealed that spontaneous on-going cortical activity in primary visual cortex frequently forms extensive patterns that strongly correlate to the geometrical shape of orientation maps. Another study demonstrated how visual context affects the emergence of activity patterns that can directly be mapped to perception: a small spot, flashed briefly before a stationary bar stimulus gave rise to propagation of cortical activity similar in time and shape to real motion. In the olfactory system VSD imaging has demonstrated that both spatial and temporal response patterns carry information about odor stimuli at a population level. In somatosensory cortex, VSD imaging has been employed to investigate the influence of sensory deprivation on the development of functional responses. Sub-threshold activity spreads strongly into neighboring cortical columns with spared sensory input at the expense of spread into columns deprived of input. In this symposium recent technical advances and new results from measurements of neuronal population activity in cortical areas of different sensory modalities will be presented.

# Voltage sensitive dye imaging in somatosensory cortex: response characteristics, spread and plasticity

Damian Haydon Wallace

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Voltage-sensitive dye (vsd) imaging is an optimal method for studying the horizontal spread of subthreshold excitation in superficial layers of the cortex. Horizontal spread is thought to be important for integration of responses within a cortical area as well as transfer for integration with responses from other modalities. Here I will discuss the characteristics of the horizontal spread of excitation measured with vsd imaging, in rat barrel cortex, the somatosensory area representing the facial whiskers. These whiskers form a large ordered array, and are used, amongst other things, for tactile discrimination. The cortical representation of a brief deflection of a single whisker is initially restricted in the superficial cortical layers to an area approximately the size of the cortical column representing the deflected whisker. Excitation then spreads horizontally over the following 50ms or so to cover a large part of the barrel cortex. The characteristics of this horizontal spread of excitation are altered by sensory deprivation during a critical period of development. Specifically, sensory deprivation (daily trimming of some of the rows of whiskers) during the second postnatal week results in asymmetrical horizontal spread of vsd signals around the non-trimmed (spared) whiskers. In deprived animals, the signal spreads preferentially towards the neighbouring columns representing the spared rather than the trimmed whiskers, whereas in normal non-deprived animals the horizontal spread is approximately symmetrical. Further examination of the characteristics of the horizontally spreading responses indicates that the asymmetry in deprived animals results primarily from a reduction in the spread of activity towards the deprived columns rather than an increase in spread of excitation towards neighbouring spared columns. Anatomical investigations using lentiviral injections of a construct expressing enhanced green fluorescent protein show that this functional change correlates well with alterations in the axonal projection pattern of pyramidal neurons in superficial cortical layers. Specifically, deprived animals have a reduced density of axons projecting to neighbouring deprived columns. In summary, vsd imaging in vivo has provided an exciting new method to examine the spatiotemporal dynamics of the activity of horizontal cortical projections in somatosensory cortex. The technique allows visualization of the representation of deflections of the facial whiskers in superficial cortical layers. These representations are highly plastic during development, and the change in the horizontal spread of vsd signals correlates well with alterations in the axonal projection pattern of pyramidal neurons in the superficial cortical layers.

# Population dynamics in cat early visual cortex evoked by local motion

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V1 neurons are located at the first cortical processing stage along the visual pathway. It is therefore reasonable that response properties of neurons in primary visual cortex vary with elementary stimulus features to provide downstream cortical areas with complete information. The characteristic layout of basic feature maps have been intensively studied in the past decades using imaging methods that allow simultaneous observation of large neuronal populations.
However, little is known about neuronal interactions when complex, dynamic, and multiple stimulus dimensions are present.
Here we used a small moving square to investigate how neuronal population dynamics act across the functional architecture of cat primary visual cortex. In order to detect population activity at both high spatial and temporal resolutions we performed voltage-sensitive dye imaging.
For several stimulus speeds tested, an initial fast spread was followed by localized emerging activity that started to propagate, gradually drawn-out in the direction of induced motion. Thus, the response was characterized by coherent "motion streak"-like activity in which the propagating front contained information about represented speed and actual stimulus location. After the stimulus has passed however, residual low-level "streak activity" formed clustered patterns that closely resembled orientation domains coding for orientation parallel to the stimulus' trajectory.
We conclude that the functional overlay of retinotopic and orientation maps allow multiplexing of information about both the present location and the orientation of a stimulus' trajectory. Moreover, since the moving square does not contain orientation information per se, the latter feature must be subsequently extracted by integration of activity along the motion path. Hence specific and perceptually useful portions of ongoing population activity in V1 may be consecutively amplified depending on actual input dynamics and stimulus history.

# Input-output transformation in the visuo-oculomotor loop: Comparison of real-time optical imaging recordings in V1 to ocular following responses.

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Early ocular following responses (OFR) are regulated by the population response in MST (Kawano et al 1994). MST neurons receive an input which is mostly the result of a complex recurrent processing between V1 and MT. It has been shown that OFR shows a context-dependent dynamic of motion integration, which can be conceptualized into a "behavioural receptive field" (BRF, Barthelemy et al 2006). To probe the possible role of V1 in the control of this BRF, we measured in behaving monkeys V1 population activity using voltage-sensitive dye optical imaging and the OFR using the electromagnetic search-coil technique. Testing the contextual dependencies of basic visual processing, we observed similar slow non-linear interactions at the level of V1 and the OFR. Such slow dynamics in V1 are the result of the gradual horizontal spread. We also measured very fast processing in V1 that are most likely explained by feedback control from MT. This result suggests that OFRs reflect the behavior of large populations of neurons within and between areas.

# Dynamics of odor representations.

Hartwig Spors

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Odors evoke distributed glomerular activity patterns in the mammalian olfactory bulb. These patterns are not static but can vary in an odor specific manner over time (Spors & Grinvald, Neuron 2002). Glomeruli activated by the same simple monomolecular odor can differ in their latency, in their decay time as well as in their modulation with respiration or sniffing. These changes of the spatial odor representations can occur within a short time window of 200 to 300 ms which is used by mice to discriminate odor pairs (Abraham et al, Neuron 2004). Using a calcium sensitive dye loaded into the olfactory receptor neurons selective imaging of the input to the olfactory bulb demonstrated that the input patterns to the olfactory bulb can already be dynamic (Spors et al, J. Neurosci. 2006). I.e. the temporal dynamics of the olfactory bulb network measured with the voltage sensitive dye do not entirely arise in the olfactory bulb. In order to compare spatio-temporal patterns of odor evoked activity on the input and the network level we used spatial independent component analysis (sICA) to extract functionally similar neuronal groups. sICA resulted in a reduction of the dimensionality of the data sets recorded with high spatial and temporal resolution without loss of the stimulus specific responses (Reidl et al, NeuroImage in press). Using sICA two or more groups of glomeruli responding with different temporal profile to the same odor could be identified. Corresponding groups of glomeruli were extracted from the responses to the same odor in the recordings of the input to the olfactory bulb and in the recordings of the network activity. This suggests that a substantial part of the spatio-temporal network activity is driven by the input dynamics from the olfactory receptor neurons. Since it is not known if the temporal dynamics of the odor representations help or hinder odor identification and odor differentiation, we tested how a simple model of olfactory performs when using dynamic input patterns. Finally we are examining which properties of the spatio-temporal patterns can predict how much time mice need in order to differentiate various odor pairs.

# Optimal Decoding of Correlated Neural Population Responses in the Primate Visual Cortex

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Even the simplest environmental stimuli elicit responses in large populations of neurons in early sensory cortical areas. How these distributed responses are read out by subsequent processing stages to mediate behavior remains unknown. Here we used voltage sensitive dye imaging to measure directly population responses in the primary visual cortex (V1) of monkeys performing a demanding visual detection task. We then evaluated the ability of different decoding rules to detect the target from the measured neural responses. We find that small visual targets elicit widespread responses in V1, and that response variability at distant sites is highly correlated. These correlations render most previously proposed decoding rules inefficient relative to one that uses spatially antagonistic center-surround summation. This optimal decoder consistently outperforms the monkey in the detection task, demonstrating the sensitivity of our techniques. Overall, our results suggest an unexpected role for inhibitory mechanisms in efficient decoding of neural population responses.

# THE DYNAMICS OF EVOKED AND ONGOING ACTIVITY IN THE BEHAVING MONKEY

Amiram Grinvald and David B. Omer

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Previous findings from Voltage Sensitive Dye Imaging (VSDI) experiments done on anesthetized cats (Grinvald et al., 1991; Arieli et al., 1995;Arieli et al., 1996; Tsodyks et al., 1999; Kenet et al., 2003) indicated that the amplitude of ongoing activity (primarily synaptic potentials) is large, suggesting that it may play an important role in cortical processing by affecting the evoked activity and therefore the final behavior itself. VSDI was recently implemented also on the awake monkey (Slovin et al., 2002; Seidemann et al., 2002;) allowing monitoring of activity from the same patch of cortex for up to a year. Several questions have been recently addressed: what are the spatial-temporal characterizations of the ongoing activity in early visual areas of the behaving monkey? Does it affect the evoked activity? How is it related to the functional architecture? We investigated the cortical activity in the primary visual cortex of a behaving monkey during both evoked and ongoing conditions. We combined simultaneous VSDI with electrophysiological recordings of the local field potential (LFP) single and multi unit activities. In the evoked condition, the monkey was trained to fixate for 10s while presented with a full field moving grating, whereas, during the ongoing condition, the monkey was required to sit quietly in a totally dark room. We found that the VSD signals in both conditions are often highly similar to the LFP, just like in the anesthetized cat. The similarity between the VSD signals and LFP was highest within the α (9-14 Hz) frequency band. For the awake monkey, the ratio between amplitude of ongoing and evoked activity was much smaller (~1/6) than what was found in the anesthetized cats. However, extensive spike triggered averaging (STA) of the VSD signals revealed coherent spontaneous activity also in the awake primate. Some cells exhibited coherent activity with large assemblies in both area V1 and V2. Cortical states related to orientation representations, if any had a short life time and short coherence length, much smaller than those found in the anesthetized cats. We also detected in the LFPs short episodes of high-energy oscillations in the ~30Hz range. Those short episodes were not detected in the evoked sessions. In contrast with the Situation reported for anesthetized cat (Gray and Singer). During the evoked sessions, cortical columns with similar orientation preference were phase coherent. We observed a clear phase shift for the orthogonal orientation columns in V1. The cortical evoked response showed maximal coherence between VSD-VSD, VSD-LFP and VSD-Spike rate within three distinct frequency bands, 4Hz, 9-14Hz & 16-19Hz. These results suggest that ongoing activity is richer in awake animal may play multiple functional role in the awake primate as well.

# Symposium

## S23: Synchronization of Circadian and Neuronal Oscillators

***Monika Stengl, Marburg***

### Slide

**S23-1** Reconfiguring cellular ensembles within the suprachiasmatic nucleus
*William Joseph Schwartz, Worcester, Massachusetts (USA)*

**S23-2** Light-induced desynchronization of neuronal populations within the mammalian SCN
*Johanna H. Meijer, Leiden (NL)*

**S23-3** Synchronization and internal desynchronization between groups of circadian pacemaker neurons in the fruit fly *Drosophila melanogaster*
*Charlotte Helfrich-Förster, Regensburg*

**S23-4** The circadian pacemaker network in the cockroach *Leucophaea maderae*
*Thomas Reischig, Göttingen*

**S23-5** The neuropeptide pigment-dispersing factor affects circadian and ultradian rhythms in the circadian system of the cockroach *Leucophaea maderae*.
*Monika Stengl, Nils-Lasse Schneider and Nico Funk, Marburg*

**S23-6** Modelling Synchronization and Phase Resetting of SCN Neurons
*Hans-Peter Herzel and Achim Kramer, Berlin*

### Poster

**TS23-1C** Slow sleep oscillations: What makes big waves in the EEG?
*M. Mukovskiy, S. Chauvette, I. Timofeev and M. Volgushev, Bochum and Quebec (Canada)*

**TS23-2C** The neuroarchitecture of a putative circadian pacemaker area in the silverfish Lepisma saccharina *Lepisma saccharina*
*A. Dann and T. Reischig, Göttingen*

**TS23-3C** Neuronal connections of putative circadian pacemaker neurons in the accessory medulla of the cockroach Leucophaea maderae
*T. Reischig, Göttingen*

**TS23-4C** Possible influences of circadian melatonin on the function of neurosecretory neurons, and serotonin-modulated behavior in Crayfish.
*AJ. Farca Luna, T. Reischig and R. Heinrich, Göttingen*

**TS23-5C** IMPROVED LEARNING AND ALTERED SLEEP PATTERNS IN SHARP-1 AND -2 DOUBLE-MUTANT MICE
*M. Rossner, K. Radyushkin, PC. Baier, F. Kirchhoff and KA. Nave, Göttingen*

**TS23-6C** Unstable attractors in a system of pulse-coupled oscillators with delay
*EN. Subramanian, K. Efstathiou and H. Broer, Groningen (NL)*

**TS23-7C** 8-Br-cAMP mimicks the effects of pigment-dispersing factor on the electrical activity of circadian pacemaker candidates in the accessory medulla of the cockroach *Leucophaea maderae*
*NW. Funk, NL. Schneider and M. Stengl, Marburg and Oldenburg*

**TS23-8C** The molecular basis of the circadian clock in the cockroach *Leucophaea maderae*
*A. Werckenthin and M. Stengl, Marburg*

**TS23-9C** Extracellular long-term recordings of the isolated accessory medulla, the circadian pacemaker center of the cockroach *Leucophaea maderae reveal*ultradian and hint circadian rhythms
*NL. Schneider and M. Stengl, Oldenburg and Marburg*

**TS23-10C** Development of light sensitivity of clock genes *Per1* and *Per2* and immediate-early gene *c-fos* within the rat suprachiasmatic nucleus
*K. Laurinová, R. El-Hennamy, M. Sládek, Z. Bendová and A. Sumová, Prague (CZ)*

**TS23-11C** Ectopic release of PDF causes internal desynchronisation of the circadian system in *sine oculis* $^{medusa}$ mutant flies
*C. Wülbeck and C. Helfrich-Förster, Regensburg*

**TS23-12C** Light and temperature synchronizations of the *Drosophila* circadian clock
*T. Yoshii, C. Wülbeck and C. Helfrich-Förster, Regensburg*

Introductory Remarks to Symposium 23

# Synchronization of Circadian and Neuronal Oscillators

Monika Stengl, Marburg

A network of circadian oscillators controls the timing of neuronal circuits in brains. While knowledge about the molecular composition of the circadian clock in the nucleus is accumulating, little is known about the neuronal network of the circadian pacemakers and their mechanisms of synchronization. Recent evidence indicates strong connections between circadian and ultradian rhythms such as gamma frequency oscillations, which are thought to underlie higher brain functions. Elucidating these connections between intercellular as well as intracellular oscillations at different time scales from milliseconds to hours will be crucial for the understanding of circadian oscillators as well as for the understanding of higher brain functions.

In this symposium W.J. Schwartz and J.H. Meijer will present new findings about mechanisms of synchronization of circadian oscillators within and between the bilaterally symmetric suprachiasmatic nucleus (SCN), the circadian pacemaker center of mammals. Both will examine with different methods the dissociation/synchronization between molecular, neuronal, and behavioural rhythms in mammals. Achim Kramer and Hans-Peter Herzel will focus on theoretical models of intracellular and intercellular synchronization of the mammalian circadian clock. C. Helfrich-Förster and T. Reischig analyze the synchronization of oscillators within the accessory medulla (AMe), the insect clock, and between AMe neurons and midbrain oscillators. Monika Stengl studies the connections between circadian scale and gamma frequency scale oscillations in neurons of the insect circadian clock. Thus, from genes to neurons and behavior this symposium will focus on the function of circadian synchronization signals which appear to orchestrate the timing of neuronal circuits and physiological processes in stable phase relationships not only on the scale of hours but also on the millisecond scale.

# Reconfiguring cellular ensembles within the suprachiasmatic nucleus

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The circadian clock in the suprachiasmatic nucleus (SCN) is composed of multiple single-cell circadian oscillators, and a challenge now is to learn how individual cells are assembled to create an integrated tissue pacemaker that can orchestrate the temporal programs of whole organisms. By measuring SCN gene expression (in situ hybridization) as an assay of clock activity, we have found that assembled cellular oscillators can assume different configurations within the SCN, giving rise to unusual locomotor activity patterns. Thus, in hamsters maintained in constant light, splitting of the single circa-24 hr activity bout into two circa-12 hr components appears to be the consequence of a paired SCN that is reorganized into two oppositely-phased, left- and right-sided circadian pacemakers. In rats exposed to an artificially short light-dark cycle, the simultaneous expression of two stable circadian motor activity rhythms with different period lengths corresponds to the desynchronization of circadian pacemakers in the ventrolateral and dorsomedial subdivisions of the SCN (as previously defined by regional differences in their cyto- and chemo-architecture and topography of afferents and efferents). These kinds of reconfigurations (left/right, dorsal/ventral) of regional oscillators should provide a powerful approach for understanding intercellular coupling, tissue organization, and differential outputs of the SCN in intact, behaving animals.

# Light-induced desynchronization of neuronal populations within the mammalian SCN

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The suprachiasmatic nucleus (SCN) of mammals functions as a circadian pacemaker. The major external stimulus that synchronizes the circadian pacemaker is light. Light information reaches the SCN via the retinohypothalamic tract (RHT), which contains several neurotransmitters, the most important ones being glutamate and PACAP. The RHT terminates predominantly in the ventral aspect of the rat SCN and more sparsely in the dorsal part. Expose of rats to shifted light-dark cycles, reveals that within the SCN, expression rhythms of Per1 reset with different speed to the new cycle (Nagano et al, 2003; Nakamura et al, 2005). We observed that following a 6 hour delay of the light-dark cycle, electrical activity rhythms in the ventral SCN reset rapidly, and in the dorsal SCN they reset slowly. We also observed that the dorsal and ventral SCN transmit electrical information to the other part of the nucleus, resulting in bimodal electrical activity patterns during resetting. Application of the $GABA_A$ antagonist bicuculline blocked the transmission of electrical activity. Interestingly, GABA was exclusively inhibitory to the ventral SCN, but appeared to be also excitatory to the dorsal SCN. We propose that GABA plays an important role in coupling between dorsal and ventral groups of oscillators within the SCN.

# Synchronization and internal desynchronization between groups of circadian pacemaker neurons in the fruit fly *Drosophila melanogaster*

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The activity rhythm of the fruit fly is controlled by a network of circadian pacemaker neurons that cyclically express the clock genes *period*, *timeless*, *clock* and cycle. These neurons are conventionally divided in 6 main groups, whereby 3 of them locate in the lateral and 3 in the dorsal protocerebrum (the so called Lateral and Dorsal Neurons). Most of these neurons send fibers into the dorsal protocerebrum and into the accessory medulla - a small neuropil that houses the circadian pacemaker center in cockroaches. The fibers of all clock neurons largely overlap, suggesting that they are functionally connected and may thus provide a common circadian output signal that is transferred from the dorsal protocerebrum to the thoracic locomotor centers via electrical or/and humoral pathways. We are interested in the question how the oscillations within different clock neurons are synchronized with each other to produce a common circadian output. Manipulations that lead to an internal desynchronization are most suited to understand the coupling mechanism between the neurons. Recently, we found that the Lateral Neurons respond differently to light, and that exposure of the flies to constant light provokes one group to free-run with short period and the others with long period (Rieger et al., 2006, J. Neurosci. 26, 2531-2543). A similar internal desynchronization can be observed on the behavioral level, if the neuropeptide pigment-dispersing factor (PDF) is ectopically expressed in the dorsal protocerebrum (Helfrich-Förster et al., 2000, J. Neurosci. 20, 3339-3353), suggesting that PDF is critically involved in coupling the oscillations of different pacemaker neurons. PDF is expressed in the small and large ventral Lateral Neurons (s-$LN_V$ and l-$LN_V$), and the lack of PDF leads to a period shortening and a gradual decrease of synchrony between all neurons (Renn et al., 1999, Cell 99, 791-802; Peng et al., 2003, PLOS Biol. 1, E13; Lin et al., 2004, J. Neurosci. 24, 7951-7957). Thus, too high or too low PDF-levels seem to be deleterious for the activity rhythms. Here, I will present the first evidence that the l-$LN_V$ cells are the most likely candidates to couple the oscillations of the other clock neurons via PDF (see also Wülbeck and Helfrich-Förster, this volume).

# The circadian pacemaker network in the cockroach *Leucophaea maderae*

Thomas Reischig

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Most bilateral animals have specialised neuronal networks that act as circadian pacemaker centres in the central nervous system. Generally, they lie in compartments that are involved in processing of visual information. This is also the case in the cockroach *Leucophaea maderae*, were mutually coupled pacemakers controlling circadian locomotor activity reside in the optic lobes. Despite of the tremendous insights into the molecular machinery of circadian rhythm generating achieved in the fruitfly *Drosophila melanogaster*, insects with larger brains as *L. maderae* are still indispensable to explore structures and functions of the neuronal networks of insect circadian pacemakers.

In *L. maderae* and other insects, neurons immunoreactive to an antiserum against pigment-dispersing factor (PDF) are thought to be circadian pacemaker and output neurons. PDF-immunoreactive medulla neurons (PDFMe) densely arborise in the accessory medulla, a small non-retinotopic neuropil of the optic lobe of the cockroach. The accessory medulla is suggested to be an integration centre for circadian timing information, since the AMe resides at the position were the pacemaker driving circadian locomotor activity was located, and since it is densely innervated by the PDFMe and other neurons that appear to be involved in the circadian system.

In my talk I will provide a view over the current knowledge about the neuronal network of the circadian system of *L. maderae*. The main interest is focussed on the neuronal pathway starting from the compound eyes, where light information for pacemaker synchronisation is received, via the accessory medulla with its putative pacemaker neurons down to the pacemaker's output elements in the central brain. A general model for the neuronal pathway from light entrainment to circadian output including neuronal circuitry in the accessory medulla is introduced.

# The neuropeptide pigment-dispersing factor affects circadian and ultradian rhythms in the circadian system of the cockroach *Leucophaea maderae*.

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Lesion and transplantation studies located the circadian clock of the cockroach *Leucophaea maderae* to the accessory medulla (AMe), ventromedially of the medulla in the brains´optic lobes. The AMe is strongly innervated by pigment-dispersing factor-immunoreactive (PDF-ir) neurons among a manyfold of associated peptide-immunoreactive neurons. The peptide PDF is assumed to control circadian locomotor activity rhythms via unknown mechanisms. Neurons of the AMe fire very regularly. In addition to circadian periods the AMe-neurons also produce ultradian action potential rhythms such as gamma frequency range (30-70 Hz) oscillations. The circadian pacemaker neurons are phase-locked via synaptic and non-synaptic mechanisms and form assemblies of synchronously spiking cells. While the cells within one assembly spike with zero phase difference, neurons belonging to different assemblies differ in phase. Injection of the neuropeptide PDF phase-locked cells belonging to different assemblies via inhibitions. Injection of cAMP-, but not cGMP-analoga mimicked the PDF-effects. We assume that the PDF-dependent phase-locking of ultradian action potential rhythms is responsible for PDF-dependent onset of circadian locomotor activity rhythms.
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# Slow sleep oscillations: What makes big waves in the EEG?

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Slow rhythms at frequencies below 1 Hz are a hallmark of the brain activity during the slow wave sleep and under some anaesthetics. The slow, large amplitude waves in the local field potential (LFP) or electroencephalogram (EEG) reflect alternating states of activity and silence in thalamocortical networks. Membrane potential of neocortical neurons fluctuates with this rhythm between a depolarized (active) and a hyperpolarized (silent) state. Although the slow rhythm is very clear both in the summary activity and in the membrane potential fluctuations of neocortical neurons, the individual events - waves in the EEG or active states in the cells - vary in their amplitude and duration. What makes the waves in the EEG so different?

Since EEG signal reflects summary activity of many cells, there are three factors, which may contribute to its variability. First, amplitude variation of the membrane potential changes in each individual neuron. Second, different degree of synchrony, with which neurons switch between states. Third, variable number of neurons, participating in that particular episode of activity, and thus contributing to that particular wave in the EEG. To disentangle the contribution of these factors, we recorded the LFP simultaneously with intracellular activity of 2-4 neocortical neurons in cats under ketamine-xylazine anesthesia. The recorded neurons were located either distantly (4 mm between recording sites) or close (within 0.5 - 1 mm) one from the other and from the LFP electrode. Totally, we analyzed 15 data sets. In these recordings, we calculated the amplitude of each slow wave in the LFP, and the respective values of the (i) amplitudes of membrane potential changes in each neuron, (ii) synchrony of the involvement of simultaneously recorded neurons in the activity and (iii) the portion of the simultaneously recorded neurons, involved in that particular episode of activity. Next, we calculated the correlations between the amplitude of the LFP wave and the above intracellular measures. We found, that the strongest factor contributing to the variability of the amplitude of slow waves in the LFP was the amplitude variability of the membrane potential changes in individual neurons. Here, significant correlation ($p<0.01$) was found in 76% of cases. The next factor was the number of neurons, contributing to the particular LFP wave. The amplitude of LFP wave was significantly correlated with the portion of neurons involved in that episode of activity in 65% of cases. Moreover, these two factors were mutually independent. The synchrony of the involvement of neurons in activity was less strong factor. It was correlated with the amplitude of the LFP wave in only 40% of cases.

We conclude, that the factors, contributing to the variability of the amplitude of slow waves in the LFP are, in decreasing strength: the amplitude of membrane potential fluctuations in neurons, the number of neurons involved in the activity episode, and the synchrony of their involvement.

# The neuroarchitecture of a putative circadian pacemaker area in the silverfish Lepisma saccharina *Lepisma saccharina*

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Insects have a small but conspicuous neuropil in the optic lobe, the accessory medulla (AMe), which is suggested to process information of circadian pacemaker neurons and environmental Zeitgeber signals to generate circadian output signals. The insect AMe is densely innervated by pigment-dispersing factor immunoreactive (PDF-ir) medulla neurons (PDFMe), which are presumptive circadian pacemaker and output neurons (Homberg et al. 2003, Chronobiol Int 20:577). To reveal a neuronal groundplan for the insect AMe and hence, the insect circadian system, we currently investigate the AMe of a an apterygote insect of the order Zygentoma, the silverfish *Lepisma saccharina*. Zygentoma are apparently the sistergroup of all pterygote insects. We are investigating the optic lobes and AMe of the silverfish with histological and immunocytochemical methods, and are developing a comprehensive three-dimensional model of the silverfish's brain to integrate anatomical data.

Due to the reduced compound eyes of the silverfish, the optic lobes are rather small compared to other insects equipped with larger eyes. A lamina and a medulla is well discernable, but the existence of a lobula is at least doubtful. The medulla consists of four consecutive layers, which are arranged almost perpendicularly to the mean visual axis of the compound eyes, contrarily to pterygote insects, were the medulla is rotated about 70-90° around its vertical axis. An area densely filled with PDF-ir fibre projections covers the whole distalmost layer of the medulla, and appears to comprise the silverfish's AMe. Interestingly, the PDFMe are not situated at the proximal medulla as in other insects, but lie further distal in a cortical area between lamina and medulla anteriorly to the first optic chiasm. We suggest that the change of the distal position of the silverfish's PDFMe to the more proximal position that is observed in Pterygota is caused by a medulla rotation in the ancestors of pterygote insects. We further propose that PDF-ir fibres and processes of other AMe-neurons in the distal layer of the medulla have condensed to form the typical AMe that is now observed in Pterygota.

Further, we performed immunostaining with antisera against FMRFamide related peptides and the transmitters GABA and serotonin in the silverfish brain. A cluster of FMRFamide-ir neurons was closely neighboured to the PDFMe. Double immunolabelling experiments revealed colocalising PDF-ir and FMRFamide-ir in about a third of the PDFMe. This situation was formerly found in cockroaches and locusts (Petri et al. 1995, Cell Tissue Res 282:3; Würden & Homberg 1995, J Comp Neurol 362:305), and emphasises the neuromodulatory function of these neurons. Also, GABA-ir and serotonin-ir neurons cluster in vicinity to the PDFMe, but with no apparent colocalisation of immunoreactivities, as in cockroaches and locusts. Our data indicate a conserved neuroarchitecture of the AMe throughout insect orders and will help to reveal the basic neuronal layout of the insect circadian system.

# Neuronal connections of putative circadian pacemaker neurons in the accessory medulla of the cockroach Leucophaea maderae

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In *L. maderae* and other insects, neurons immunoreactive (-ir) to an antiserum against pigment-dispersing factor (PDF) are thought to be circadian pacemaker and output neurons (Homberg et al. 2003, Chronobiol Int 20:577). PDF-ir medulla neurons (PDFMe) densely arborise in the accessory medulla (AMe), a small neuropil of the optic lobe of the cockroach, which is suggested to be an integration centre for circadian timing information. Further, the PDFMe form large arborisation fields in various areas of the optic lobes and central brain, which appear to be output targets of phase information transmitted by the PDFMe. The aim of this study is to reveal the connectivity of the PDFMe to putative input and output elements.

No direct photoreceptor input into the AMe exist, and a set of gamma-aminobutyric acid (GABA)-ir neurons near the AMe is proposed to comprise a part of the neuronal entrainment pathway between compound eyes and PDFMe (Petri et al 2002, J Exp Biol 205:1459). Within the AMe, input synapses to PDF-ir fibres were demonstrated in an earlier study (Reischig and Stengl 2003, Cell Tissue Res 314:421). Now, anti-GABA and anti-PDF labelling on ultrastructural level suggest that this synaptic input to the PDFMe is partly performed by GABA-ir neurons, thus supporting the hypothesis that GABA-ir neurons contribute to the light entrainment of the PDFMe.

In the central brain, PDF-ir neurons innervate large areas where the target neurons for circadian phase information are assumed. Although output of the PDFMe neurons obviously is performed by paracrine neuropeptide release, it is not unlikely that target neurons are additionally contacted by synapses. To find postsynaptic partners of the PDFMe in the central brain, anti-PDF stainings on ultrastructural level were performed, and all central arborisation areas of the PDFMe were evaluated. However, contrarily to observations in the AMe, no output synapses of PDF-ir terminals were found so far in the central brain, thus suggesting that circadian phase information is transmitted to central targets exclusively by peptide release.

Even if PDFMe are not connected to target neurons via synapses in the central brain, they should arborise in vicinity to the PDF-ir terminal to be in diffusion reach of peptide released from their varicosities. To test whether these targets are descending neurons that control motor patterns in the ventral ganglia to elicit specific behaviour, tracer backfills of the cervical and oesophageal connectives of *L. maderae* where combined with anti-PDF immunolabelling. Only minor overlap of PDF-ir terminals with processes of PDFMe was observed. Further, neurons of the pars intercerebralis (PI) projecting to the corpora cardiaca (CC), which are neurohemal organs in insects, are also likely targets of circadian output of the PDFMe, since internal clocks are often closely connected to the neuroendocrine systems in animals. However, backfills from the CC revealed no conspicuous overlap of PI-neurons with PDF-ir terminals. The data suggest that the central effector pathway of the circadian system of *L. maderae* downstream to the PDFMe consists of local interneurons that are exclusively modulated by peptide release.

# Possible influences of circadian melatonin on the function of neurosecretory neurons, and serotonin-modulated behavior in Crayfish.

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Circadian rhythmicity in crustaceans has been documented extensively but until now no anatomical structure has been shown to harbour the complete features of a circadian pacemaker. In this study, we used confocal microscopy to identify elements of the possible pacemaker in the eyestalk and the subesophageal ganglion SEG, the main pacemaker candidates in crayfish.

We first describe the distribution of PER protein, an important element in the negative transcription-translation loop which regulates circadian rhythms, in the crayfish nervous system. In the second part we tried to map the central nervous distributions of the indolamine melatonin, its precursor serotonin, and Pigment Dispersing Hormone (PDH). All of them have been suggested to function as potential output signals that convey time information from the pacemakers to other tissues.

Strong PER- immunoreactivity was observed in cells of the Median Protocerbral Neuropil and fibers of the SEG, as well as in fibers of the Medulla Externa, Medulla Interna and Medulla Terminalis of the eyestalks. PDH-immunofluorescent cells with fibers projecting to the Sinus Gland were located in cells of the X-organ, the main neurosecretory organ of the crayfish. PDH immunofluorescence was also found in cells of the Lamina Ganglionaris and in fibers of the Medulla Externa and Medulla Interna of the eyestalk which extend as far as to the SEG. Serotonin-containing cells were located in the X-organ, the Medulla Terminalis. Serotonin was also detected in Giant cells of the SEG and in fibers that emerge from the Medulla external and enter the circumesophagial connectives, presumably projecting to the ventral nerve cord. Double immunostainning against PDH and serotonin revealed no colocalization in particular cells. However, close contact of serotonin-containing fibers with fibers containing PDH in the Medulla Terminalis and Sinus Gland was observed. This may suggest mutual influences of these neurons that could influence the release of their signalling molecules. Melatonin-related immunofluorescence was found in some, but not all, preparations. If seen, labelling was restricted to some cells of the eyestalk and retina. The indolamine melatonin, its precursor, serotonin, and the Pigment Dispersing Hormone (PDH) seem to be involved in the control of circadian functions in crustaceans. Potential interactions between signalling pathways using these substances to control circadian rhythms are possible. Our results support the idea of the eyestalk of the crayfish as an integral part of the circadian pacemaker.

# IMPROVED LEARNING AND ALTERED SLEEP PATTERNS IN SHARP-1 AND -2 DOUBLE-MUTANT MICE

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Emerging evidence suggests that higher cognitive processing and balanced sleep-wake cycles are interdependent. Here, we show that the basic Helix-Loop-Helix (bHLH) transcription factors SHARP-1 and -2 are essentially involved in learning and memory formation and in the control of sleep. We analysed SHARP-1 and -2 double mutant (S1/2-/-) mice in behavioural tests monitoring learning and memory formation. In hippocampus and cortex dependent aversive and non-aversive tasks, S1/2-/- mice show improved learning compared to wild-type animals. Moreover, hippocampal synaptic plasticity is enhanced in S1/2-/- mice. Quantitative analyses of EEG recordings reveal profound changes in the relative distribution of different vigilance states during light and dark periods. Sleep deprivation leads to a reduced rebound REM sleep and shifts theta oscillations significantly in S1/2-/- mice. Finally, sleep requirement and memory formation seem to be partially uncoupled in S1/2-/- mice. In sharp contrast to wild-type controls, sleep deprivation does not lead to a dramatic reduction in fear memory in S1/2-/- mice. Thus, SHARP-1 and -2 likely serve a dedicated role in the control of sleep and memory formation.

# Unstable attractors in a system of pulse-coupled oscillators with delay

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We consider a coupled cell network of one dimensional harmonic oscillators. The coupling between the cells is defined using the Mirollo-Strogatz (MS) model with non-zero delay. Since this is a `delay' system its dynamics is described in an infinite dimensional phase space. Previous research has shown that Mirollo-Strogatz systems with sufficiently large number of oscillators have unstable attractors. These are saddle points whose stable set has non-empty interior and it turns out that they occur generically in the class of systems we consider. When a system has unstable attractors, there are open sets of initial conditions that converge to one of the attractors and stay there for indefinite time until, given the instability, a small perturbation enables the state of the system to switch from one attractor to another. In addition, it is possible to have heteroclinic connections between unstable attractors which could be a useful way for information processing in neural systems. In the current study, we provide a rigorous mathematical proof of the fact that networks that consist of three or more oscillators have unstable attractors for an open set of parameter values and we provide exact estimates of the parameter values for which unstable attractors exist. The orbits converge to the unstable attractor through the following mechanism: an open region of the phase space collapses to the stable manifold of the attractor and then converge to the attractor in finite time. The unstable attractors that we study here, correspond to the synchronization of n-1 oscillators in a system of n oscillators and they co-exist with stable attractors.

# 8-Br-cAMP mimicks the effects of pigment-dispersing factor on the electrical activity of circadian pacemaker candidates in the accessory medulla of the cockroach *Leucophaea maderae*

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In the cockroach *Leucophaea maderae* transplantation studies located the circadian pacemaker center which controls locomotor activity rhythms to the accessory medulla (AMe). The AMe is a small, nodular neuropil at the ventromedial edge of the medulla in the optic lobe. It is densely innervated by a multitude of peptidergic neurons, among them 12 pigment-dispersing factor immunoreactive (PDF-ir) neurons. These neurons are circadian pacemaker candidates, controlling locomotor activity rhythms in *L. maderae* and *Drosophila melanogaster*. PDF was shown to act as a non-photic input signal into the clock, delaying the phase of locomotor activity at the late subjective day. Extracellular recordings of the excised AMe revealed that circadian pacemaker candidates produce circadian as well as ultradian rhythms. The ultradian oscillating cells produce very regular interspike-intervals and are coupled via synaptic (mostly GABAergic) and nonsynaptic interactions, forming different assemblies of cells that share the same frequency and the same phase (timing of spikes). Cells belonging to different assemblies differ in phase, but can share the same period. Application of PDF was shown to synchronize phase and period of cells belonging previously to different assemblies mostly via inhibition of electrical activity.

This study examined whether the PDF-receptor is coupled to a $G^S$-protein stimulating adenylyl cyclase, resulting in an increase of cAMP levels. Therefore, PDF and membrane-permeable, hydrolysis-resistant cyclic nucleotide analogues were applied in extracellular recordings of excised accessory medullae (AMae). The application of 8-Br-cAMP, but not 8-Br-cGMP mimicked the effects of PDF application, namely inhibition of action potential activity in absence of synaptic interactions. Thus, apparently in the cockroach PDF acts via cAMP rises. In current studies we are searching for further components of the PDF signalling pathway. We are testing, whether cAMP rises lead to an activation of the cAMP dependent protein kinase (PKA) or to an activation of the exchange protein directly activated by cAMP (Epac), a guanine nucleotide exchange factor (GEF) for the Ras-like small GTPases Rap1 and Rap2. [Supported by DFG STE531/15-1,2].

# The molecular basis of the circadian clock in the cockroach *Leucophaea maderae*

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In *Drosophila melanogaster* as well as in the cockroach *Leucophaea maderae* different groups of circadian pacemakers such as the pigment-dispersing factor immunoreactive (PDF-ir) neurons were identified, but the interactions of these pacemakers are still not fully resolved. To analyze the circadian network, which orchestrates the timing of physiological processes in the brain as well as in the remaining body, electrophysiological as well as molecular investigations are necessary. Because electrophysiological studies are very tedious and limited in the fruitfly *D. melanogaster*, we turned to the large cockroach *L. maderae* as an alternative circadian model system. With molecular genetics and immunocytochemistry using antibodies against clock genes and neuropeptides we attempt to identify the circadian network in the cockroach. We started to clone circadian clock genes such as *period* from *L. maderae* and focus on the analysis of the functions of the PDF-ir neurons within this network. Thus, we search for the PDF-receptor in *L. maderae*, next to mapping the synaptic connectivity of the PDF-ir neurons within the accessory medulla, the circadian pacemaker center of the cockroach. So far, *L. maderae period* has been cloned and an antibody has been generated. In western blots PER-immunoreactive bands varied at different circadian times. In addition, immunostainings identified circadian pacemaker candidates. [Supported by DFG STE531/15-1]

# Extracellular long-term recordings of the isolated accessory medulla, the circadian pacemaker center of the cockroach *Leucophaea maderae reveal*ultradian and hint circadian rhythms

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In the cockroach *Leucophaea maderae* transplantation studies located the circadian pacemaker center which controls locomotor activity rhythms to the accessory medulla (AMe), ventromedially to the medulla of the brain´s optic lobes. The AMe is densely innervated via GABA- and manyfold peptide-immunoreactive neurons. They express ultradian action potential oscillations in the gamma frequency range and form phase-locked assemblies of synchronously spiking cells. Peptide application resulted in transient rises of extracellularly recorded activity. It remained unknown whether transient rises in spontaneous electrical activity as a possible indication of peptide release occurs in the isolated circadian clock in a rhythmic manner.
In extracellular glass electrode recordings of the isolated AMe in constant darkness, which lasted at least 12 hours, the distribution of daytime-dependent changes in activity independently of the absolute action potential frequency were examined. Rapid, transient changes in activity preferentially occurred at the mid-subjective night, with a minimum at the middle of the subjective day, hinting the presence of circadian rhythms in the isolated circadian clock. Additionally, ultradian rhythms in activity change that are multiples of a fundamental 2 hours period were observed. We hypothesize that circadian rhythms might originate from coupled ultradian oscillations, possibly already at the single cell level.

# Development of light sensitivity of clock genes *Per1* and *Per2* and immediate-early gene *c-fos* within the rat suprachiasmatic nucleus

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Many behavioral, physiological and molecular processes exhibit diurnal rhythms. In mammals is the center which governs circadian (period ± 24h) rhythms located in the supachiasmatic nuclei of hypothalamus (SCN). Light entrains circadian rhythms to a 24h period of solar day. Previous studies show that light pulse administration during the first part of subjective night delays and during the second part advances the phase of circadian rhythms. Light pulses during the subjective day are without any effect. Studies of light sensitive immediate-early gene *c-fos* show that time-gate enabling the phase resetting is controlled by the clock itself. Research of molecular mechanism of endogenous circadian rhytmicity (clock genes) shows that *Period1* (*Per1*) and *Period2* (*Per2*) are important for light resetting of circadian clock. In SCN of adult rats light may induce high expression of *Per1* during the whole subjective night, but not during subjective day. *Per2* expression in SCN is inducible by light during the first part of subjective night, but not during the second part and during subjective day. Development of time-gate mechanism was still studied only on the light sensitivity of *c-fos*. 1st day after birth is the expression of *c-fos* in SCN of rat inducible by light at any time of day and the gating mechanism which is present in adult SCN is still not developed.

The aim of this study is to elucidate when in the postnatal development clock genes *Per1* and *Per2* start to be light-sensitive and how develops the time-gate mechanism of *Per1*, *Per2* and *c-fos* within the SCN of early postnatal rat. Pregnant rats and rats with their pups were maintained under light-dark regime with 12h of light and 12h of darkness. The day of birth was noted as postnatal day 0 (P0). Rats with their pups were released into darkness at the expected dark-light transition at P1, P3, P10 or P20. The pups were exposed to a 30 min light pulse during the subjective day or during the first or the second part of subjective night. Control pups were left untreated in the constant darkness. Both groups were sampled 30min, 1h and 2h after start of each pulse. Levels of *Per1*, *Per2* and *c-fos* mRNA on brain slices containing SCN were assessed by in-situ hybridization with consequent autoradiography. Some slides were covered with autoradiographic emulsion to view the localization of mRNA signal within SCN.

The expression of *Per1* and *c-fos* seems to be inducible by light pulse at P1. The time-gate mechanism of light sensitivity of *Per1* and *c-fos* expression in SCN starts to appear at P3 and matures gradually in further development. *Per2* expression in SCN became light sensitive later than expression of *Per1* and development of gating mechanism seems to be slower. Our results show that the time-gates of light sensitivity of *Per1*, *Per2* and *c-fos* expression develops individually. The development of gating of light sensitivity is also accompanied by localization of light-induced mRNA signal in the ventral part of SCN.

# Ectopic release of PDF causes internal desynchronisation of the circadian system in *sine oculis* $^{medusa}$ mutant flies

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*Drosophila melanogaster's* rhythmic activity is controlled by six groups of circadian pacemaker cells: five small and four large ventral Lateral Neurons (s-$LN_v$ and l-$LN_v$), six to seven dorsal Lateral Neurons ($LN_d$) and three groups of dorsal neurons ($DN_1$, $DN_2$, $DN_3$). The ventral cell goups appear to communicate with the dorsal cell goups via the neuropeptide PIGMENT DISPERSING FACTOR (PDF) leading to a coherent rhythm in all neurons (Lin et al., 2004, J Neurosci. 24, 7951-7957). PDF is secreted rhythmically into the dorsal protocerebrum by four of the five s-$LN_v$ cells (Park et al., 2000, PNAS 97, 3608-3613). Furthermore, PDF is released in a paracrine manner by all four l-$LN_v$ cells on the surface of the medulla, probably controlling sensitivity rhythms in the compound eyes (see Pyza and Meinertzhagen, 1999, J Neurobiol 40, 77-88). Additionally, the l-$LN_v$ cells connect the Lateral Neurons of both brain hemispheres with each other (Helfrich-Förster et al., 2006, J Comp Neurol, in press).

Here we show that *sine oculis* $^{medusa}$ ($so^{mda}$) mutants display a behavioral desynchronization into two free-running components ($\tau 1$ = 21.5h, $\tau 2$ = 24.9h) as soon as the flies are transferred to constant conditions. $so^{mda}$ flies lack Bolwig's Organ and Nerve, which pioneers the axonal outgrowth of the photoreceptor axons into the optic anlage and is essential for normal organization of the optic lobes (Serikaku and O'Tousa, 1994, Genetics 138, 1137-1150). Therefore $so^{mda}$ mutants possess tiny optic lobes forcing the l-$LN_v$ cells -devoid of their normal aborization targets on the surface of the medulla- to aberrantly project into the dorsal protocerebrum. These aberrant projections may interfere with the normal connectivity of the pacemaker neurons leading to the observed internal desynchronization between different clock cell groups. This reminds on similar results found after ectopic PDF-expression in the dorsal protocerebrum (Helfrich-Förster et al., 2000, J. Neurosci. 20, 3339-3353). In order to test, whether PDF secretion from the misguided l-$LN_v$ neurites is the cause for the internal desynchronization, we generated $so^{mda};pdf^{01}$ double mutants. The doubly mutant flies showed weak rhythms with a period around 23 hours that is typical for $pdf^{01}$ mutants. Not a single fly exhibited an internal desynchronization into long and short periods, suggesting that increased PDF levels in the dorsal protocerebrum are responsible for this behavior. Our results also suggest that the l-$LN_v$ cells are crucial for coupling the rhythms of the other clock cells. Future studies will show, which neurons become out of synchrony in $so^{mda}$ mutants.

# Light and temperature synchronizations of the *Drosophila* circadian clock

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Most organisms display daily rhythms in physiology and behaviour. The rhythms are driven by an endogenous time-keeping mechanism, the so-called circadian clock. In *Drosophila melanogaster*, the pacemaker clock neurons controlling locomotor activity rhythm have been identified. Two of main components of the clock, PER and TIM proteins, are rhythmically expressed in different groups of neural clusters in the brain. Previous studies suggested that these clusters play different roles in the control of the locomotor rhythm (Veleri et al., 2003, Curr. Biol. 13, 1758-1767; Klarsfeld et al., 2004, J. Neurosci. 24, 1468-1477; Stoleru et al., 2004, Nature 431, 862-868; Grima et al., 2004, Nature 431, 869-873; Rieger et al., 2006, J. Neurosci. 26, 2531-2543).

The locomotor rhythm can be synchronized by light-dark cycles and temperature cycles, while light-dark cycles are the more dominant environmental cue (zeitgeber) for the clock. Recent studies found that certain PER-positive neurons, the LPNs and the DN3s, may be especially important for the temperature synchronization of the locomotor rhythm (Yoshii et al., 2005, Eur. J. Neurosci. 22, 1176-1184). This data suggest that some of the pacemaker neurons are involved in the temperature synchronization and others are important for the synchronization to light-dark cycles. CRYPTOCHROME (CRY) is a primary component for the light synchronization of the clock and many but not all pacemaker neurons express CRY. By *in situ* hybridization, immunohistochemistry and *cry*-driven GFP expression, we found that the DN2s and some of the DN1s and DN3s are CRY-negative pacemaker neurons. Therefore, these neurons might be the pacemaker neurons for the temperature synchronization.

Here we investigated the synchronization patterns of the locomotor rhythm under conflicting conditions of light-dark cycles and temperature cycles. When exposed to the different periods of light-dark cycles and temperature cycles, wild-type flies showed strong synchronization to the light-dark cycles and only a weak response to the temperature cycles. But the loss-of-function mutant flies of CRY, $cry^{b}$, showed more synchronization to the temperature cycles than to the light-dark cycles. This underlines our hypothesis. In future experiments, we will investigate on the cellular level whether the CRY-negative pacemaker neurons cycle in synchrony to the temperature cycles.

# Symposium

## S24: Do We Know What the Early Visual System Computes?

*Matthias Bethge and Christoph Kayser, Tübingen*

### Slide

**S24-1** Opening remarks for Symposium 24
*Matthias Bethge and Christoph Kayser, Tübingen*

**S24-2** Understanding retinal output with an encoding model of multi-neuron responses
*Jonathan W Pillow, London (UK)*

**S24-3** The hidden clock in LGN -- is phase coding employed in early vision?
*Kilian Koepsell, Berkeley, CA (USA)*

**S24-4** The role of contrast adaptation in the coding of natural visual stimuli
*Nicholas Lesica, Martinsried*

**S24-5** A saliency map in the primary visual cortex for bottom up visual selection --- theory and experiments
*Li Zhaoping, London (UK)*

**S24-6** Masking by Mach Bands
*Bruce Henning, Oxford (UK)*

### Poster

**TS24-1C** Contrast dependent spatial integration in V1: BOLD, LFP, and spikes
*A. Thiele, D. Hunter, MA. Gieselmann and L. Sun, Newcastle upon Tyne (UK)*

**TS24-2C** Identifying temporal population codes in the retina using canonical correlation analysis
*M. Bethge, JH. Macke, S. Gerwinn and G. Zeck, Tübingen and Martinsried*

**TS24-3C** Bayesian neural system identification: error bars, receptive fields and neural couplings
*S. Gerwinn, M. Seeger, G. Zeck and M. Bethge, Tuebingen and Martinsried*

**TS24-4C** The Functional Role of Patchy Cortical Circuits
*RR. Rohrkemper Jr. and RJ. Douglas, Zurich (CH)*

Introductory Remarks to Symposium 24

# Do We Know What the Early Visual System Computes?

Matthias Bethge and Christoph Kayser, Tübingen

Decades of research provided much data and insights into the mechanisms of the early visual system. Currently, however, there is great controversy on whether these findings can provide us with a thorough functional understanding of what the early visual system does, or formulated differently, of what it computes. At the Society for Neuroscience meeting 2005 in Washington, a symposium was held on the question "Do we know that the early visual system does", which was accompanied by a widely regarded publication in the Journal of Neuroscience. Yet, that discussion was rather specialized as it predominantly addressed the question of how well neural responses in retina, LGN, and cortex can be predicted from noise stimuli, but did not emphasize the question of whether we understand what the function of these early visual areas is. Here we will concentrate on this neuro-computational aspect of vision. Experts from neurobiology, psychophysics and computational neuroscience will present studies which approach this question from different viewpoints and promote a critical discussion of whether we actually understand what early areas contribute to the processing and perception of visual information.

# Understanding retinal output with an encoding model of multi-neuron responses

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Neural circuits are known to exhibit correlated spiking activity, whose origin and significance is a topic of much current interest. I will discuss using a generalized linear model, fit using maximum likelihood, to account for the stimulus-dependence, history-dependence, and correlation structure in the spike responses of a group of nearby neurons in primate retina. I will discuss implications for the multi-neuronal coding of visual information.

# The hidden clock in LGN -- is phase coding employed in early vision?

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The retinal network encodes information about the visual stimulus into neural activity. Retinal ganglion cells transmit this information in form of spikes via the thalamus to visual cortex. Although the spike rate of individual retinal ganglion or thalamic cells in response to visual stimuli is well understood, it is still an open question how the visual information is encoded within the whole population of retinal ganglion cells. For example, it is still controversial, if retinal ganglion cells use rates or precisely timed spikes to convey information and if they act as independent encoders or if they encode additional information on the population level.

Intrinsic oscillatory network activity in the retina which persists without stimulation causes variability between repeated stimulus representations. In the framework of retinal ganglion cells as independent rate encoders, ongoing retinal activity is a source of noise. We explore the alternative possibility that this oscillatory activity (50-75 Hz) serves as a reference 'clock' signal for a spike timing code. Analogous coding strategies are employed by other neural systems, such as olfactory and somato-sensory system as well as the hippocampus.

To explore how the stimulus vs. the intrinsic oscillations influence thalamic responses, we analyzed the timing of retinal inputs (EPSPs) and thalamic output (spikes) obtained by whole-cell recordings from relay cells in cat's lateral geniculate nucleus. We showed that much of the variability of responses from one trial to the next could be explained by intrinsic retinal oscillations that are not time-locked to the stimulus. Specifically, we found that spike latencies with respect to the stimulus were far more variable than spike timing with respect to retinal EPSPs, which showed millisecond fidelity. [1]

We proposed that this disparity in temporal precision gives rise to separate channels for information to reach cortex which operate on distinct time scales: On a slow time scale (<50 Hz), spike rates of individual ganglion cells encode information about stimuli falling in the center of the receptive field, whereas spike timing on fast time scales (>50 Hz) encodes additional information which is not necessarily spatially restricted to lie within the receptive field. Interestingly, both the precise spike timing and the oscillatory reference signal are reliably relayed by thalamus to cortex. [2]

Here, we propose a novel code for visual information that utilizes both firing rates and precise spike timing relative to ongoing network oscillations. While spike rate encodes receptive field content, temporal spike phases encode spatial phase information. We show, how network dynamics in the retina might give rise to such a coding strategy, and how the code can be read out by downstream neurons. Furthermore, we discuss evidence from electro-physiological data for this type of code.

This work has been supported by NIH (EY09593) and Redwood Center for Theoretical Neuroscience at UCB

[1] K. Koepsell, X. Wang, Q. Wang, Y. Wei, V. Vaingankar, J. A. Hirsch, F. T. Sommer, SFN abstract (2005).
[2] K. Koepsell, X. Wang, Q. Wang, Y. Wei, V. Vaingankar, J. A. Hirsch, F. T. Sommer, SFN abstract (2006).

# The role of contrast adaptation in the coding of natural visual stimuli

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Adaptive processing is essential for sensory function in dynamic natural environments. Here, we characterize the processing of natural visual stimuli in the cat LGN under different viewing conditions. We observe adaptive changes in spatiotemporal filtering, sensitivity, and selectivity in responses to the same natural scene movies at high and low contrast. Our analysis shows that changes in spatiotemporal filtering are responsible for contrast-dependent differences in the timing and occurrence of firing events, while changes in sensitivity and selectivity control the overall firing rate. Using a simple model of the early visual pathway, we illustrate how contrast adaptation modulates a set of tradeoffs, with spatiotemporal filtering controlling signal to noise ratio and redundancy, and sensitivity and selectivity controlling mean firing rate and sparseness. These results provide the first direct characterization of contrast adaptation during natural stimulation and support the hypothesis that adaptive mechanisms serve to enhance sensory processing of natural stimuli.

# A saliency map in the primary visual cortex for bottom up visual selection --- theory and experiments

Li Zhaoping

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Given the attentional bottleneck, the visual system must select a limited aspects of inputs for detailed processing. Much of the selection is by bottom up mechanisms to direct gaze to the selected or most salient visual locations for detailed processing. While saliency has been investigated extensively in behavioral studies, it's physiological basis remain controversial. I will present theoretical and experimental evidences to argue that the primary visual cortex (V1) creates a saliency map of the visual space, such that the receptive field location of the most responsive V1 neuron to a scene is most likely selected for attentional processing.

# Masking by Mach Bands

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The masking effects of Mach bands are complicated because Mach bands are distorted by the signals to be detected (Ratliff, 1983; Henning et al., 2001; 2004): Narrow luminance increments widen dark Mach bands and narrow the bright bands; decrements narrow the dark bands and widen the bright bands. [Indications of the effect are also seen in one observer's results in (Fiorentini et al., 1955).] The subtle distortion cue affects the shape of psychometric functions relating detection performance to signal magnitude. Fortunately, the opposing effects of incremental and decremental signals mean that randomizing signal polarity within blocks of trials effectively removes the distortion because observers are then unable to use differences in the widths of the Mach bands to indicate the signal interval; the psychometric functions become approximately parallel on semilogarithmic co-ordinates (Henning et al., 2004). However, an intriguing phenomenon is observed in random polarity experiments. Separate analysis of trials with increments and decrements reveals that near Mach bands, there are large 8-c/deg ripples or oscillations in performance as a function of location across the masking stimulus. Oscillations with increments are 180° out-of phase with those for decrements. The oscillations, much larger than measurement error, and are likely to be related to the weighting function associated with the spatial-frequency-tuned channel through which the broadband signals are detected. The ripples vanish at stimulus durations below 25 msec implying either that the site of masking has changed or that the weighting function - and hence spatial-frequency tuning -- has yet to develop.

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Ratliff, F., Milkman, N. and Rennert, N. (1983). "Attenuation of Mach bands by adjacent stimuli," Proc. Natl. Acad. Sci. (USA), **80**, 4554-4558.
Henning, G.B., Millar, R.W. and Hill, N.J. (2000). "Detection of incremental and decremental bars at different locations across Mach bands and related stimuli," J. Opt. Soc. Am. A, **17**, 1147-1159.
Henning, G,B., Hoddinott, K.T., Wilson-Smith, Z.J., and Hill, N.J. (2004). "Masking effect produced by Mach bands on narrow bars of random polarity," J. Opt. Soc. Am A, **21**, 1379-1387

# Contrast dependent spatial integration in V1: BOLD, LFP, and spikes

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Responses of neurons in primary visual cortex (V1) to stimuli presented inside their classical receptive field (centre stimuli) can be modulated by stimuli presented outside the receptive field (surround stimuli). Whether these influences are facilitatory or inhibitory depends on the details of centre and surround stimuli. The contrast of the centre stimulus strongly determines whether surround stimuli are facilitatory or inhibitory. Responses to low contrast centre stimuli are facilitated by high contrast surround stimuli, while responses to high contrast centre stimuli are suppressed (e.g. Polat et. al., 1998, Nature, 391:580-584). It is likely that GABAergic mechanisms mediate the suppression of neuronal activity. It is unclear whether fMRI can be used to measure the dichotomy between surround facilitation and suppression, as this depends on whether a) the suppression of neuronal activity results in decreased metabolic rates or b) the GABAergic synaptic transmission itself causes sufficient increased metabolic demand such that the reduction of neuronal activity is concealed. To investigate this we measured BOLD responses in V1 of a passively fixating awake behaving macaque monkey, while 'centre' moving Gabor patches (spatial frequency=0.5 cyc/º, temporal frequency= 6 Hz, σ= 0.5, Michelson contrast=4,8,16,48, or 98%) were presented in isolation or flanked by 2 iso-oriented Gabors (fixed at 48% contrast, otherwise identical to the centre stimulus). Centre stimuli were presented in the right visual hemifield at 6.5º along the horizontal meridian. Flanker stimuli were presented along this meridian, separated from the centre stimulus by 5 times the Gabor wavelength. Data were acquired using a 4.7T Bruker scanner, with a surface coil placed over the left V1. A blocked design (18 sec stimulus on, followed by 18 sec stimulus off periods, TR 3s, TE 20 ms, 16 slices, 1*1.25*1.5 mm resolution) was used. We found that the BOLD response in V1, V2, and MT was parametrically reduced by flanker stimuli at medium and high centre contrast (16-96%). In V1 it was facilitated by flanker stimuli at low (4-8%) contrast. This is in line with predictions from single unit studies in anesthetized animals. We compared these V1 findings to local field potential (LFP) and spike data where spatial integration was measured electrophysiologically. Surprisingly, neither spike, nor LFP data mapped to the BOLD data measured by means of fMRI. Importantly, LFP and spike data also showed different behaviour depending on contrast and flanker interactions. These data demonstrate that the BOLD signal cannot always be mapped to either spiking or local field potential, and its precise interpretation requires further scrutiny. (Supported by Wellcome Trust and BBSRC).

# Identifying temporal population codes in the retina using canonical correlation analysis

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Right from the first synapse in the retina, the visual information gets distributed across several parallel channels with different temporal filtering properties (Wässle, 2004). Yet, the prevalent system identification tool for characterizing neural responses, the spike-triggered average, only allows one to investigate the individual neural responses independently of each other. Here, we present a novel data analysis tool for the identification of temporal population codes based on canonical correlation analysis (Hotelling, 1936). Canonical correlation analysis allows one to find `*population receptive fields*' (PRF) which are maximally correlated with the temporal response of the entire neural population. The method is a convex optimization technique which essentially solves an eigenvalue problem and is not prone to local minima.

We apply the method to simultaneous recordings from rabbit retinal ganlion cells in a whole mount preparation (Zeck et al, 2005). The cells respond to a 16 by 16 pixel m-sequence stimulus presented at a frame rate of 1/(20 msec). The response of 27 ganglion cells is correlated with each input frame in an interval between zero and 200 msec relative to the stimulus. The 200 msec response period is binned into 14 equal-sized bins. As shown in the figure, we obtain six predictive population receptive fields (left column), each of which gives rise to a different population response (right column). The x-axis of the color-coded images used to describe the population response kernels (right column) corresponds to the index of the 27 different neurons, while the y-axis indicates time relative to the stimulus from 0 (top) to 200 msec (bottom). The six population receptive fields do not only provide a more concise description of the population response but can also be estimated much more reliably than the receptive fields of individual neurons.

In conclusion, we suggest to characterize retinal ganglion cell responses in terms of population receptive fields, rather than discussing *stimulus-neuron* and *neuron-neuron* dependencies separately.

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W"assle H (2004) Parallel processing in the mammalian retina. Nature Reviews Neuroscience 5:1-11.
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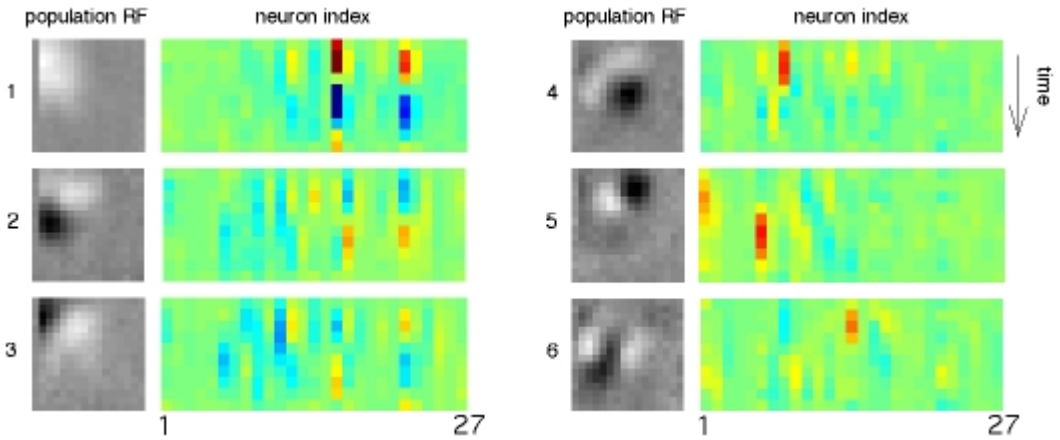

# Bayesian neural system identification: error bars, receptive fields and neural couplings

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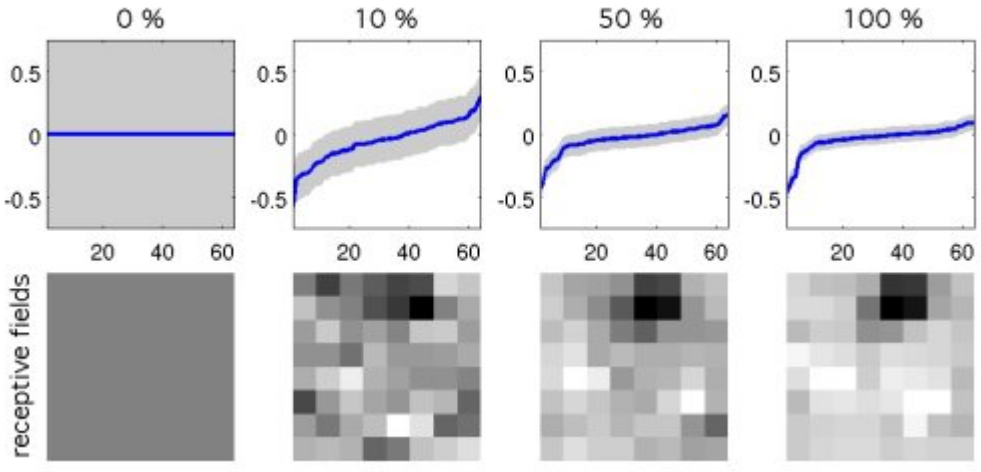
The task of system identification lies at the heart of neural data analysis. Bayesian system identification methods provide a powerful toolbox which allows one to make inferences over stimulus-neuron and neuron-neuron dependencies in a principled way. Rather than reporting only the most likely parameters, the posterior distribution obtained in the Bayesian approach informs us about the range of parameter values that are consistent with the observed data and the assumptions made. In other words, Bayesian receptive fields always come with error bars. Since the amount of data from neural recordings is limited, the error bars are as important as the receptive field itself.

Here we apply a recently developed approximation of Bayesian inference to a multi-cell response model consisting of a set of coupled units, each of which being a Linear-Nonlinear-Poisson (LNP) cascade neuron model. The instantaneous firing rate of each unit depends multiplicatively on both the spike train history of the units and the stimulus. Parameter fitting in this model has been shown to be a convex optimization problem (Paninski 2004) that can be solved efficiently, scaling linearly in the number of events, neurons and history-size. By doing inference in such a model one can estimate excitatory and inhibitory interactions between the neurons and the dependence of the stimulus. In addition, the Bayesian framework allows one not only to put error bars on the inferred parameter values but also to quantify the predictive power of the model in terms of the marginal likelihood.

As a sanity check of the new technique, and also to explore its limitations, we first verify for artificially generated data that we are able to infer the true underlying model. Then we apply the method to recordings from retinal ganglion cells (RGC) responding to white noise (m-sequence) stimulation. The figure shows both the inferred receptive fields (lower) as well as the confidence range of the sorted pixel values (upper) when using a different fraction of the data (0,10,50, and 100 %). We also compare the results with the receptive fields derived with classical linear correlation analysis and maximum likelihood estimation.

Paninski L (2004) Maximum likelihood estimation of cascade point process neural encoding models.



# The Functional Role of Patchy Cortical Circuits

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In Layer 2/3, axons of pyramidal neurons make non-uniform projections to distant regions. The resulting patchy structure suggests several functional advantages. The high performance of the human brain may be aided by certain conditions which "set the stage" for this processing to occur. In particular, the architecture of the cortex should allow for highly correlated activity in some regions, less correlated activity in others, and also complete isolation in others still. The inputs and processing stages should not corrupt each other. With this relative isolation, each piece of information may then be processed in the most efficient manner possible. Simultaneously, there need to be additional mechanisms, which increase the reliability of information processing. One example would be multiple neurons which may be spatial close together are wired in such a fashion that their activity is made more reliable and resistant to interference.

The architecture of the cortex, though it's patchy structure, with many neurons within each patch, suggests a compromise. There may be a tradeoff between the above described computational characteristics of computational power though either parallelism or robustness. Parallelism could be enhanced by using separate patches to act on different inputs or to perform different computations. Robustness may be implemented with correlated activity between the neurons in a cluster.

In our simple model of the superficial cortical layers, neurons are represented as integrate and fire neurons that have a single circular central cluster of output with synaptic boutons, and further circular clusters disposed about this central cluster. The overall connectivity between neurons in a single layer is computed for various settings of their bouton clustering parameters. Then, we examine the neurons' subdivision into separate correlation groups as a function of their bouton clustering pattern. Correlation groups may act as discrete units which enhance the computational redundancy and richness.

# Satellite Symposia

Sat1 Neurotropic Viruses
*Bernd Heimrich and Martin Schwemmle, Freiburg*

Sat2 Ion transport in the brain and beyond: from function to genes
*Eleni Roussa, Göttingen*

Sat3 Neural stem cells and neuronal specification
*Tanja Vogel and Andreas Wodarz, Göttingen*

# Satellite Symposium

## Sat1: Neurotropic Viruses

***Bernd Heimrich and Martin Schwemmle, Freiburg***

### Slide

**Sat1-1** Opening remarks for Sat. Symposium 1
*Bernd Heimrich and Martin Schwemmle, Freiburg*

**Sat1-2** Neurotropic viruses - a serious problem for patients, clinicians and virologists
*Thomas Mertens, Ulm*

**Sat1-3** Brain infections by measles virus: human disease and mouse model
*Jürgen Schneider-Schaulies, Sabine Schubert, Katrin Singethan, Paul W. Duprex and Bert K. Rima, Würzburg and Belfast (UK)*

**Sat1-4** Molecular Aspects of the Life Cycle of the Flavivirus Tick-Borne Encephalitis Virus
*Christian W Mandl, Wien (A)*

**Sat1-5** Dissecting determinants of mumps virus neurovirulence
*Christian Sauder, Steven Rubin, Tahir Malik, Candie Wolbert, Paul Duprex and Kathryn Carbone, Bethesda (USA) and Belfast (UK)*

**Sat1-6** Recombinant rabies viruses as a tool to study neurobiology
*Karl-Klaus Conzelmann, Stefan Finke, Ian Wickersham, Edward M. Callaway, Yvonne Klingen and Krzysztof Brzózka, München and La Jolla (USA)*

**Sat1-7** RAT STRAIN SPECIFIC DIFFERENCES IN DENTATE GRANULE CELL DEATH AFTER NEONATE BORNA DISEASE VIRUS INFECTION IN VIVO AND IN VITRO
*Martin Schwemmle, Heike Fischer, Daniel Mayer, Sonja Schmid and Bernd Heimrich, Freiburg*

**Sat1-8** Use of electrophysiology and proteomics to assess the bases of Borna disease virus interference with neuronal plasticity
*Daniel Gonzalez-Dunia, Romain Volmer, Elsa Suberbielle, André Garenne, Christine Prat and Gwendal Le Masson, Toulouse (F) and Bordeaux (F)*

**Sat1-9** Bad Virus - Good Guy. Usability of Pseudorabies Viruses as "Live-Cell" Tracers in the Trigeminal System
*Markus Rothermel, Nils Damann, Nicole Schöbel, Thomas C. Mettenleiter, Hanns Hatt and Christian H. Wetzel, Bochum and Insel Riems*

### Poster

**TSat1-1A** The role of myelin in Theiler's virus persistence in the central nervous system of mouse
*JP. Roussarie, C. Ruffié and M. Brahic, Paris (F)*

**TSat1-2A** Rapid onset of simian NeuroAIDS is associated with disruption of excitatory amino acid transporters
*E. Koutsilieri, F. Meisner, E. Neuen-Jacob, S. Sopper, D. Vosswinkel and C. Scheller, Würzburg, Düsseldorf and Göttingen*

Introductory Remarks to Satelite Symposium 1

# Neurotropic Viruses

Bernd Heimrich and Martin Schwemmle, Freiburg

Neurotropic viruses have attracted increasing attention not only because some of them cause severe human diseases, but also because they serve as model agents to study virus-induced disorders of the central nervous system.

In this symposium we will cover clinical aspects of human neurotropic viruses and efforts to establish animal models to identify the molecular determinants of invasiveness and neurotropism that are central for the understanding of virus-induced pathogenesis in the central nervous system. Furthermore, studies will be presented that illustrate the possibilities to study basic neuronal functions by using genetically manipulated neurotropic viruses. In addition, animal and ex-vivo models will be discussed that allow the study of neuronal plasticity and death.

# Neurotropic viruses - a serious problem for patients, clinicians and virologists

Thomas Mertens

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A huge number of viruses from very different virus families can be "neurotropic" and can provoke neurological disease in man, starting from connatally neurological infections of the very young to the varicella-zoster-virus reactivation with slowly progressing encephalitis in old patients.
The spectrum of disease manifestations comprises quite different entities like encephalitis/pan encephalitis, meningitis, encephalopathy, myelopathy, neuritis, pareses and other more rare manifestations.
The potential of some viruses to induce neurological disease is strictly dependant on the immune status of the infected person (e.g. Cytomegalovirus, JC-virus), so the definition of neurotropism may also depend on the host.
Pathogenesis of virus induced neurological disease varies greatly from "simple" cytopathogenicity of poliovirus to the induction of PML, Reye- or Guillain-Barré-Syndrome.

For clinical management of patients it is often very important to differentiate between bacterial or viral infections, so we need rapid viral diagnosis, especially since therapeutic intervention is different and e.g. early therapy in herpes-simplex-virus encephalitis is absolutely decisive for the clinical outcome.

Rapid viral diagnosis has been much improved by the introduction of techniques from molecular biology and is no longer dependent of antibody detection or virus isolation. These improvements enable the clinical virologist to better help the clinician in diagnosis, indication for antiviral therapy, monitoring of success of therapy, and identification of the emergence of resistant virus populations.

# Brain infections by measles virus: human disease and mouse model

Jürgen Schneider-Schaulies[1], Sabine Schubert[1], Katrin Singethan[1], Paul W. Duprex[2] and Bert K. Rima[2]

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Measles remains an important cause of childhood mortality especially in developing countries leading to approximately 700000 deaths annually worldwide. In a joint strategic plan WHO and UNICEF target 45 priority countries to achieve mortality reduction by high routine immunization coverage and ensuring that all children receive a second opportunity for measles immunization. During acute infections measles virus (MV) causes a transient immunosuppression and a variety of complications such as otitis media, pneumonia, diarrhoea, acute postinfectious measles encephalomyelitis (APME, incidence 0.1%) and blindness. MV may also persist for 5 to 15 years after acute infection and cause the invariably lethal brain disease subacute sclerosing panencephalitis (SSPE, incidence 0.01%). In brains of SSPE patients viral nucleocapsids are abundantly present in neurons and glial cells.

In order to investigate possibilities to modulate a persistent CNS infection, we established a mouse model using the recombinant MV MV-EGFP-CAMH expressing the haemagglutinin (H) of the rodent adapted MV-strain CAM/RB and the enhanced green fluorescent protein (EGFP). In newborn rodents the virus infects neurons and causes an acute lethal encephalitis. From two weeks on, when the immune system of the genetically unmodified animals is maturating, intracerebral infection is overcome subclinically, however, a focal persistent infection in groups of neurons remains. The complete brain can be analysed in 50 or 100 µm slices, and infected autofluorescent cells are readily detected. Virus persists in approximately 81% of mice and is detectable for more than 50 days post infection. Intraperitoneal immunisation with MV one week before infection, but not after the primary infection, protects and prevents persistence. The high percentage of persistence demonstrates that this is a reliable and useful model of a persistent CNS infection in fully immunocompetent mice, which allows the investigation of factors determining the fate of the persistent infection.

# Molecular Aspects of the Life Cycle of the Flavivirus Tick-Borne Encephalitis Virus

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The genus Flavivirus (family Flaviviridae) comprises a number of arthropod-transmitted human pathogens of global importance. Prominent neurotropic flaviviruses are the mosquito-transmitted Japanese encephalitis and West Nile viruses as well as the tick-transmitted tick-borne encephalitis virus (TBEV), which is endemic in parts of Europe and Asia and causes several thousand cases of severe neurological illness every year. Effective vaccines are available against some flaviviruses, such as TBEV, but new vaccines are needed for others such as West Nile virus, an emerging disease agent in North America. No specific antiviral treatments are available for any of these viruses.

Understanding the molecular details of the flavivirus life cycle is instrumental for developing new antiviral strategies. Flaviviruses are small enveloped particles with a positive-stranded RNA genome. The viral RNA is replicated in the cytoplasm of the host cell. Newly formed RNA is engulfed by the viral capsid protein C and acquires its lipid membrane by an intracellular budding process. We have studied molecular elements of TBEV which are involved in RNA replication and the assembly of virus particles. Using reverse genetics, specific mutations were introduced into the TBEV genome that modulated the assembly of infectious virus particles as well as non-infectious subviral particles. Key determinants of the TBEV life cycle were identified and characterized by specific mutagenesis studies. These include specific elements which mediate cyclization of the viral genome during replication, protease cleavage sites which are utilized during assembly and maturation of the viral particle, and the role of the natural gene order for viral assembly. These studies revealed features which are characteristic for the TBEV life cycle and differ from mosquito-borne flaviviruses. In addition, general principles which are useful for generating new vaccines against various flaviviruses, such as self-replicating RNA vaccines could be deduced.

# Dissecting determinants of mumps virus neurovirulence

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Mumps virus is a highly neurotropic negative stranded enveloped RNA virus, infecting the central nervous system in about 50% of cases. In the pre-vaccine era, mumps virus was the leading cause of viral meningitis in the developed countries. In rare cases, lethal encephalitis occurs. Use of live attenuated mumps virus vaccines has dramatically reduced the incidence of mumps in countries achieving high vaccination coverage. While the efficacy of mumps virus vaccine strains has been well-characterized, assessing safety has been challenging. For example, despite demonstrating the safety of the Urabe-AM9 and the Leningrad-Zagreb mumps vaccines during clinical development, both vaccine strains have been causally linked to aseptic meningitis in vaccinees. This problem can largely be attributed to the poorly-predictive value of the standard monkey neurovirulence safety test, which is widely used to assess vaccine neurotoxicity. To address this issue, our laboratory developed a rat-based neurotoxicity test wherein the degree of virus-induced hydrocephalus is used as a marker for neurotoxicity severity. By means of repeated passaging of both neurovirulent wild type and neurovirulent vaccine (Urabe-AM9) mumps viruses in suitable cell lines, attenuation was achieved as demonstrated in rats. Sequencing of the genomes of parental and attenuated viruses revealed single point mutations in several virus genes that are associated with attenuation. To determine which nucleotide changes in which genes are critical for attenuation, we used reverse genetics technology. In a first step to identify markers of neurovirulence, we constructed a panel of chimeric viruses where individual or several genes from a non-neurovirulent mumps virus strain were replaced with corresponding genes from two neurovirulent mumps viruses. In addition, an infectious clone of a neurovirulent mumps virus was generated and single point mutations associated with attenuation were inserted. Our data obtained by testing of the various viruses in the rat model point towards a multigenic mechanism of attenuation / neurovirulence.

# Recombinant rabies viruses as a tool to study neurobiology

Karl-Klaus Conzelmann[1], Stefan Finke[1], Ian Wickersham[2], Edward M. Callaway[2], Yvonne Klingen[1] and Krzysztof Brzózka[1]

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Rabies virus (RV) is an enveloped negative strand RNA virus that efficiently infects neuronal cells of animals and man. Using reverse genetics, we are generating recombinant RV to identify the viral factors responsible for neurotropism in neuronal cells as well as the mechanisms of host cell defense against RV infection. This knowledge is also being used to generate safe RV vectors useful as vaccines, and tools to study neurobiology.

RV infection of neuronal cells is characterized by high level virus gene expression and astonishingly little damage of neurons, making recombinant RV vectors ideal long-term tracers to study morphology of neurons and neural connectivity. A deletion mutant RV lacking the spike glycoprotein (G) gene and encoding fluorescent proteins like EGFP is particularly useful for studying the detailed anatomy and physiology of individual projection neurons. On stereotaxic inoculation, the virus efficiently labels large numbers of distant cells that project to the injection sites within the brain. Missing its G protein, the virus is unable to spread beyond initially infected cells. Since infected neurons survive and efficiently express EGFP for more than two weeks, cells can be targeted on the basis of their detailed morphology for physiological investigations. Other RVs have been engineered to further allow a single transsynaptic crossing, unambiguously identifying cells directly connected to the starting population.

Live imaging of RV expressing fluorescent core or envelope fusion proteins is being used to reveal details of the mechanisms underlying the characteristic retrograde axonal transport of RV. Our data show that complete enveloped virions can be carried within neuronal transport vesicles along microtubules by dynein motor complexes. The selection of transport vesicles appear to depend on the origin of the envelope G protein. Thus, G is responsible not only for entry into neurons in the periphery, but also for rapid long-distance axonal transport.

Efficient replication of RV in the cytoplasm of neurons requires that the host interferon system is inhibited. This is achieved by two independent functions of the RV phosphoprotein P. P prevents phosphorylation of the transcription factor IRF3/7 to abolish transcriptional induction of IFN, and retains tyrosine-phosphorylated STAT1 and STAT2 in the cytoplasm to prevent IFN-mediated induction of antiviral and immune genes. In the absence of IFN-antagonistic functions of P, RV can establish infection after i.c. injection into brains of IFN receptor knock-out mice, but not in wt mice with an intact IFN system, demonstrating the critical function of IFN antagonistic functions in vivo.

# RAT STRAIN SPECIFIC DIFFERENCES IN DENTATE GRANULE CELL DEATH AFTER NEONATE BORNA DISEASE VIRUS INFECTION IN VIVO AND IN VITRO

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In the hippocampus of Borna disease virus (BDV)-infected newborn rats, dentate granule cells undergo progressive cell death. BDV is non-cytolytic and the pathogenesis of this neurodevelopmental damage in the absence of immunopathology remains unclear. A model system to study early events of the pathology is lacking. We represent herein that organotypic hippocampal slice cultures of newborn rat pups are a suitable an ex vivo model to examine BDV neuropathogenesis. After challenging hippocampal slice cultures with BDV, we observed a progressive loss of calbindin-positive granule cells 21-28 days post infection. This loss was accompanied by reduced numbers of mossy fiber boutons when compared to mock-infected cultures. Similarly, the density of dentate granule cell axons, the mossy fiber axons, appeared to be substantially reduced. In contrast, hilar mossy cells and pyramidal neurons survived, although BDV was detectable in these cells. Despite infection of dentate granule cells two weeks post infection, the axonal projections of these cells and the synaptic connectivity patterns were comparable to mock-infected cultures, suggesting that BDV-induced damage of granule cells is a post-maturation event that starts after the mossy fiber synapses are formed. In summary, BDV infection of rat organotypic hippocampal slice cultures results in selective neuronal damage similar to that observed in newborn-infected rats and is therefore a suitable model to study BDV-induced pathology in the hippocampus.

Next, we tested whether rats with other genetic backgrounds show differences in pathology after BDV infection and whether such differences are reflected in our hippocampal slice system. For this purpose we infected newborn Lewis, Wistar and Sprague-Dawley (SD) rats with BDV. While infection of Wistar and Lewis rats resulted in only mild disease symptoms, BDV-infected SD rats developed severe disease symptoms. Despite the presence of perivascular lymphocyte infiltrations, no or only moderate DG damage could be detected in SD rats 43 days after virus challenge. At this time point most of the Lewis and Wistar showed pronounced neuronal loss in the dentate gyrus. Infection of hippocampal slices reflected the onset of neuronal damage in infected animals. Although viral replication efficiency and spread was comparable in the cultures of either rat strain at 28 days p.i., most calbindin-positive granule cells disappeared only in slice cultures of Lewis and Wistar rats. With a delay of 14 days, however, loss of granule cells was also evident in cultures from SD rats. Thus, our data indicate that genetic host factors can determine both the cellular immune response and the susceptibility of dentate granule cells to BDV-induced damage.

(Supported by the DFG: He1520, SCHW 632)

# Use of electrophysiology and proteomics to assess the bases of Borna disease virus interference with neuronal plasticity

Daniel Gonzalez-Dunia[1], Romain Volmer[1], Elsa Suberbielle[1], André Garenne[2], Christine Prat[1] and Gwendal Le Masson[2]

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Infection by Borna disease virus (BDV) enables the study of the molecular mechanisms whereby a virus can persist in the central nervous system and lead to altered brain function, in the absence of overt cytolysis and inflammation. This neurotropic virus infects a wide variety of vertebrates and causes behavioral diseases. The basis of BDV-induced behavioral impairment remains largely unknown. Recently, by studying synaptic vesicle recycling, we have demonstrated that BDV specifically blocks activity-dependent enhancement of synaptic activity, suggesting defects in long-term potentiation (Volmer et al, PloS Pathogens, 2006). We have now performed an analysis of the electrical activity of BDV-infected neuronal networks grown on microelectrode arrays. In addition, we have applied a global proteomic analysis on primary cultured cortical rat neurons infected with BDV. This approach is based on protein fractionation by two-dimensional liquid chromatography (PF2D), followed by the identification of the proteins of interest by nanoLC MS/MS. This analysis has helped to pinpoint neuronal signaling pathways affected by BDV infection and may contribute to unravel the mechanisms whereby this virus can induce synaptic dysfunction and cause behavioral disorders.

# Bad Virus - Good Guy. Usability of Pseudorabies Viruses as "Live-Cell" Tracers in the Trigeminal System

Markus Rothermel[1,3], Nils Damann[1], Nicole Schöbel[1], Thomas C. Mettenleiter[2], Hanns Hatt[1] and Christian H. Wetzel[1]

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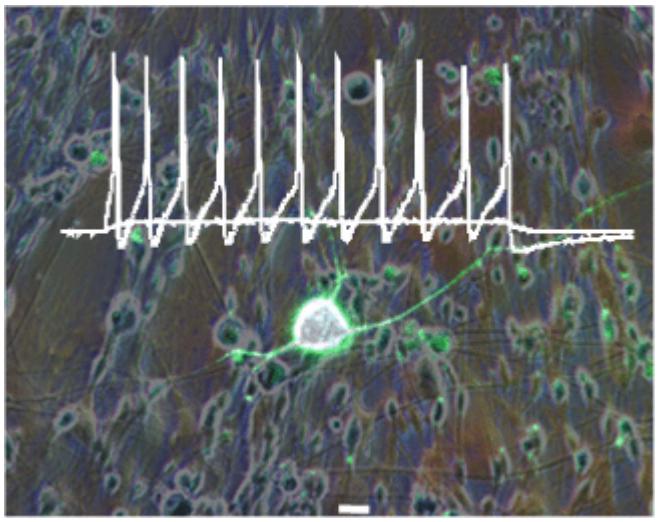
The trigeminal nerve is the major mediator of sensations from the mammalian head and comprises neurons that transduce mechanical, thermal and chemical stimuli. Thereby single neurons mediate sensory input from selective areas of the head. Physiological features of individual trigeminal neurons depending on their somatosensory function and area of innervation remain largely unclear. Availability of a labelling technique to identify and to physiological characterise defined peripheral neuronal subpopulations and their central projections would be of great advantage in understanding somatosensory perception. We already showed that the Bartha strain of the Pseudorabies virus (PrV) can be used as a "live-cell" tracer for physiological investigations of selectively labelled peripheral neurons (Damann *et. al.,* 2006). However this approach was limited to primary sensory neurons, because PrV-Bartha has lost its capability to spread in anterograde direction of information processing within the nervous system. Here we report the use of the bidirectional tracer PrV-Kaplan as a "live-cell"-tool for characterisation of defined neuronal populations within the trigeminal ganglion and synaptically connected higher order neurons in the brainstem.

We used marker protein expressing variants of the Pseudorabies virus to identify neurons in the trigeminal system of mice. Plating of ganglionic and brainstem tissue (10 hours and 36 hours after intranasal inoculation, respectively) and pharmacological inhibition of viral replication allowed physiological analysis of identified neurons *in vitro*. First characterisations revealed that resting membrane potential, $I_h$, and $Na_V$ currents of these cells were indistinguishable from non-labelled, randomly chosen neurons. Biophysical properties as well as ATP or KCl-induced signals in calcium imaging measurements suggest that neurons are not significantly affected by viral infection, making PrV-Kaplan eligible for functional analysis of somatotopically defined and synaptically connected neurons *in vitro*.



The detailed characterisation of sensory properties of peripheral neurons and mechanisms of synaptic and cellular plasticity in central neurons of the trigeminal-cortical system should lead to a better understanding of somatosensory and chemosensory perception.

# The role of myelin in Theiler's virus persistence in the central nervous system of mouse

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Theiler's virus, a picornavirus, persists for life in the CNS of mouse and causes a demyelinating disease. The virus infects neurons first but persists in white matter glial cells, mainly oligodendrocytes and macrophages. The mechanism of viral traffic from neurons to glial cells and the respective roles of oligodendrocytes and macrophages in persistence are poorly understood. We took advantage of the *shiverer* mouse, a myelin mutant resistant to persistent infection, to examine the role of myelin in persistence. We showed that the resistance of *shiverer* mice is not mediated by immune responses and that the mutation does not impair the permissiveness of oligodendrocytes and macrophages to the virus. Using the optic nerve as a model, we showed that the virus traffics from axons to the cytoplasmic channels of myelin and that this traffic is impaired in *shiverer* mice. Using another resistant myelin mutant, *rumpshaker*, we confirmed that mutations affecting myelin structure prevent the virus from infecting myelin and oligodendrocytes and from persisting. These results uncover an unsuspected axon to myelin traffic of Theiler's virus and the essential role played by the infection of myelin/oligodendrocyte in persistence.

# Rapid onset of simian NeuroAIDS is associated with disruption of excitatory amino acid transporters

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Glutamate-mediated neurodysfunction in HIV infection has been primarily suggested by in vitro studies. The regulation of glutamatergic neurotransmission in inflammation is a complex interaction between activation of immune mediators and adaptive changes of the functional elements of the glutamatergic synapse. We have used the most relevant animal model for HIV infection, the simian immunodeficiency virus (SIV)-infected macaques, to answer the questions a) whether perturbation of glutamate neurotransmission is evident during progression of immunodeficiency disease, b) what are the mechanisms underlying this impairment and c) what are the consequences on neuronal function. Disease progression both in the periphery and in the brain was documented by clinical and general pathological examination, plasma and CSF viral RNA load, T-cell analysis and brain histopathology. We report for the first time disruption of excitatory amino acid transporters (EAATs) during SIV infection and a break down of EAATs associated with development of rapid AIDS. This great impairment is accompanied by increases in glutamate levels and changes in the expression of NMDA subunits during disease progression. In accordance to a recent study reporting that TNF-alpha downregulates EAAT2, we found higher TNF-alpha production in microglia isolated from these animals. TNF-alpha production was correlated with activation status of microglia. In these settings, we found dramatic decrease in function of cholinergic neurons without an effect on GABA neurons in the putamen of animals with AIDS. Our data on SIV-infected macaques support the glutamate hypothesis for HIV dementia and suggest that the pathogenetic mechanism for the neurodysfunction is the break down of glutamate clearing which occurs in the stage of AIDS and which is associated with high levels of TNF-alpha produced by activated microglia.

# Satellite Symposium

## Sat2: Ion transport in the brain and beyond: from function to genes

***Eleni Roussa, Göttingen***

### Slide

**Sat2-1** Opening remarks for Sat. Symposium 2
*Eleni Roussa, Göttingen*

**Sat2-2** Extraneuronal pathophysiology of K-Cl cotransport.
*Seth Leo Alper, Boston (USA)*

**Sat2-3** On the mechanisms of developmental up-regulation of KCC2
*Claudio Rivera, Helsinki (FIN)*

**Sat2-4** Compartment-dependent colocalization of G-protein-coupled inwardly rectifying $K^+$ channels and $GABA_B$ receptors in hippocampal pyramidal cells.
*Akos Kulik, Freiburg*

**Sat2-5** Cytosolic $Ca^{2+}$ oscillations and store-operated $Ca^{2+}$ entry in astrocytes and neurons
*Joachim W. Deitmer, Kaiserslautern*

**Sat2-6** Developmental changes in CO2 sensivity and brain pH regulation: Effects on neuronal excitability.
*Sebastian Schuchmann, Berlin*

Introductory Remarks to Satelite Symposium 2

# Ion transport in the brain and beyond: from function to genes

Eleni Roussa, Göttingen

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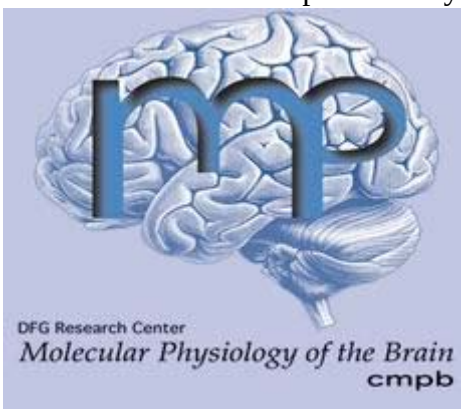


Ionic and pH homeostasis are prerequisites for normal cerebral activity. Electrical signaling in neurons is based on the coordinated parallel or sequential action of ion pumps, carriers and channels and is associated with rapid pH shifts. In the post-genomic era, molecular insights have complemented early biophysical studies and revealed new additions to the list of physiological functions of key molecules in the brain and beyond.

This symposium attends to highlight important recent findings on key players for ionic homeostasis and pH regulation in the brain from a functional and molecular viewpoint. In this context, developmental, physiological and pathophysiological aspects will be addressed.

# Compartment-dependent colocalization of G-protein-coupled inwardly rectifying $K^+$ channels and $GABA_B$ receptors in hippocampal pyramidal cells.

Akos Kulik

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COMPARTMENT-DEPENDENT COLOCALIZATION OF G-PROTEIN-COUPLED INWARDLY RECTIFYING $K^+$ CHANNELS AND $GABA_B$ RECEPTORS IN HIPPOCAMPAL PYRAMIDAL CELLS

Akos Kulik

Institute of Anatomy and Cell Biology, University of Freiburg

The G-protein-coupled inwardly rectifying $K^+$ channels (Kir3 channels), coupled to metabotropic $GABA_B$ receptors ($GABA_B$Rs), are essential for the control of neuronal excitation. To determine the distribution of Kir3 channels and $GABA_B$Rs, as well as, their spatial relationship over axonal and somato-dendritic membranes of hippocampal pyramidal cells, we used high-resolution immunocytochemical approaches. Immunoreactivity for the most abundant Kir3.2 and $GABA_{B1}$ subunits was mainly localized postsynaptically to the extrasynaptic plasma membrane of dendritic shafts and spines of principal cells. Quantitative analysis of immunogold particles for the proteins revealed an enrichment of the subunits around glutamatergic synapses in dendritic spines. Consistent with this observation, a high-degree of coclustering of Kir3.2 and $GABA_{B1}$ was found around excitatory synapses by the highly sensitive SDS-digested freeze-fracture replica immunolabeling method. In contrast, in dendritic shafts channels and receptors were found to be mainly segregated. These results suggest that Kir3.2-containing $K^+$ channels in dendritic spines preferentially mediate the effect of GABA, whereas channels in dendrtic shafts are likely to be activated by other neurotransmitters as well. Thus, Kir3 channels, localized to different subcellular compartments of hippocampal pyramidal cells, appear to be differentially involved in synaptic integration in principal cell dendrites.

# Satellite Symposium

## Sat3: Neural stem cells and neuronal specification

*Tanja Vogel and Andreas Wodarz, Göttingen*

### Slide

**Sat3-1** Opening remarks for Sat. Symposium 3
*Tanja Vogel and Andreas Wodarz, Göttingen*

**Sat3-2** Cell adhesion molecules, stem cells and neural repair
*Melitta Schachner, Hamburg*

**Sat3-3** Development of autonomic neurons: extrinsic signals and transcriptional control
*Hermann Rohrer, Frankfurt/Main*

**Sat3-4** Mechanisms regulating proliferation and fate decisions in neural stem cells
*Lukas Sommer, Zurich (CH)*

**Sat3-5** Different roles for Bmi1 in the control of neural stem cell competence
*Yvan Arsenijevic, Lausanne (CH)*

**Sat3-6** Ski in Neural Development and Repair
*Suzana Atanasoski, Basel (CH)*

**Sat3-7** ON THE ORIGIN OF BRAIN TUMORS - CRITICAL EVALUATION OF THE NEOPLASTIC STEM CELL PREMISE
*Bjorn Scheffler, Anthony T. Yachnis, Shanshan Wang, Daniel J. Silver, Tong Zheng, Timothy M. Shepherd, A. Katrin Goetz, David Pincus, Oliver Brüstle and Dennis A. Steindler, Bonn and Gainesville, FL (USA)*

Introductory Remarks to Satelite Symposium 3

# Neural stem cells and neuronal specification

Tanja Vogel and Andreas Wodarz, Göttingen

Neural stem cells are considered to have a high therapeutical potential for treatment of neurodegenerative diseases. But so far their actual application to patients is only limited, since it is a prerequisite to understand first the basis of stem cell biology. Great efforts are undertaken to explore the biochemical functions of stem cells, whose extraordinary potential is apparently mediated through a complicated network of multiple factors. Not only the identification of participating proteins of such networks but also their functional interaction is a major task in todays investigation of stem cell biology.

This symposium will give insight into fundamental findings regarding key processes of neural stem cell behaviour. We will focus on neural stem cells that have been isolated from different sources like the retina and forebrain, as well as neural crest derived cells implicated in the development of the peripheral and autonomic nervous systems. We will discuss recent results unraveling e.g. the dependence of neural stem cells on signals of different growth and transcription factors for survival and differentiation. Furthermore we will highlight the involvement of extracellular matrix proteins for the functioning of stem cells and their implication in neuronal repair. The investigation of CNS stem cell implication into tumor formation, recurrence and progression will bring together basic and clinical research.

# Different roles for Bmi1 in the control of neural stem cell competence

Yvan Arsenijevic

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The polycomb group transcriptional repressor Bmi1 has recently been shown to be essential for neural stem cell (NSC) renewal and brain development. We observed a strong increase in the astrocyte number in vivo in the brain of Bmi1 knockout (Bmi1-/-) mice and showed in vivo that astrocyte progenitor proliferation was independent of the absence of Bmi1, whereas the number of the other progenitor/stem cells decreased in the cortex and the striatum at late developmental stages. By RNA interference, we demonstrated in vitro that the action of Bmi1 is intrinsinc to the neural stem cells and that the loss of Bmi1 led the neural stem cell to generate preferentially astrocytes. In view of these results, we sought to dissect the role of Bmi1 and the effects of Bmi1 loss in the retina, as well as in retinal stem cells (RSCs).

Bmi1 is expressed in proliferating cells of the newborn retina and in in vitro expanded RSCs. Bmi1-/- mice show an impaired response to light stimuli, as assessed by electroretinogram (ERG) recordings. The main observations are a reduction in the b-wave amplitude and a lack of oscillatory potentials. However, the global morphology and cell pattern of the adult (P30) Bmi1-/- retina show no marked difference compared to wild-type controls. Ganglion cells (stained by NeuN) are present in normal numbers, while Müller cells and rod photoreceptors (labeled respectively by CRALBP and Rho4D2 antibodies) show no striking difference in their pattern compared to wild-type retinas. Nevertheless, a 21% reduction in calbindin-positive horizontal cells is detectable in the Bmi1-/- retina at P30, accompanied by a 23% reduction in the number of PKCa-positive bipolar cells. This observation correlates with the type of defect observed with the ERG recordings.

These data show that Bmi1 is necessary for proper CNS development and function, and that Bmi1 has different actions depending on its localization in the CNS: in the cortex Bmi1 controls the cell number at the end of neurogenesis and, in the eye, Bmi1 appears to control the final number of certain retinal cell types at different developmental stages.

# Ski in Neural Development and Repair

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During development of the central nervous system, multipotent neural stem cells rise to neural progenitor cells (NPCs) and finally lead to the formation of neurons, astrocytes and oligodendrocytes. Various intrinsic factors and extracellular signalling molecules, such as members of the transforming growth factor (TGF) β family, act at different time points to regulate proliferation and differentiation of progenitor cells. TGFβ signals through activation of its respective receptors and phosphorylation of its signaling effectors, Smad2 and Smad3. Recent findings have identified the proto-oncogene Ski as an inhibitor of the TGFβ pathway. Ski physically interacts with Smads and represses TGFβ signalling through additional recruitment of N-CoR and the histone deacetylases, thereby modulating gene transcription in a broad way. Depending on the cellular context, Ski may promote either cell proliferation or differentiation. We hypothesize that Ski, by virtue of its ability to bind to different partners, will be critically involved in the regulation of the different effects of TGFβ in neural stem and progenitor cells. This hypothesis is substantiated by our findings that Ski is expressed in NPCs and that loss of Ski induces premature differentiation of neural stem cells. Moreover, Ski-deficient mice show a cranial neural tube defect with a reduced level of nestin expression, a marker for NPCs.

# ON THE ORIGIN OF BRAIN TUMORS - CRITICAL EVALUATION OF THE NEOPLASTIC STEM CELL PREMISE

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Diagnosis, treatment, and prognosis of most human brain tumors, today, are based almost exclusively on histological criteria such as growth patterns and cytological appearance, tumor cell and endothelial proliferation, or necrosis. Very little fundamental information is available on the cell or cells of origin of primary brain tumors. This lack of basic knowledge on the cellular origins of human central nervous system (CNS) neoplasia hinders efforts to develop effective therapies and may account for the continued poor prognosis of certain brain tumor entities. Recent evidence suggests that stem-like cells are present in some human malignant gliomas and that such cells could be responsible for disease recurrence and/or progression. It remains unclear whether tumorigenesis results from loss of regulatory mechanisms that influence normally occurring CNS stem cells or whether tumors arise from populations of cells that acquire stem-like identities. In the period of one year, we have randomly collected tissue samples from 20 pediatric brain surgeries, covering a range of primary brain tumors (n=15) and CNS tissue from epilepsy surgery or diagnosed otherwise (n=5). Applying classic and novel methods for the isolation and observation of adult neural stem cells in culture, we have identified three primary brain tumors (two malignant gliomas and one primitive neuroectodermal tumor) that appeared to originate from cells with stem-like characteristics. An initial set of data is presented on the biologic behavior and characteristics of these cells, and on an in vivo transplantation paradigm. This study suggests that there are subtypes of CNS tumors within the spectrum of histologically defined entities that arise from alterations of multipotent, self-renewing stem-like cells.

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|---|---|
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# Poster Topic

## T1: Development I: Neural stem cells and neurogenesis

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**T1-2A** An Induction Gene Trap Screen for Tenascin-C Target Genes in Neural Stem Cells
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**T1-4A** Survival Promoting Peptide (SPP) alters the neruotrophin expression, retards somatodendritic differentiation but enhances cell migration.
*S. WAGH, C. Grote-Westrick, P. Landgraf, MR. Kreutz, HC. Pape and P. Wahle, Bochum, Madgeburg and Münster*

**T1-5A** Neural Stem Cells form Human Embryonic Stem Cells
*P. Koch, T. Opitz, J. Steinbeck, J. Ladewig, J. Driehaus, A. Biegler, B. Seinfarz and O. Brüstle, Bonn*

**T1-6A** Lineage selection of doublecortin-positive migratory human ES cell-derived neuroblasts
*J. Ladewig, P. Koch, B. Meiners, B. Steinfarz, S. Couillard-Despres, L. Aigner and O. Brüstle, Bonn and Regensburg*

**T1-7A** Embryonic stem cell-derived neurons develop functional neuronal networks on microelectrode arrays.
*S. Illes, W. Fleischer, C. Bernreuther, M. Schachner, HP. Hartung, M. Siebler and M. Dihné, Düsseldorf and Hamburg*

**T1-8A** *In vitro* differentiation of human umbilical cord blood progenitor cells
*C. Ganser, A. Papazoglou, G. Lepski and G. Nikkhah, Freiburg*

**T1-9A** Impaired neurogenesis and astroglial gap junction coupling in BDV infected differentiated P19 embryonic carcinoma cells
*BM. Reuss and C. Köster-Patzlaff, Göttingen*

**T1-10A** Expression of epigenetic factors in mouse brain development
*T. Vogel, Göttingen*

**T1-1B** Control of neuronal differentiation by SATB genes
*MdC. de Juan, O. Britanova and V. Tarabikyin, Göttingen and Moskaw (RUS)*

**T1-2B** Developmental deficits in central and peripheral mouse nervous system after knockout of membrane fusion proteins.
*AJ. Kunwar, M. Rickmann, G. Fischer von Mollard and K. Krieglstein, Göttingen and Belefeld*

**T1-3B** Sip1 regulates proliferation, differentiation, maturation and migration in the embryonic mouse neocortex
*A. Nityanandam, A. Miquelajauregui, E. Seuntjens, D. Huylebroeck and V. Tarabykin, Göttingen and Leuven (B)*

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*N. Osterberg, E. Roussa and K. Krieglstein, Göttingen*

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*A. Pekcec, VM. Stein, JP. Bankstahl, A. Tipold, W. Baumgärtner and H. Potschka, Hannover*

**T1-6B** Influence of phosphoinositide-3-kinase gamma on neurogenesis in adult dentate gyrus
*E. Chanina, C. König, M. Grün, OW. Witte, R. Wetzker and C. Redecker, Jena*

**T1-7B** Layer-specific expression of multiple (proto-)cadherins in the cerebral cortex of adult ferret
*K. K., M. Nürnberger and C. Redies, Jena*

**T1-8B** Comparative analysis of the cerebellar systems of chicken, mouse and ferret using cadherins as molecular markers
*F. Neudert, M. Nuernberger, K. K., J. Luo and C. Redies, Jena*

**T1-9B** Regional expression of multiple (proto-)cadherins in the developing cerebral cortex of the ferret
*M. Nuernberger, K. K. and C. Redies, Jena*

**T1-10B** Radiation induced alteration of proliferative activity in the forebrain of exposed male rats and their offspring
*S. Balentova, E. Racekova and E. Misurova, Kosice (Slovakia)*

**T1-1C** The expression of embryonic tau protein isoforms persist during adult neurogenesis in the hippocampus
*T. Bullmann, R. de Silva, M. Holzer, H. Mori and T. Arendt, Leipzig, London (UK) and Osaka (J)*

**T1-2C** Stem cell transplantation in rat model cognitive dysfunction: Neurobehavioral, Neurochemical and Immunohistochemical assessment
*N. Srivastava, K. Seth and AK. Agrawal, Lucknow (India)*

**T1-3C** Pax6-instructed neurogenesis from astrocytes:
Molecular analysis of Pax6 function and functional features of newborn neurons
*R. Blum, B. Berninger, A. Lepier, J. Ninkovic, H. Wohlfrom and M. Götz, München and GSF, Neuherberg*

**T1-4C** Transforming growth factor-beta1 inhibits adult neurogenesis
*L. Aigner, FP. Wachs, B. Winner, S. Couillard-Despres, J. Winkler and U. Bogdahn, Regensburg*

**T1-5C** Imaging of Neurogenesis using the Doublecortin Promoter in Transgenic Mice.
*S. Couillard-Despres, B. Winner, U. Bogdahn, J. Winkler, J. Bischofberger and L. Aigner, Regensburg and Freiburg*

**T1-6C** Bone Marrow Stromal Cells Instruct Oligodendrogenic Fate Decision on Adult Neural Stem Cells
*FJ. Rivera, S. Coiullard-Depres, X. Pedre, S. Ploetz, M. Caioni, C. Lois, U. Bogdahn and L. Aigner, Regensburg and Cambridge (USA)*

**T1-7C** Glioblastoma-initiating cells: Identification by stem cell properties and tumorigenity but not CD133
*D. Lemke, G. Tabatabai, K. Rauner, M. Tatagiba, N. Hopf, M. Weller and W. Wick, Tuebingen and Stuttgart*

**T1-8C** Brain development in the Marbled crayfish: analysing cell proliferation and neuronal expression of engrailed
*S. Sintoni, K. Fabritius-Vilpoux and S. Harzsch, Ulm*

**T1-9C** Is Nato3 a Novel Regulator of Floor Plate and Spinal Cord Development?
*AA. Mansour, E. Nissim-Eliraz, S. Zisman, T. Golan-Lev, O. Schatz, A. Klar and N. Ben-Arie, Jerusalem (Israel)*

# Gene Regulation of Tenascin C and its Isoforms in Neural Stem Cells and the Developing Mouse Central Nervous System

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We are interested in the regulation and function(s) of the extracellular matrix glycoprotein Tenascin C (Tnc) during central nervous system development. Tnc occurs in the adult neural stem cell niche and in the ventricular zone of the developing brain, where it controls aspects of neural stem cell (NSC) development. Studies in Tnc knockout mice revealed its involvement in neural progenitor maintenance and maturation. Structurally, Tnc consists of several protein domains including 8 constitutive fibronectin type-III (FNIII) domains. By independent alternative splicing of six additional FNIII domains, theoretically, up to 64 different Tnc isoforms can be generated, and in cerebellum 27 different Tnc isoforms have been detected. The analysis of the complexity of Tnc isoform expression in NSCs that were grown as free-floating neurospheres revealed the presence of 20 different Tnc isoforms. The isoform pattern was comparable to the one documented in P6 cerebellum that contains granule cell precursor cells. One novel Tnc isoform could be identified in neural stem cell cultures. Its expression pattern in the developing brain was analysed by in situ techniques.

In order to study the regulation of Tnc and its isoforms in neurospheres grown from embryonic brain cell suspensions we transfected different plasmids coding for the transcriptional regulators Pax6, Otx2, Emx2 and Tlx, which resulted in the overexpression of these factors. When Pax6 or Otx2 was overexpressed the large Tnc isoforms were upregulated whereas the small isoforms without any or with one additional domain were downregulated. We also analysed the Tnc isoform complexity after Pax6 overexpression, but to our surprise we did not observe any significant change in the combinatorial code of Tnc isoform expression after the analysis of several hundred clones. The overexpression of Pax6 also resulted in the upregulation of a Tnc binding integrin receptor subunit in neural stem cells.

Neurospheres were cultured in the presence of different growth factors known to control the expression level of Tnc in various cell types. Epidermal growth factor and basic fibroblast growth factor upregulated the expression of Tnc under proliferative conditions. Transforming growth factor beta showed no regulatory effect on Tnc in neurosphere cultures, which differs from the situation found in primary cortical rat astrocytes.

These findings show that the expression of the extracellular matrix molecule Tnc and its isoforms is regulated by defined intrinsic and extrinsic factors. We conclude that the differential expression of Tnc isoforms modulated by specific transcriptional regulators and growth factors can be observed in the developing central nervous system and in neural stem cells.

# An Induction Gene Trap Screen for Tenascin-C Target Genes in Neural Stem Cells

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The self-renewal and differentiation of neural stem cells (NSCs) is regulated by intrinsic and extrinsic factors. Within the last years the stem cell niche concept emerged underlining the importance of environmental cues for NSC behaviour/responses including interactions of the NSCs with extracellular matrix (ECM) components. Tenascin-C (Tnc) is a modular ECM glycoprotein expressed in the ventricular and subventricular zone during embryonic forebrain development where NSCs reside. Postnatally, expression persists in the neural stem cell niche of the adult CNS. For Tnc several interaction partners (e.g. phosphacan, neurocan) including cell surface receptors have been described (e.g. integrin αvβ3, RPTP-β, F3/Contactin). Moreover many of the known receptors have been mapped to specific Tnc domains but in most cases neither signalling pathways nor Tnc regulated target genes are known. Recently, it has been shown that Tnc regulates the NSC pool during embryonic development by modulating growth factor responses of NSCs. It increases the sensitivity of NSCs to FGF2 and interferes with the BMP4 signalling pathway, which results in the correct temporal EGFR expression during development. It is, however, incompletely understood which genes are regulated by Tnc signalling in NSCs and how the latter modulates responses of NSCs to intrinsic and/or extrinsic signals.

To identifiy and characterize genes which are regulated by Tnc we applied the induction gene trap technology to mouse NSCs grown as free-floating neurospheres, which serve as a widespread model system for NSC maintenance and neural differentiation. The pt1βgeo gene trap vector was electroporated into cells derived from 3rd passage neurospheres with an transfection efficiency of 56±9% as determined 1 d after electroporation. Upon integration into the genome, transfected neural stem cells were selected with G418 (neomycin) giving rise to clonal neurospheres. Using our library we have detected 2 integrations that responded to Tnc treatment and identified the trapped genes using 5`-RACE as a guanidyl exchange factor (GEF) and a dynein light chain, respectively. The regulation of the identified candidates was confirmed in non transfected cells via RT-PCR, which also shows that the lacZ expression of the induced clones reflects the endogenous expression of the trapped gene in response to Tnc in vitro. In situ hybridisation analysis of the GEF showed that it is expressed throughout the telencephalon with most prominent signals in the ventricular and subventricular zone of the developing mouse brain. Corresponding analysis will be carried out for the other candidate and for both gain and loss of function approaches will be performed using overexpression or RNAi techniques, respectively.

In summary, we successfully applied the induction genetrap technology to mouse NSCs and developed a screening strategy for Tnc responsive target genes, which led to the identification of reasonable and promising candidates. The discovered candidates are likely to be important for understanding the cell biology of NSCs, which will help to evaluate their function in development and disease to generate new improved therapeutic concepts.

# Developmental expression patterns of NMDA and delta receptors in differentiating embryonic stem cells

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Ionotropic glutamate receptors play an essential role in neuronal development and are expressed in neural precursor cells. Some studies have demonstrated the critical role of glutamate in early development of the ventral telencephalon, particularly in promoting the proliferation of striatal progenitors via an NMDA receptor-dependent mechanism, while the proliferation of cortical progenitors derived from the dorsal telencephalon is regulated by activation of AMPA/KA receptors. AMPA receptors mediate fast excitatory neurotransmission while NMDA receptors play a more complex role in conferring synaptic plasticity. It also appears that activation of NMDA receptors could inhibit proliferation of progenitor cells expressed in the adult mouse brain.

Therefore, the aim of the present project is to study the developmental expression patterns of NMDA receptors in well-defined neural stem cells (NSCs) derived from embryonic stem cells (ESCs), and in the terminally differentiated progeny of the NSCs, the neurons and glial cells. An additional glutamate receptor subfamily, the orphan or delta receptors, will also be studied.

The NMDA receptor subfamily comprises three different types of subunits, namely one NR1, four NR2 and two NR3 subunits, while there are two mammalian orphan receptor subunits. The expression patterns of these subunits are examined and compared by real time RT-PCR and on the protein level by immunoblotting.

We established the expression of all four NR2 subunits, of the NR3A subunit and the delta receptors in NSCs which extends the expression profile of ESCs. The expression of the NR1 subunit is remarkably reduced in comparison with the positive control (p2 mouse cortex) and does not exceed the expression level of NR1 in ESCs. These results are consistent with other studies indicating that the presence of functional NMDA receptors inhibit NSC proliferation. As a next step we will examine of the effects of NR1 overexpression in NSCs on the proliferation and differentation which may demonstrate a the direct influence of NMDA receptors on brain development.

# Survival Promoting Peptide (SPP) alters the neruotrophin expression, retards somatodendritic differentiation but enhances cell migration.

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Survival Promoting Peptide, SPP/Y-P30, has recently been shown to promote the survival of thalamic neurons, the growth of thalamocortical axons and neurite growth in cerebellar microcultures. SPP is produced by peripheral blood monocnuclear cells of pregnant rats and humans, becomes imported into the brain, and enriches in cortical neurons of early postnatal rat and fetal humans (Landgraf et al., FASEB J, 2004). The presence of SPP during corticogenesis suggests multiple functions. The present study was designed to characterize the role of SPP on neurotrophin expression, cell differentiation and cell migration.

For survival of neurons, the most important factors are the neurotrophins. As SPP promote the survival of neuron, we thought to analyze the effect of SPP on neurotrophin expression levels be RT-PCR, the results shows that a transient pulse of SPP on cortical slice culture significantly upregulate the expression of NGF while reduces NT-3 and has no effects on BDNF and NT4/5 mRNA. Characterization of the role of SPP in morphological differentiation using organotypic cultures from postnatal rat cortex shows that the addition of exogenous SPP to the medium has no effect on somatic growth of GABA-immunoreactive neurons nor the dendritogenesis of pyramidal neurons transfected with EGFP, as assessed by quantitative morphometry, Strikingly, the neutralization of endogenous cortical SPP with anti-SPP antibodies seems to increase the size of GABAergic somata, and it promotes the elongation and branching of pyramidal cell dendrites.

Secondly, we tested if SPP influences the cell migration using transformed human umbilical vein endothelial cells and neural PC12 cells. In a Boyden Chamber and in an in-vitro wound healing assay with time-lapse video imaging. SPP transiently enhances the migration rate of cell. The involvement of small GTPases is currently under investigation.

The results suggest that SPP alters the expression of neurotrophins, increases cell migration but retards differentiation. Supported GRK 736

# Neural Stem Cells form Human Embryonic Stem Cells

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Human embryonic stem cells (hESC) are expected to have far reaching applications in regenerative medicine. An intriguing question is whether these pluripotent cells can be used as a source of somatic stem cells for tissues with limited regenerative potential and whether such in vitro generated somatic stem cells display intrinsic and physiological properties comparable to primary cells. We have generated stably proliferating neuroepithelial stem cells from human ES cells (hES-NSCs). These cells can be extensively proliferated, show multipotentiality at a clonal level and maintain a constant neuro- and gliogenic differentiation pattern. In vitro, they give rise to functional neurons of mostly GABA(+)/GAD(+) phenotypes. RT-PCR and immunofluorescence data indicate that spontaneously differentiating hES-NSCs exhibit a regionally restricted identity compatible with an anterior hindbrain fate. Remarkably, even after long-term propagation, hES-NCSs remain responsive to instructive regionalization cues. Exposure of hES-NSCs to Shh and FGF8 induces ventral midbrain markers and enables the derivation of large numbers of TH-positive neurons. Retinoic acid treatment promotes further posteriorization with expression of caudal Hox genes. The neurogenic potential of hES-NSCs is conserved in vivo. Four months after transplantation into newborn SCID beige mice, hES-NSCs-derived neurons can be detected in various brain regions where they functionally integrate and exhibit spontaneous postsynaptic currents. Thus, stably proliferating hES-NSCs may provide a useful tool for studying mechanisms of human neural lineage segregation and a donor source for nervous system repair.

Supported by DFG, BMBF and the Hertie Foundation

# Lineage selection of doublecortin-positive migratory human ES cell-derived neuroblasts

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Human embryonic stem cells (hESC) represent a virtually unlimited donor source for cell transplantation. Key prerequisite for a clinical application of hESC-based neuronal replacement are highly purified populations of immature migrating neurons capable of integrating into host tissue. These properties are featured by doublecortin-positive neuronal progenitors in the developing CNS. Upon in vitro differentiation of hESC-derived neural stem cells (hES-NSCs), DCX is expressed by immature neurons co-expressing the early neuronal markers beta-III-tubulin and MAP2ab. We have combined controlled in vitro differentiation and genetic lineage selection of doublecortin-positive cells to derive transplantable neuronal precursors from hESC. Specifically, hES-NSCs were transfected with a construct carrying EGFP under the control of the human DCX promoter. Transfected clones showed faithful recapitulation of DCX expression by the EGFP reporter. FACSorting of immature DCX-EGFP-positive cells yielded neuronal populations at purities exceeding 95%. Sorted cells were amenable to replating and further in vitro differentiation. In vitro migration assays in transwell chambers as well as transplants in hippocampal slice cultures and the living rodent brain revealed an enhanced migration potential of the DCX-positive cells. DCX-EGFP-based lineage selection may thus provide a useful tool for deriving transplantable immature neurons from human ES cells.

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# Embryonic stem cell-derived neurons develop functional neuronal networks on microelectrode arrays.

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Embryonic stem (ES) cells can be differentiated into neurons of diverse neurotransmitter-specific phenotypes. They are known to express voltage dependent ion channels and their electrophysiological activities were demonstrated on single cell level in several *in vitro* and *in vivo* studies. However, it is unclear if populations of ES cell-derived neurons are able, similar to primary neurons, to generate a functional neuronal network *in vitro* that depends on the density and appropriate function of a multiplicity of neurons and their synapses. To clarify this issue we performed extracellular recordings by using the microelectrode array (MEA) technique that allows to observe electrophysiological function of populations of ES-derived neurons over a longer time period *in vitro*. ES cell-derived neural precursors were seeded onto MEAs and grown to confluency under the influence of fibroblast growth factor-2. Then, neural precursors were induced to differentiate into neurons and glial cells by growth factor withdrawal. Immunocytochemical investigations illustrated expression of diverse neuronal markers within 4 weeks after initiation of differentiation. MEA-recordings revealed first spontaneous spike activities ~2 weeks after initiation of differentiation. During the following 2 weeks the spatial and temporal density of spike activities increased and ~3 weeks after initiation of differentiation we observed spontanous bursts of spikes. Four weeks after initiation of differentiation we observed synchronously oscillating bursts of spike activity on spatially separated electrodes. Synchronously oscillating bursts were sensitive for synaptically acting drugs. These data indicate that populations of ES-derived neurons are able to generate functional neuronal networks. (supported by BMBF `Cell-based regenerative medicine´)

# *In vitro* differentiation of human umbilical cord blood progenitor cells

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There is an increasing interest in stem cell research from both basic science and clinical perspective since *in vitro* as well as *in vivo* data demonstrate the wide potentials of these cells. Human Cord Blood (HCB) is considered a new valuable pool in stem cell research. HCB is the blood remaining in the placenta and umbilical cord after the baby is born, and contains in its mononuclear cell fraction a certain number of mesenchymal stem cells (MSC) that are able to proliferate and to differentiate *in vitro* into many different cell types. MSC derived from HCB have the same potential as the MSC derived from bone marrow. However, since the HCB cells derive from newborns, they have longer telomeres resulting in higher proliferation capacity. Our objective for this project is to propagate HCB derived MSC and to study their differentiation development *in vitro*.

HCB probes are collected after informed consent of mothers according to the German guidelines for blood donation. Mononuclear cells were isolated from HCB by Ficoll density-gradient centrifugation and cultured in MSC medium. The MSC are growing as adherent cell fraction and they are cultured until their morphology changes from flattened to spindle-shaped. In this stage, the cells are pre-differentiated with bFGF and EGF containing medium for one week. Pre-differentiated cells are plated on fibronectin coated cover slips and cultured in different media for further differentiation. Cells are fixed at several differentiation time points (24 hours up to 16 days) and processed for immunocytochemistry against Vimentin, Nestin, β-tubulin, MAP2, NeuN, GFAP, Neuro D, Neurofilament 200, Neurofilament M, Tyrosine Hydroxylase, and Glutamate Decarboxylase 65.

The different HCB samples exhibit a wide range in number as well as in survival pattern of mononuclear cells. After three weeks in MSC culture medium the cells reach the stage of morphology of spindle-shape and start proliferating faster whereas some probes keep growing in flattened morphology. At this stage, bFGF and EGF exposure changes their morphology towards neuronal phenotype. Our results show that it is possible to isolate MSC out of HCB, proliferate and differentiate them into cells expressing stem cell markers as well as neuronal markers. A detailed analysis of the differentiation potential of HCB derived MSC will be presented at the meeting.

# Impaired neurogenesis and astroglial gap junction coupling in BDV infected differentiated P19 embryonic carcinoma cells

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Borna disease virus (BDV) is a single stranded neurotropic non-segmented RNA virus which upon neonatal infection in the rat leads to degeneration of the dentate gyrus granule cells and of cerebellar Purkinje neurons. Underlying cellular mechanisms are only partially understood but seem to be primarily mediated by an activation of the immune system. In order to asses immune-independent effects of BDV on neuronal development and functioning we investigated here actions of a latent BDV infection on neuronal differentiation and on gap junction coupling in P19 embryonic carcinoma cells. P19 cells upon embryoid body formation and retinoic acid treatment undergo neural differentiation resulting in mixed cultures of neurons and astroglial cells but without any immune cells being present. Using this cell culture model we could demonstrate that in BDV infected P19 cell cultures retinoic acid induced neuronal differentiation is greatly impaired as revealed by a reduction of cells positive for the neuronal differentiation markers Doublecortin and NeuN. In parallel, we could demonstrate a significant reduction in gap junction coupling in astrocytes of BDV infected retinoic acid treated cultures as revealed by the spread of the gap junction permeant fluorescent dye Lucifer Yellow. In addition expression of the gap junction connexin cx43 was reduced. Our results therefore demonstrate, that besides the well documented immune mediated effects of BDV on the nervous system, BDV seems to affect affect neuronal differentiation and functioning also in an immune independent manner.

# Expression of epigenetic factors in mouse brain development

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Polycomb group (PcG) genes are well described regulators of body segmentation and cell growth, being therefore important players during development. PcG proteins form large complexes (PRC) and are involved in various epigenetic phenomena, such as maintenance of Hox gene expression patterns, X chromosome inactivation and chromosomal imprinting. Methylation of histone H3 and subsequent ubiquitination of histone H2A are among the epigenetic functions of PcG proteins that lead to the specific inheritance of a transcriptional program. Recently, epigenetic chromatin modifications are discussed in the context of neurodevelopmental diseases such as schizophrenia.

Although expression of PcG genes in the brain has been noticed, the involvement of PcG genes in the processes of brain development is not understood. In this study we analysed the expression patterns of PRC1 complex members to reveal PcG proteins that might be relevant for mouse brain development. Using in situ hybridisation we show PRC1 activity in proliferative progenitor cells during neurogenesis, but also in maturated neuronal structures. PRC1 complex compositions vary in a spatial and temporal controlled manner during mouse brain development, providing cellular tools to act in different developmental contexts of cell proliferation, cell fate determination and differentiation.

# Control of neuronal differentiation by SATB genes

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Satb1 is a transcription factor that seems to be involved in the regulation of tissue-specific organization of chromatin. It is a nuclear protein that regulates transcription by binding matrix attachment regions (MAR) of genomic DNA.

We identified a close homologue of Satb1, Satb2 in a subtraction hybridization based screen of genes controlling neural differentiation. Satb2 and Satb1 expression was detected in mutually exclusive subpopulations of developing mouse CNS. We found Satb2 to be expressed exclusively in a subpopulation of postmitotic neurons of the upper layers (II- IV) of the cerebral cortex.

In order to characterize the two subpopulations of cells, two separate approaches have been used. First, we generated Satb2 mouse knockout line-Satb2-KO. For the second approach we have generated a mouse line where the Satb2 coding sequence has been replaced by Satb1 gene- Satb1-KI . In these mice all cells that normally express Satb2 will ectopically expresses Satb1.

Total knockout of Satb2 leads to craniofacial abnormalities and malformations in the cerebral cortex. The phenotype of the Satb2 KO and Satb1 KI will be presented and discussed.

# Developmental deficits in central and peripheral mouse nervous system after knockout of membrane fusion proteins.

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In eukaryotic cells, molecules need to be transported to their correct intracellular destination without compromising the structural integrity of cellular compartments. To achieve this, transport vesicles bud from intracellular donor organelle and then target, dock and fuse with an acceptor organelle. Members of a family of membrane fusion proteins have been implicated as central in membrane trafficking events studied so far. The two proteins studied in this investigation have distinct but overlapping subcellular localization. One has been associated with recycling process after endocytosis and early fusion events whereas the other is involved in late fusion and lysosomal degradation.

Mice deficient in both membrane fusion proteins, die during intrauterine life just before birth, whereas single knockouts and triallelic mice survive and reach normal age without difficulty. The reason for this differential behaviour is completely unknown. The homozygous KO mice have various changes in central (CNS) as well as peripheral nervous system (PNS). In CNS they show wide ventricles, lack several fiber tracts and some axons do not reach to their targets suggesting a deficit in axonal guidance and neurite outgrowth. Additionally, the homozygous KO mice also show more neurons in inner cortical layers. This could be due to altered productivity in the ventricular zone during cortical layer development or defects in neuronal migration along radial glia cells. On the other hand in PNS, KO mice show various degrees of neurodegeneration in different types of ganglia. Our data provide the neuroanatomical basis for ongoing work on possible mechanism in which membrane fusion proteins are involved during nervous system development.

# Sip1 regulates proliferation, differentiation, maturation and migration in the embryonic mouse neocortex

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Sip1, or Smad- interacting protein 1, is a transcriptional repressor known to play a role in several neurodevelopmental events. These include patterning of the neuroepithelium, differentiation and migration of the neural crest in mammals, and neural induction in Xenopus. Mutations in Sip1 are known to cause the Mowat- Wilson Syndrome in humans. In the developing mouse cortex, Sip1 is expressed mostly in the differentiating and post- mitotic fields of the cortex and weakly in the proliferative zones. Our analysis of conditional knockouts of Sip1 in the cortex revealed gross morphological defects like absence of a corpus callosum, and a progressively degenerating hippocampal formation. We also observed several defects in the development of the neocortex. Specific deletion of Sip1 in cortical progenitors and subsequently the post-mitotic neurons they generate, led to a depletion of deep layers of the cortical plate, and premature generation of upper layer neurons. We also found abnormal proliferation, and hampered migration of neurons and astrocytes born in the cortex. Deletion of Sip1 led to a decrease in the expression of Reelin in the marginal zone. At the molecular level, the expression of the secreted Wnt antagonist Sfrp1 was ectopically upregulated in the entire cortical plate (including the germinal zone) during late embryonic stages of development. When we deleted Sip1 exclusively in the postmitotic cortical plate neurons, we were able to reproduce some of the phenotypic effects seen earlier, but to a lesser extent, leading us to believe that some, but not all, of the consequences of Sip1 deletion might be caused due to non- cell autonomous effects, possibly mediated by Sfrp1. Our goal is to identify other targets of Sip1 in the neocortex, to further investigate the cell autonomous versus the non- cell autonomous roles of Sip1 in neocortical development, and the molecular cause of the migration defects seen in the Sip1 mutant.

# Induction and specification of sertonergic neurons of the ventral rhombencephalon

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Serotonin (5-HT) producing neurons of the formatio reticularis are involved by their projections in the modulation of behaviour as anxiety, sleep and mood. Dysfunction of the 5-HT system is associated with disorders as depression, schizophrenia and migraine. Their development depends critical on the floor plate signal Shh, on FGF8 from the isthmus-organizer and the pre-patterning signal FGF4 during early development. So far the transcription-factors Nkx2.2., Lmx1b, Gata2 and Pet1 have been described to act downstream of Shh, leading to the differentiation of progenitor cells and specification of the 5-HT phenotype. In order to complete this network, we performed a cDNA micro-array, comparing the gene expression pattern of ventral mesencephalon and ventral rhombencephalon of mice E11, a time point where 5-HT neurons are first generated. Subsequently, micro-array results were validated by RealTime PCR and in situ hybridization.
The results show differential expression of several genes in ventral hindbrain, compared to ventral midbrain. These results together with the observation of the selective agenesis of paramedian raphe 5-HT neurons in TGFβ2 knock-out mice at E18.5 indicate that TGFβ2 is an essential member of this network. Current in vitro experiments focus on the effects of TGFβ1-treatment, of functional blocking of endogenous TGFβ as well as the inhibition of TGFβ by receptor-blocking on differentiation of progenitor cells towards serotonergic fate.
Supported by the DFG through Center of Molecular Physiology of the Brain (CMPB)

# EFFECT OF AGING ON HIPPOCAMPAL NEUROGENESIS IN THE CANINE BRAIN

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Throughout life, neuronal progenitor cells located in the subgranular zone of the dentate gyrus give rise to new neurons for the granule cell layer. The continuous generation of newborn neurons is however declining with age. This consideration is mainly based on data from rodent models, whereas there is almost no data available from patient brain tissue. Therefore, we determined the effect of aging on neurogenesis in brains of canine patients.

Using post mortem tissue, the expression of the neurogenesis marker doublecortin was analyzed in dogs without brain-derived clinical dysfunctions (n=32) in the age between 2 and 216 months. Moreover, doublecortin expression was specifically studied in the aged canine brains of dogs (n=7) which display diffuse, senile plaques within the hippocampus.

In dogs without brain-derived clinical dysfunctions, expression of doublecortin clearly correlated with age, declining to very low levels in the aged canine brain. Preliminary evaluations indicated that doublecortin expression rates of aged dogs with senile plaques tended to be lower than expected from the data of age-matched control dogs.

These data give first proof that an age-dependent decline of hippocampal neurogenesis is not only present in laboratory animals, but also dominates hippocampal neurogenesis rates in animals kept under a variety of environmental conditions. In view of a putative role of neurogenesis for learning and memory, it has been hypothesized that this decline may be one reason for senile dementia. Thus, enhancement of neurogenesis may be a suitable strategy to ameliorate the outcome of cognitive dysfunction syndromes associated with senile dementia in elder dogs.

The study was supported by a scholarship (to Anton Pekcec) of the Center of System Neuroscience

# Influence of phosphoinositide-3-kinase gamma on neurogenesis in adult dentate gyrus

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Phosphoinositide-3-kinases (PI3K) mediate numerous cellular functions including cell survival and proliferation. Increasing evidence indicates that they are crucially involved in the regulation of adult neurogenesis. Here we analyzed the role of PI3K gamma, an isoform important for survival and function of immune cells, on neurogenesis in the dentate gyrus. It has been recently shown that immune cells contribute to neurogenesis and spatial learning. Using PI3K gamma knockout mice (PI3Kγ-/-) we analyzed the survival of new born cells in subgranular zone of the dentate gyrus. PI3Kγ-/- and control animals received daily intraperitoneal injection of 5-bromo-2-desoxiuridine for 1 week (BrdU, 50 mg/kg, twice daily). Spatial learning was tested for 7 days at the 5th week after the last BrdU injection using the Morris water maze. Animals survived for 7 weeks after beginning of the experiment. Immunocytochemistry with antibodies against BrdU and mature neuronal and glial markers demonstrated a significant increased number of new born neurons (+46%) in animals deficient for PI3Kγ compared with controls. This finding was not associated with a better performance in the Morris water maze. Further experiments were performed to analyze the role of microglia in the neurogenic niche of the subgranular zone. Our data provide evidence that lack of PI3Kγ increased neurogenesis in the dentate gyrus. The underlying mechanisms are currently under investigation.
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# Layer-specific expression of multiple (proto-)cadherins in the cerebral cortex of adult ferret

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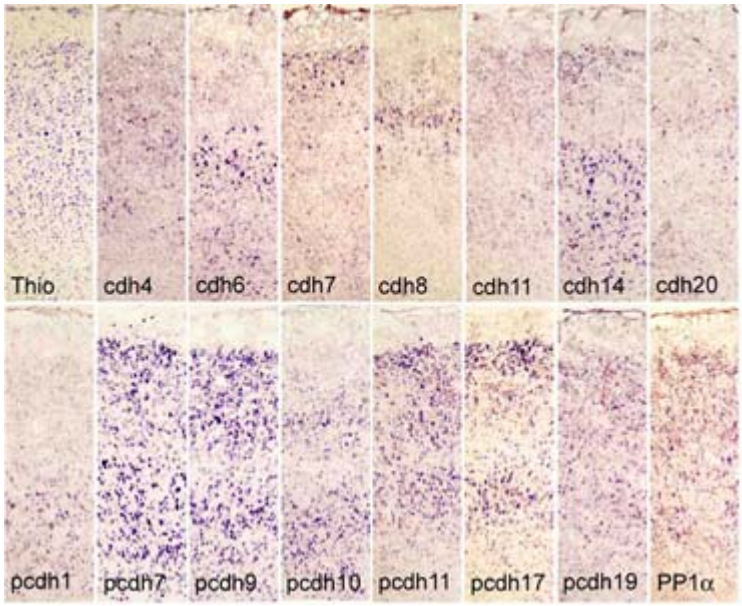
Cadherins are Ca2+-dependent cell adhesion proteins mediating cell-cell adhesion. Dozens of cadherins are expressed in the vertebrate brain where they have been implicated in a variety of developmental processes. Previous findings have demonstrated that each cadherin is expressed in a specific subset of developing gray matter structures, fiber tracts and neural circuits. For example, the layers of the chicken tectum differentially express multiple cadherins. In the deeper tectal layers, subsets of projection neurons express cadherins differentially. Their axons take separate paths to their respective target areas.

To gain insight into the molecular mechanisms regulating layer formation in the cerebral cortex, we cloned 8 classic cadherins and 8 delta-protocadherins for the first time from ferret brain. Their expression patterns were investigated by in situ hybridization in developing cortex (see companion poster by Nürnberger et al.) and in adult cortex.

The figure illustrates that most (proto-)cadherins are expressed in layer-specific manner in adult parietal cortex. The expression of some (proto-)cadherins is restricted to particular cortical layers while others are expressed more widely. A few cadherins (cdh4, cdh7 and cdh20) are expressed by dispersed populations of cells scattered across different cortical layers.

Thus, our results suggest that cadherins are molecular markers for subsets of neurons in specific cortical layers and may be involved in their formation.



# Comparative analysis of the cerebellar systems of chicken, mouse and ferret using cadherins as molecular markers

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The cerebellum is an evolutionary ancient structure that shows similarities in histology, morphology and functional organization in higher vertebrates. The cerebellar cortex can be divided into several functionally distinct parasagittal domains, which project to specific parts of the deep cerebellar nuclei and the inferior olive. From medial to lateral, the cerebellar cortex consists of the vermis, the intermediate zone and the hemispheres in birds and mammals (Larsell, 1948; Goodman et al., 1964). Hodologically (and phylogenetically), the cerebellum has been divided into the vestibulo- (archi-), spino- (paleo-) and pontocerebellum (neocerebellum).

It has been suggested that the cerebellar hemispheres (neocerebellum) have emerged and increased in size in parallel to the enlargement of neocortex in mammals. In the present study, we compared the cerebellar systems of three vertebrates with large differences in cortical size (chicken, mouse and ferret) by mapping gene expression. A similar molecular approach to define phylogenetically homologous regions has been successfully applied to other brain regions, for example to the forebrain (Puelles et al., 2000). We mapped the expression of three members of the cadherin superfamily of adhesion molecules that are markers of functional brain structures. Several cadherins are expressed differentially in parasagittal domains of the cerebellar cortex, the deep cerebellar nuclei and the inferior olive divisions.

The expression of a classic cadherin (cdh8) and two protocadherins (pcdh7 and pcdh10) was mapped at comparable stages of late cerebellar development (E12 in chicken, P3 in mouse, and P2 in ferret). In all three species, the cadherins show characteristic parasagittal stripes in cerebellar cortex and a regionally restricted expression in the deep cerebellar nuclei and the inferior olive. In mouse and ferret, expression patterns show a similar number, complexity and arrangement of stripes in the cerebellar cortex and in the deep cerebellar nuclei. In contrast, the parasagittal cortical pattern in chicken is more distinct and less complex with fewer stripes in some areas, although the overall rostrocaudal sequence of expression is similar in all three species. A lateralmost part of the hemispheres does not show expression of the three cadherins; it has about the same relative size in all three species. In the inferior olive, the expression patterns are difficult to compare because the laminar structure of the olive is less clear in chicken than in mouse and ferret.

Our results do not support the notion that the cerebellar hemispheres have enlarged during evolution in parallel to the growth of the cerebral cortex. Rather, the enlargement of cerebral cortex may be reflected in an increase in complexity of all parts of the cerebellum.

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# Regional expression of multiple (proto-)cadherins in the developing cerebral cortex of the ferret

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Cadherins are mediators of cell adhesion, known to play a key role in tissue and organ development. In the brain, multiple cadherins provide an adhesive code for developing brain structures. Classic cadherins are expressed differentially in developing brain nuclei, cortical layers, fiber tracts and neural circuits. For example, in the postnatal mouse brain, classic cadherins (cdh2, cdh4, cdh6, cdh8, and cdh11) show distinct regional expression patterns in cerebral cortex, thalamic nuclei and other subcortical regions (for review, see Redies, 2000). Little is known about the expression of other classic cadherins or protocadherins in the mammalian cortex. In addition, most gene expression studies have been carried out in mammals with relatively small cerebral cortices.

In the present study, we mapped the expression of multiple (proto-)cadherins in the developing ferret, a carnivore that has a large cerebral cortex. Eight classic cadherins (cdh4, cdh6, cdh7, cdh8, cdh11, cdh14/18, cdh19, cdh20) and 7 delta-protocadherins (pcdh1, pcdh7, pcdh9, pcdh10, pcdh11, pcdh17, pcdh19; Redies et al., 2005) were newly cloned with RT-PCR using degenerate primers. Expression patterns were examined in embryonic brain (E23, E30, E38), postnatal brain (P2, P15, P25, P38, P46, P60) and adult brain by in situ hybridization.

Most of the cadherins studied display gradients of expression in the different layers of ferret neocortex, as shown previously for cdh6, cdh8 and cdh11 in the mouse (Suzuki et al., 1997). For example, cdh7 shows an expression gradient increasing from dorsal to basolateral, cdh14/18 from medial to lateral, cdh20 from dorsal to basolateral, pcdh1 and pcdh10 from occipital to frontal, and pcdh7 and pcdh19 from lateral to medial (see Figure: ferret postnatal day 2). Expression is restricted to distinct cortical layers and differs between (proto-)cadherins (see also companion poster by Krishna et al.). Moreover, there are sharp expression boundaries, for example, at the neo/allocortical transition (for example, for cdh20 and pcdh19, see Figure). These results suggest that (proto-)cadherins provide multiple adhesive cues for the arealization and layering of developing cerebral cortex.

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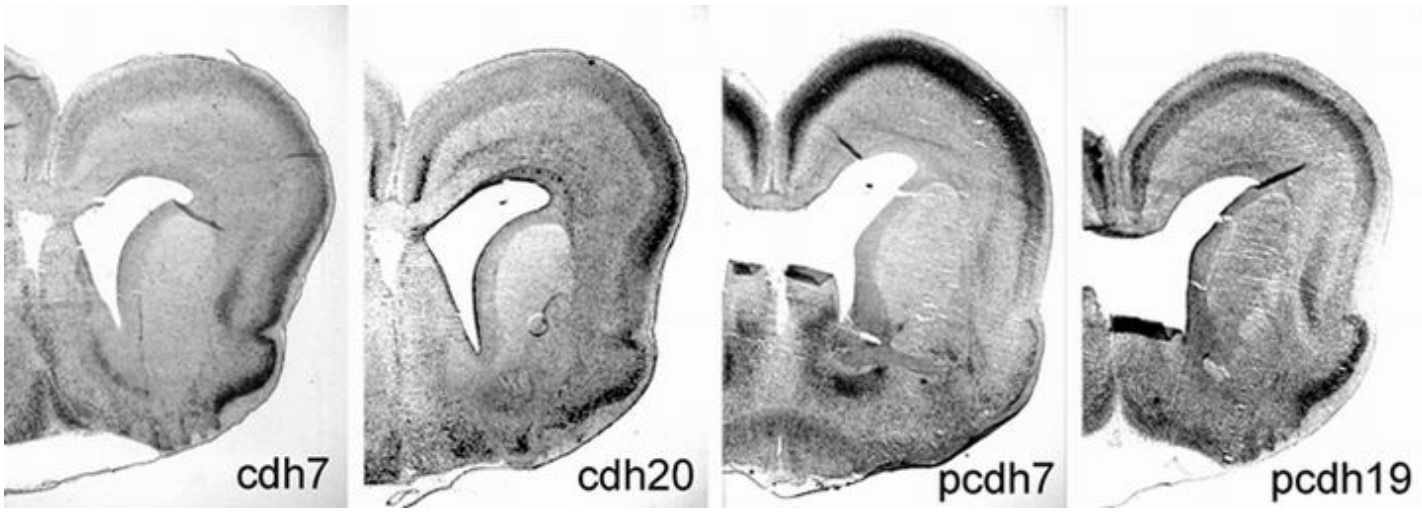

# Radiation induced alteration of proliferative activity in the forebrain of exposed male rats and their offspring

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The transgenerational effect of gamma radiation on the proliferative activity of cells in the forebrain rostral migratory stream (RMS) was studied in parental generation and offspring of male rats exposed to the dose of 3 Gy 25 days before conception with intact control females. Proliferation was quantified by stereological assessment of bromodeoxyuridine (BrdU) labelling. The number of BrdU-positive cells was counted in three parts of the RMS, i.e. in the vertical arm, elbow and horizontal arm in irradiated males (25 days after irradiation) and in their offspring at the 3rd, 7th, 14th, 21st and 28th postnatal days. In exposed male rats of parental generation, an increase in the BrdU-positive cells was found in the elbow and mainly in vertical arm of the RMS. In the progeny of irradiated rats, the age-related dynamics of the changes was similar to that in the corresponding control groups, however, the number of BrdU-positive cells was significantly higher along the whole RMS at all intervals of investigation. The current results suggest that paternal exposure to ionizing radiation caused decrease in the stability of genome; the decrease in the radiation-induced genome stability manifested itself in the progeny by alteration in proliferative activity or slackening of cell migration in the RMS, which was even more conspicuous than in the exposed males of parental generation.
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# The expression of embryonic tau protein isoforms persist during adult neurogenesis in the hippocampus

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Tau is a microtubule-associated protein with a developmentally regulated expression of multiple isoforms. The neonatal isoform is devoid of two amino terminal inserts and contains only three instead of four microtubule binding repeats (0N/3R-tau). We investigated the temporal expression pattern of 0N-tau and 3R-tau in the rat hippocampus. After the decline of 0N- and 3Rtau immunoreactivity during the postnatal development both isoforms remain highly expressed in a few cells residing beneath the granule cell layer. Coexpression of the polysialylated neuronal cell adhesion molecule, doublecortin and incoporated bromodeoxyuridine showed that these cells are proliferating progenitor cells. In contrast mature granule cells express the adult tau protein isoform containing one aminoterminal insert domain (1N-tau). Therefore a shift in tau isoform expression takes place during adult neurogenesis which might be related to migration, differention and integration in the granule cell layer. Providing a model to study shifts in tau isoform expression in a defined subset of neurons might contribute to understand the pathomechanism of tauopathies were isoform composition is of critical importance for the mechanism of neurodegeneration, such as Pick's disease or FTDP-17.

# Stem cell transplantation in rat model cognitive dysfunction: Neurobehavioral, Neurochemical and Immunohistochemical assessment

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Neural Progenitor cells (NPCs), the multipotent stem cells derived from from early embryonic fetal brain have emerged as suitable alternative for fetal neural cells in cell replacement therapy specially in neurodegenerative diseases. NPCs are capable of expension in culture and can be differentiated into desired neuronal phenotype through specific trophic factor support.

Keeping this in mind, in the present study an attempt was made to derive NPCs from rat fetuses of embryonic day 11, expend them in culture and differentiate into specific cholinergic type by exposure to basic fibroblast growth factor and epidermal growth factor. These cells were then transplanted into kainic acid induced rat model of cognitive dysfunction (four weekspost lesioning)to see funtional restoration.

The cultured cells were characterized immunocytochemically for choline acetyltransferase (ChAT), acetyl choline estrase (AChE) and acetyl choline receptors (AChR) expression before transplantation. Neurobehavioral, neurochemical and Immunohistochemical assessmentwere made four weeks post transplantation. NPCs transpanted animals had shown significant($p>0.005$) learning and memory recovery on Y Maze as compared to lesioned animals. More neurochemical restotation was observed in transplanted animals where a 70%recovery in Cholinergic receptor binding and high immunoreactivity for choline acetyltransferase (ChAT) was evident as compared to lesioned animals.

The present findings suggest that NPCs differentiated into cholinergic neurons are capable of functional restoration when transplanted in rat model of cognitive dysfunction and appeared to be potential cell source for cell replacement in various neurodegenerative diseases .

# Pax6-instructed neurogenesis from astrocytes: Molecular analysis of Pax6 function and functional features of newborn neurons

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While radial glial cells acting as adult neural stem cells up-regulate Pax6 and generate neurons, most astroglial cells in the adult mammalian brain fail to do so. Thus, instruction of astrocytes towards neurogenesis by ectopic Pax6 expression provides a powerful tool to initiate repair in the adult mammalian forebrain (Buffo *et al.*, 2005). Here, we examined the functional maturation of neurons derived from astrocytes isolated from the postnatal mouse cortex by virally mediated expression of Pax6, its different isoforms and various posttranslationally modified forms. Ectopic expression of the canonical isoform of Pax6 directed cortical astroglia towards neurogenesis, as detectable by expression of the neuronal markers Doublecortin (Dcx), Tau, and β-Tubulin III, 7-10 days after transduction. 13-17 days after transduction, more mature neuronal markers such as NeuN appeared, while Dcx was down-regulated, consistent with neuronal maturation *in vivo*. After 7-10 days, the neurons had already acquired TTX-dependent $Na^+$-channels and fired action potentials. They could also receive functional synaptic input as evident in co-cultures with primary cortical neurons. These electrophysiological data are consistent with the acquisition of neuronal polarity observed after 20 days. While most neurites were still co-labelled for Tau, β-Tubulin III and Map2, some neurites acquired axonal features by maintaining Tau, but losing the dendritic marker Map2. Surprisingly, however, we could not detect a variety of presynaptic markers, consistent with the finding that Pax6-instructed neurons were not able to form functional presynaptic compartments.

Given the role of Pax6 in instructing neurons to receive synaptic inputs, we next set out to determine the molecular domains critical for this potent function. Preliminary data of ectopic expression of different Pax6 isoforms in astrocytes suggested that the paired domain plays a crucial role in its neurogenic effect. Moreover, we established stable neuroblastoma cell lines expressing recombinant Pax6. In these cells, phosphorylation sensitive residues were identified in the transactivation domain of Pax6. Preliminary results show that the mutation of most of these residues does not affect the principle neurogenic property of Pax6. We will discuss the role of posttranslational modification of Pax6 and its DNA-binding domains with regard to its potent neurogenic effects.

Supported by the DFG and the BMBF.

Buffo *et al.* (2005) PNAS 102: 18183 ff.

# Transforming growth factor-beta1 inhibits adult neurogenesis

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Transforming growth factor (TGF)-beta1 has multiple functions in the adult central nervous system (CNS). It modulates inflammatory responses in the CNS and controls proliferation of microglia and astrocytes. In the diseased brain, TGF-beta1 expression is up-regulated and, depending on the cellular context, its activity can be beneficial or detrimental regarding regeneration.

Here, we focus on the role of TGF-beta1 in adult neural stem cell biology and neurogenesis. In adult neural stem and progenitor cell cultures and after intracerebroventricular infusion TGF-beta1 induced a long-lasting inhibition of neural stem and progenitor cell proliferation and a reduction in neurogenesis. In vitro, although TGF-beta1 specifically arrested neural stem and progenitor cells in G0/1 phase of the cell cycle, it did not affect the self-renewal capacity and the differentiation fate of these cells. Also, in vivo, TGF-beta1 did not influence the differentiation fate of newly generated cells as shown by bromo-deoxyuridine incorporation experiments. Based on these data we suggest that TGF-beta1 is an important signalling molecule involved in the control of neural stem and progenitor cell proliferation in the CNS. This might have potential implications for neurogenesis in a variety of TGF-beta1 associated CNS diseases and pathological conditions.

# Imaging of Neurogenesis using the Doublecortin Promoter in Transgenic Mice.

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The use of thymidine analogues, e.g. BrdU, over the last decades revealed the presence of neurogenesis in the mammalian adult central nervous system, including in human. Although valuable, this approach presents important limitations such as the need to perform *in vivo* labeling and the need to identify *a posteriori* the phenotype of newly generated cells using various markers. Thus far, this limited the analysis of *in vivo* neurogenesis as an ongoing process. Recently, we reported that the abundance of doublecortin (DCX)-expressing cells reflected levels on ongoing neurogenesis. DCX is a protein specifically and transiently expressed in newly generated neurons. Hence, we generated transgenic mice bearing fluorescent reporter genes under the control of the human DCX promoter. We described here that developmental and adult transient patterns of expression of the transgene reflected the endogenous DCX gene expression. Within the adult active neurogenic areas, the human DCX promoter drove expression of the fluorescent proteins in newly generated neurons, allowing for their direct identification on acute slices. Electrophysiological analysis on the reporter-expressing cells in the dentate gyrus revealed properties previously described for PSA-NCAM-expressing cells. A central feature of newly generated neurons was a very high current input resistance as well as an increased excitability as compared to surrounding mature granule cells. In contrast to young individuals, aged animals have a dramatically decreased neurogenic activity. Using the expression of fluorescent reporters to identify the very few newly generated neurons in the aged dentate gyrus, we analyzed electrophysiological properties of these cells and compared them to the new neurons observed in young transgenic animals. Our observation suggested that newly generated neurons in aged animals were not differing from those produced in young animals. Therefore, although levels of neurogenesis decreased during aging, the intrinsic properties of the newly added cells remained similar. Moreover, this suggests that neural stem cells, the source of new neurons, maintained their properties during the individual whole lifespan. Hence, stimulation of neurogenesis may constitute a valid approach to counteract age-associated neuronal loss and/or cognitive decline.

# Bone Marrow Stromal Cells Instruct Oligodendrogenic Fate Decision on Adult Neural Stem Cells

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Adult stem cells reside in different tissues and organs of the adult organism. Among these cells are BMSCs that are located in the adult bone marrow and NSCs that exist in the adult central nervous system (CNS). In transplantation experiments, BMSCs demonstrated neuroprotective and neuroregenerative effects that were associated with functional improvements. The underlying mechanisms are largely unidentified. Here, we reveal that the interactions between adult BMSCs and NSCs, mediated by soluble factors, induce oligodendrogenic fate decision in NSCs at the expense of astrogenesis. This was demonstrated (a) by an increase in the percentage of cells expressing the oligodendrocyte markers GalC and myelin basic protein, (b) by a reduction in the percentage of glial fibrillary acidic protein (GFAP)-expressing cells, and (c) by the expression pattern of cell fate determinants specific for oligodendrogenic differentiation. Thus, it involved enhanced expression of the oligodendrogenic transcription factors Olig1, Olig2, and Nkx2.2 and diminished expression of Id2, an inhibitor of oligodendrogenic differentiation. Results of (a) 5-bromo-2´-deoxyuridine pulse-labeling of cells, (b) cell fate analysis, and (c) cell death/survival analysis suggested an inductive mechanism and excluded a selection process. A candidate factor screen excluded a number of growth factors, cytokines, and neurotrophins that have previously been shown to influence neurogenesis and neural differentiation from the oligodendrogenic activity derived from the BMSCs. This work might have major implications for the development of future transplantation strategies for the treatment of degenerative diseases in the CNS.

# Glioblastoma-initiating cells: Identification by stem cell properties and tumorigenity but not CD133

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Recent evidence suggests that tumor stem cells exist in human glioblastomas and can be isolated based on the expression of CD133. CD133+ cells formed tumors which mirrored the growth pattern of human glioblastomas when transplanted into SCID mice and had the ability to self renew and proliferate. Finally only CD133+ cells were multipotent when differentiated.
We corroborate here that specimens from human glioblastomas contain cells which form neurospheres and display characteristic stem cell features in vitro. These features are multipotency with evidence of astroglial and neuronal differentiation, self-renewal capacity at single-cell level, as well as a stable proliferation potential. Most importantly, these cells are tumorigenic in vivo: 50 cells suffice to establish tumors after orthotopic intracerebral implantation and serial orthotopic transplantations in vivo resemble the histological features of the original human glioblastomas. In contrast to previously published data CD133 was not a discriminating marker in 9 tumor specimens analyzed in this project, as we find stem cell characteristics in a subset of CD133+ as well as CD133- cells.
When neurospheres of different tumors were differentiated with the help of FCS not all of them lost their stem cell properties. Moreover, there was no significant difference between the more differentiated cells and the neurospheres in respect to their resistance towards radio-chemotherapy in vitro.

Thus, human glioblastomas contain cells with features typical of neural stem cells. These cells are identified by a characteristic sphere formation in vitro when kept in serum free medium supplemented with EGF and FGF. Differentiation with the help of FCS does not consistently provoke the loss of stem cell properties and neurosphere cells did not demonstrate exquisite resistance mechanisms towards radio-chemotherapy in vitro. The sensitivity and specificity of CD133 as a stem cell marker is low.

# Brain development in the Marbled crayfish: analysing cell proliferation and neuronal expression of engrailed

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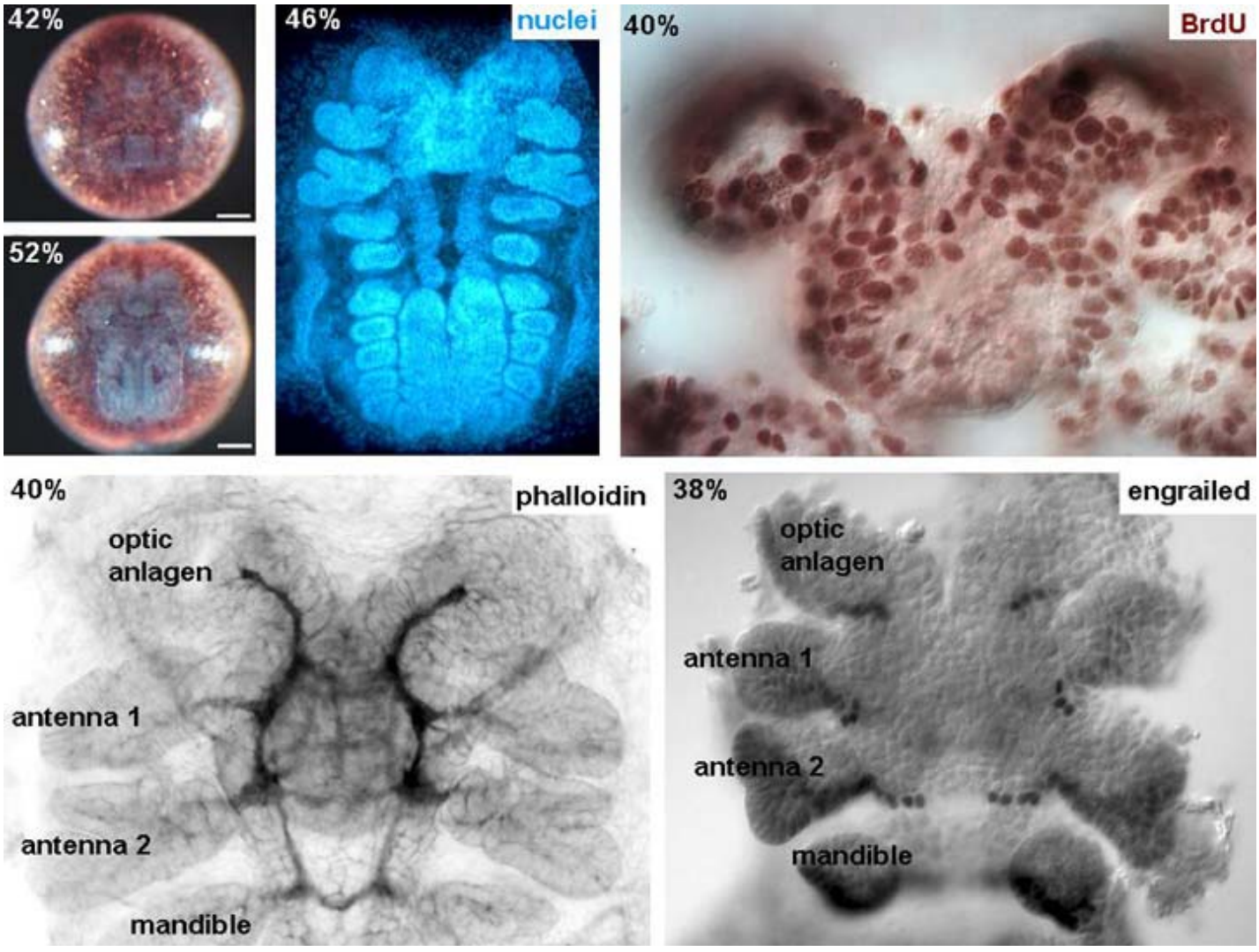
Development of the crustacean ventral nerve cord recently was analysed with regard to similarities between insect and crustacean neurogenetic mechanisms (Harzsch S. 2003. Arthr. Struct. Dev. 31:17). In the present project we analysed brain development in the Marbled crayfish (Seitz et al. 2005. J. Exp. Zool. 303A:393) in order to compare these ontogenetic processes to insects in an evolutionary context. The structure of the embryonic brain was analysed by histochemical labelling with phalloidin (Vilpoux et al. 2006. Dev. Genes. Evol. 216:209). Neurogenesis was monitored with the mitosis marker BrdU and the neuronal expression of engrailed was studied immunohistochemically (see Fig.). Our results indicate that the mitotic activity of neuronal stem cells is most intense between 38% and 45% of embryonic development. These neuroblasts can be identified individually based on their position within the developing brain. Engrailed immunolocalization labelled only a small number of cells at the posterior margin of the proto-, deuto-, and tritocerebrum. Double labelling experiments will show if there are any neuroblasts within the population of engrailed expressing cells. Acknowledgements: Silvia Sintoni is the recipient of a EC fellowship under the 6th Framework Programme 'Marie Curie Host Fellowships for Early Stage Research Training (EST)' ("MolMorph"). This project was also funded by the DFG (Ha 2540) and a Heisenbergfellowship to S.H.



# Is Nato3 a Novel Regulator of Floor Plate and Spinal Cord Development?

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The floor plate is one of the key organizers of the central nervous system in vertebrates, and plays important roles in both ventral patterning and axonal guidance in the neural tube. The floor plate is induced at the ventral midline of the neural tube through the action of Sonic hedgehog (Shh), which in turn activates the transcription of downstream transcription factors. Nato3 is a member of the basic Helix-Loop-Helix (bHLH) family of transcription factors, which regulate the specification/differentiation of specific cell lineages during neurogenesis. We found that within the developing spinal cord MNato3 and CNato3 expression is restricted to the floor plate in mouse and chick, respectively. However the signaling pathway through which MNato3 acts is currently unknown. Here, we aimed at identifying cis control elements and molecular mechanisms that target MNato3 expression to the floor plate.

To identify the DNA regions that direct MNato3 expression to the floor plate we applied a reporter gene assay in chick embryos using in ovo electroporation. Our results reveal a short genomic DNA region upstream of MNato3, which is necessary and sufficient to derive mouse Nato3 expression in the chick floor plate. We next looked for evolutionarily conserved transcription factor binding sites within this region, which may regulate the expression of MNato3. In vitro studies confirmed that the putative conserved binding sites identified in vivo and in silico are indeed functional, and relate MNato3 to the Sonic hedgehog signaling pathway. Our results provide the first linkage between MNato3 and the signaling pathway these factors regulate, and suggest that MNato3 may have an important role in spinal cord development.

# Poster Topic

## T2: Development II: Migration and path finding

**T2-1A** Phenotypic analysis of Sulf1 and Sulf2 deficient mice
*I. Kalus, M. Padva, R. d`Hooge and T. Dierks, Bielefeld, Göttingen and Leuven (B)*

**T2-2A** Identification of novel intracellular interaction partners of RPTPbeta/zeta using a Bacterial two-hybrid System
*T. Sobik, A. Horvat-Bröcker, G. Zoidel, R. Dermietzel and A. Faissner, Bochum*

**T2-3A** Comparison of embryonic rostral and caudal serotonin neuron gene expression profiles.
*CJ. Wylie, T. Hendricks, M. Goulding and E. Deneris, Cleveland OH (USA) and La Jolla CA (USA)*

**T2-4A** Early experience alters hippocampal reelin gene expression in a gender-specific manner
*CM. Gross, A. Flubacher, A. Heyer, M. Scheller, I. Herpfer, M. Frotscher, K. Lieb and CA. Haas, Freiburg*

**T2-5A** AXON GUIDANCE IN THE DOPAMINERGIC SYSTEMS OF ZEBRAFISH
*J. Schweitzer, S. Ryu, E. Kastenhuber and W. Driever, Freiburg*

**T2-6A** Reelin is secreted by the classical secretory pathway, but independent of neuronal activity
*S. Tinnes, F. Armin, S. Zhao, M. Frotscher and CA. Haas, Freiburg*

**T2-1B** Opposite roles of Reelin in the lamination of dentate granule cells by binding to different receptors
*S. Zhao, X. Chai, H. Bock, B. Brunne, E. Förster and M. Frotscher, Freiburg*

**T2-2B** Neuronal cell migration in an insect embryo.
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**T2-4B** Developmental strategies for the assembly of callosal connections
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**T2-6B** Effects of soluble semaphorin gradients on cortical fibers
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**T2-1C** EphrinA5 is involved in the regulation of the tangential migration of cortical interneurons
*G. Zimmer, P. P. Garcez, R. Niehage, F. Weth and J. Bolz, Jena and Rio de Janeiro (Brazil)*

**T2-2C** Temporal expression of *sequoia* defines target layer identity of *Drosophila* photoreceptor cells
*M. Petrovic and T. Hummel, Münster*

**T2-3C** Axonal guidance and cerebellar lobe formation in NrCAM-, CHL1-,and NrCAM-CHL1-double-deficient mice
*A. Heyden, F. Rathjen and D. Montag, Magdeburg and Berlin*

**T2-4C** The histone deacetylase SIRT2 in neurite outgrowth and axon guidance
*KV. Harting, R. Pandithage, B. Lüscher and B. Knöll, Tübingen and Aachen*

**T2-5C** Analysing the role of SRF in axon guidance
*C. Stritt and B. Knöll, Tübingen*

# Phenotypic analysis of Sulf1 and Sulf2 deficient mice

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Sulfatases belonging to a large protein family that is not only responsible for the degradation of sulfated macromolecules but also involved in the modification of sulfated compounds, cell-signaling processes and embryonic development. Most of the known sulfatases are lysosomal enzymes with the exception - amongst others - of the enzymes Sulf1 and Sulf2. Sulf1 and Sulf2 have been identified as extracellular heparansulphate (HS) 6-o-endosulfatases. To analyze the function of these enzymes knock out mice were generated. The phenotypic analysis of Sulf1, Sulf2 and double knockout mice is under investigation including histological analysis, behavioural and electrophysiological studies. Preliminary results give the indication that especially Sulf2 seems to be involved in the development of the central nervous system. Double knockout mice die mostly pre-natal. Surviving beyond birth they are smaller in size than control animals.

# Identification of novel intracellular interaction partners of RPTPbeta/zeta using a Bacterial two-hybrid System

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Receptor-type protein tyrosine phosphatase beta/zeta (RPTPβ/ζ) is a member of the R5 subgroup of protein tyrosine phosphatases and due to glycosaminoglycan side chains it belongs also to the group of chondroitin sulphate proteoglycans. It is characterized by the presence of a carbonic-anhydrase-like domain and fibronectin type III (FNIII) domain in the extracellular region and two intracellular phosphatase domains. RPTPβ/ζ is mainly expressed in the CNS and regulates different signal transduction pathways. Since RPTPβ/ζ is expressed in early embryonic stages, it is therefore likely that RPTPβ/ζ is involved in neuronal migration and differentiation. Four isoforms of RPTPβ/ζ are known: two isoforms are secreted into the extracellular matrix whereas the two other isoforms represent transmembrane proteins. Although several extracellular ligands of RPTPβ/ζ such as Tenascin-C/R, Pleiotrophin, F3/contactin are identified, our knowledge about the intracellular signalling molecules is still not satisfactory.

To investigate the intracellular interacting partners we performed a two-hybrid assay with the complete cytoplasmatic part of RPTPβ/ζ For this approach a bacterial two-hybrid system (BacterioMatchII$^{TM}$, Stratagene®) was used. The whole intracellular part of RPTPβ/ζ was cloned into the pBT Plasmid and served as a bait. The screen was performed against a rat brain cDNA library which contained 3,05 x $10^6$ target plasmids (40 pooled brains, 7-10 weeks old) according to the manufacturers description. The screening procedure was performed twice and 192 independent clones were subjected to sequence analysis. The results showed 40% positive and 60% false positive clones. Under the positive clones scaffolding proteins, tumor suppressor proteins, and proteins of the proteasomal degradation pathway were found.

The most enriched clone was named TS5.1 and represents a scaffolding protein, which is involved in axon guidance, synaptic morphology and synaptic size. To verify the interaction between RPTPβ/ζ and TS5.1 classical molecular approaches like colocalization studies, RT-PCR and precipitation studies were carried out. Further experiments should give answers to the assumption, if the interaction of RPTPβ/ζ and the putative interactor TS5.1 have substantial functional implications in the development of neural tissue connections.

# Comparison of embryonic rostral and caudal serotonin neuron gene expression profiles.

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The serotonin neurons of the mammalian brain are comprised of two subdivisions with distinct anatomical positions and axonal trajectories in the developing hindbrain. Serotonin neuron cell bodies in the rostral subdivision migrate to the midbrain and pons and extend ascending projections throughout the forebrain. Cell bodies in the caudal subdivision migrate to the ventral medulla and caudal half of the pons and provide descending projections to the brainstem and spinal cord. We hypothesize that distinct molecular programs are responsible for their disparate migration patterns and axonal trajectories. To investigate this hypothesis we set out to determine which genes are differentially expressed in rostral and caudal serotonin neurons during critical stages of their development. To facilitate this approach we have generated a transgenic mouse line in which developing rostral and caudal serotonin neurons are marked with enhanced yellow fluorescent protein (eYFP). We used fluorescent activated cell sorting (FACS) to purify these genetically labeled rostral and caudal neurons for gene expression profiling. Time points were chosen during embryonic development when migration and axonal pathfinding are underway. Our microarray studies suggest the differential expression of several genes in rostral versus caudal serotonin neurons. These include transcription factors and molecules involved in axon pathfinding. In addition, we also define a set of genes that are selectively expressed by both rostral and caudal serotonin neurons but not other surrounding cells in the developing neural tube. Verification of our microarray findings is currently underway using in situ hybridization and real-time PCR. Future loss and gain of function studies of verified differentially expressed genes will be aimed at defining their roles in the differentiation, migration, and axonal pathfinding of rostral and caudal serotonin neurons.

# Early experience alters hippocampal reelin gene expression in a gender-specific manner

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Early-life experience has long-term consequences on behavior and stress responsiveness of the adult. Environmental influences during sensitive time windows of early postnatal life interfere with the development of emotional and cognitive functions. Recent studies in rodents have shown that functional maturation of higher associative cortical regions, in particular those of the limbic system are strongly influenced by emotional experience. In rats chronic environmental impoverishment results in decreased brain size, cortical thickness, dendritic complexity and spine density. Since little is known how these processes are regulated at the molecular level, we used an early separation paradigm in mice followed by a hippocampal expression analysis of molecules important for cortical development (reelin and brain-derived neurotrophic factor, BDNF) and synapse formation (synaptopodin, synapsin I). We compared four experimental groups: NH (not handled), H (handled), MS (maternal separation, intact litter separated from the mother for three hours daily), ED (early deprivation, individual isolation of each pup for three hours daily). The separation paradigms were performed from postnatal day (PND) one until PND fifteen followed by immediate or delayed real time RT-PCR analysis at PND 70. At PND fifteen male mice responded to handling and early deprivation with a strong and significant increase in the expression of reelin, BDNF and synaptopodin mRNA as compared to non-handled ones. Female pups showed the same expression pattern for BDNF mRNA, but they did not respond to handling with a significant up-regulation of reelin and synaptopodin mRNA expression. In the adult mice (PND 70) none of the separation paradigms elicited any changes in gene expression. Taken together, our data show that the expression of molecules important for cortical development can be modified by environmental stimuli in a gender-specific fashion during early postnatal life.

# AXON GUIDANCE IN THE DOPAMINERGIC SYSTEMS OF ZEBRAFISH

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The cellular and molecular mechanisms orchestrating axonal outgrowth and guidance of dopaminergic (DA) neurons are largely unknown. However, from a biomedical point of view, such knowledge may be the base for more successful treatments of Parkinson´s and other diseases using cell transplantation and regenerative approaches, as the formation of proper axonal connections is required for proper reconstitution of dopaminergic regulatory circuits. Here we make use of the zebrafish as a model system to analyze axonal outgrowth and pathfinding of DA neurons. To that aim, we have characterized DA projections by anti-Tyrosine Hydroxylase (TH) immunohistochemistry as well as transient expression of *th:gfp* BACs in individual DA neurons to specifically visualize DA axonal outgrowth in vivo. Our focus is the ventral diencephalon, where specific DA groups have descending projections into hindbrain and spinal cord, while other DA groups with ascending projections may have functions similar to mammalian mesencephalic DA neurons. In addition we are examining the function of classical guidance molecules during axonal outgrowth of DA neurons. In a first step, we have characterized expression of axon guidance signals and their receptors with respect to DA axons and DA somata, respectively. For the Robo/Slit guidance system, our studies demonstrate, that *robo2* mRNA is transiently expressed by DA neurons during their initial phase of axon pathfinding, Furthermore, DA axons display premature midline crossing in the diencephalon of the *robo2* mutant *astray*. Four zebrafish Slit homologes are expressed at the midline, consistent with their role as potential repulsive ligands for Robo2. Further functional studies are underway to reveal the contribution of Robo/Slit mediated signaling to control midline crossing of DA axons.

# Reelin is secreted by the classical secretory pathway, but independent of neuronal activity

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The extracellular matrix protein reelin controls neuronal migration during brain maturation. In layered structures such as hippocampus and neocortex, it is synthesized and secreted by Cajal-Retzius cells during development and by GABAergic interneurons in the adult. Until now, very little is known, whether and how reelin secretion is regulated. To address this question, we used organotypic rat hippocampal slice cultures as a model to study reelin secretion. First, we investigated whether reelin release is influenced by neuronal activity. To this end we either blocked neuronal activity in hippocampal slice cultures by tetrodotoxin (TTX) or stimulated them with KCl or kainic acid. Subsequently, the reelin content of tissue and of supernatants was analyzed by quantitative Western blot analysis. Addition of 5 mM KCl and 5 µM kainic acid for 3, 24 and 48 h induced a transient c-fos expression peaking at 3 h, but did not elicit any changes in the reelin content of slices or supernatants at any time point studied. Similarly, TTX treatment did not have any effect. In order to investigate whether reelin is secreted in a $Ca^{2+}$-dependent manner, we blocked presynaptic, voltage-dependent Ca2+- channels of the P/Q-, N- and R-type with subtype-specific neurotoxins (ω-agatoxin IVA, ω-conotoxin GVIA and SNX-482). Treatment of hippocampal slice cultures with these three compounds for 3, 24, 48 h did not affect reelin secretion either. To exclude a secretion mechanism independent from the ER/Golgi-complex, hippocampal slice cultures were incubated for 12 h with Brefeldin A, which interferes with the ER/Golgi apparatus. Addition of Brefeldin A to the medium resulted in an accumulation of full length reelin (400kDa) in slices and in a significant reduction of the reelin content in supernatants. These data indicate that reelin is processed and secreted by the classical secretory pathway (Brefeldin A-sensitive), but it is released independent of neuronal activity and presynaptic, voltage-dependent $Ca^{2+}$- channels do not play a role.(Supported by the DFG: TR3).

# Opposite roles of Reelin in the lamination of dentate granule cells by binding to different receptors

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Control of neuronal migration is essential for the correct formation of neuronal layers during brain development. Previous studies have shown that Reelin, an extracellular matrix protein, is required for the proper positioning of neurons. Reelin binds to the ApoE receptor 2 (ApoER2) and the VLDL receptor (VLDLR) and induces the phosphorylation of the adaptor protein Dab1. In reeler mutant mice lacking Reelin, layer formation is severely altered in the hippocampus, neocortex and cerebellum. ApoER2/VLDLR double knock-out mice and Dab1 knock-out mice show a phenotype similar to that of reeler mutants. Recently, we have shown that Reelin secreted by different cell types in various brain regions of wild-type animals could induce layer formation of granule cells in the reeler dentate gyrus when the reeler hippocampus was cocultured with different wild-type tissue containing Reelin. In the present study, we performed an in vitro assay using PP2 that inhibits the phosphorylation of Dab1 and examined the layer formation of dentate granule cells in wild-type mice, VLDLR knock-out mice, and ApoER2 knock-out mice by immunostaining for prox-1, a marker of dentate granule cells. Our results indicate that Reelin plays opposite roles in the layer formation of dentate granule cells at different stages during neuronal migration by binding to two different receptors. We hypothesize that during early stages of neuronal migration Reelin binds to ApoER2 and acts as an attractive signal to promote granule cell migration towards the Reelin-rich marginal zone. As soon as the migrating granule cells reach this zone, Reelin binds to VLDLR and acts as a stop signal to arrest the migration of granule cells that form a densely packed cell layer.
(Supported by the DFG: SFB 505, Transregional SFB TR3)

# Neuronal cell migration in an insect embryo.

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During formation of the nervous system, neuronal cell migration is controlled by multiple guidance cues. The enteric nervous system of locusts provides a useful, robust, and manageable model to study cell motility. Here, we show evidence that not only released or extracellular matrix bound molecules are able to regulate cell motility, but also the gaseous messenger molecules carbon monoxide (CO) and nitric oxide (NO) affect migration in an insect enteric nervous system (ENS).

In the embryonic grasshopper, the nerve plexus of fore- and midgut including the enteric ganglia are formed by neurons that arise in three neurogenic zones on the stomodeum. The midgut plexus in particular is established from neurons of the ingluvial ganglia that travel in posterior direction on defined migratory pathways. This process employs extensive cell migration over several hundred micrometers. The midgut neurons exhibit NO-induced cyclic guanosine-monophosphate immunoreactivity (cGMP-IR) throughout the whole phase of migration and formation of the midgut plexus (Haase & Bicker (2003) Development 130: 3977-3987). During earlier phases of ENS development, cGMP-IR can be first detected in neurons of the frontal ganglion. In the hypocerebral and ingluvial ganglia somatic cGMP-IR is preceeded by cGMP positive neurites. Intriguingly, cells of the ingluvial ganglia remain cGMP negative until the onset of midgut neuron migration, although the neurons migrating on the foregut already exhibited a strong NO-dependent cGMP-IR.

Using an in vivo culturing system, it could be demonstrated that pharmacological inhibition of endogenous NO synthase, its effector enzym guanylyl cyclase (sGC) and protein kinase G (PKG) activity results in significant reduction of midgut neuron migration. Enzymatic inhibition of the NO/cGMP/PKG cascade combined with pharmacological rescue experiments implicate NO as a positive regulator of cell migration.

CO is produced by heme oxygenase (HO) enzymes during the conversion of heme to biliverdin (Boehning & Snyder (2003) Annu Rev Neurosci 26: 105-131) and has the potential to signal via the sGC/cGMP pathway. Here, we also report that after onset of migration midgut neurons show immunoreactivity to the constitutive isoform HO-2 while the enteric ganglia do not exhibit a specific HO-2 immunoreactivity. Since pharmacological inhibition of the CO releasing enzyme heme oxygenase-2 enhances midgut neuron migration, carbon monoxide may be a negative regulator.

The cellular distribution of NO and CO biosynthetic enzymes together with the results of the pharmacological manipulations suggest that CO and NO act as antagonistic regulators of neuronal migration in the enteric nervous system.

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# Influence of NO/cGMP on the growth of peripheral pioneer neurons in the grasshopper

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The grasshopper embryo has been used as a convenient system with which to investigate mechanisms of axonal navigation and pathway formation at the level of individual neurons. In this study we focus on the developing metathoracic limb bud of the grasshopper embryo (*Schistocerca gregaria*) where two siblings of pioneer neurons establish the first axonal pathway into the CNS. We detected nitric oxide (NO) -induced synthesis of cGMP in the pioneer neurons of the limb bud by immunocytochemistry. NO synthase (NOS) was detected by Western Blot analysis of whole embryos at the stage of axonogenesis. To investigate the role of the NO/cGMP signalling system during pathfinding, we examined the pattern of outgrowing pioneer neurons in embryo culture. Pharmacological inhibition of soluble guanylyl cyclase (sGC) and NOS resulted in a significantly slowed axonogenesis and in an abnormal pattern of pathway formation within the limb bud. The observed effects include completely abnormal axon patterns as well as a delay of growth. In both cases the growth cones do not reach the CNS. Adding membrane permeant cGMP or a direct activator of NOS into the culture medium recovered the inhibition of growth significantly. These findings indicate that the NO/cGMP signalling is involved in directed axonal elongation of pioneer neurons in the developing limb bud of the grasshopper.

# Developmental strategies for the assembly of callosal connections

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The main output routes of adult cerebral cortical axons are the internal capsule and the corpus callosum. How neurons during development choose on of these axonal trajectories is still not known. We hypothesized that axonal bifurcation followed by elimination of one branch might be a developmental strategy to accomplish this aim. To test this idea, we used embryonic and postnatal mice, labeled cortical projecting neurons with callosal and subcortical axons, and quantified their axonal bifurcations in correlation with the position of their somata in the cortex. In addition, we quantified bifurcations formed by dissociated green-fluorescent cells plated onto cortical slices. Bifurcating axons were numerous in the younger brains, and their number declines during further development. Most of the bifurcating axons belong to neurons located in the dorsolateral cortex, whereas bifurcating axons were less frequent in neurons located in the medial cortex. Moreover, callosal neurons bifurcate more than subcortically-projecting cells. In slices overlay assays, neurons grown over dorsolateral cortex bifurcate more often than those grown over medial cortex, irrespective of their positional origin in the donor. To analyse whether intermediate targets have a influence on axonal bifurcation, neurons were plated on slices without midline, internal capsule, or both targets. In all cases, cells bifurcate less comparing to control slices with targets. We conclude that transient bifurcation is a strategy employed by developing dorsolateral callosal axons in search of their targets. Axons bifurcate and send collaterals medially and laterally, the majority then choose one of these cortical output routes, eliminating the other. As cell body position and intermediate targets determines axon behavior, we suggest that bifurcations are regulated by cues expressed in the environment. (Supported by DAAD and PROBRAL).

# Expression of ADAM10 during chicken brain development

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ADAMs (a disintegrin and metalloproteases) are a family of transmembrane proteins possessing a disintegrin and a metalloprotease domain, which play roles in cell-cell and cell-matrix adhesion, in proteolysis and in signaling transduction (White, 2003; Blobel, 2005). ADAMs are involved in morphogenesis and tissue formation during embryonic development (Hall and Erickson, 2003). In the present study, a chicken cDNA encoding ADAM10 was cloned after RT-PCR and identified by sequencing. The expression of ADAM10 was investigated by in situ hybridization during chicken brain development. Our results show that ADAM10 is widely expressed in the developing brain. ADAM10 is transcribed prominently in the hyperstriatum accessorium and neostriatum, in the dorsal thalamus, in the oculomotor nuclei and isthmic nuclei, in the stratum griseum centrale of tectum, in Purkinje cells and in the internal granular layer. Our data suggest that ADAM10 plays a role in the development of specific brain structures in chicken embryos. For example, ADAM10 may regulate neuronal migration or axon outgrowth, as previously shown for Kuzbanian, the homologous gene in Drosophila (Fambrough et al., 1996).

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# Effects of soluble semaphorin gradients on cortical fibers

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During development of the nervous system, axonal growth cones navigate through a complex environment containing both diffusible and membrane-associated guidance cues. It is not clear whether diffusible signals exert a chemotactic influence (i.e. whether they act as soluble guidance cues) or a haptotactic influence (i.e. whether they act as membrane or ECM bound guidance cues). As reported previously, diffusible Sema3A exerts a repellent and Sema3C an attractive effect on cortical axons (Bagnard et al., J. Neurosci. 20, 1030-1035, 2000). Using different cell lines, we re-examined the growth of cortical fibers in soluble semaphorin gradients, Sema3A and Sema3C. Cortical explants obtained from E15 mice were cultured next to aggregates of HEK 293 cells secreting AP-Sema3A or AP-Sema3C. Untransfected HEK 293 cells were used as a control.

Our results indicate that the number and the length of fibers growing towards increasing concentrations of Sema3A were reduced compared to the control. In contrast, more and longer fibers grew in the direction of Sema3C-expressing cells; demonstrating the chemoattractive effect of Sema3C. Strikingly, fibers growing away from the chemorepulsive Sema3A-cell aggregates, were significantly longer and more numerous than fibers under control conditions. In addition, we observed a basic trophic effect on cortical fibers extending out from explants: increased number of fibers correlates with increased length of fibers. Thus, the raised cortical fiber length caused by decreasing Sema3A concentrations could due to Sema3A itself and/or the trophic effect of an explant culture we observed. A quantitative analysis however indicated, that we have to consider both described mechanisms.

We believe that this constitutes an efficient and robust mechanisms to steer axonal growth cones in the developing nervous system. (Supported by the IZKF Jena and INSERM).

# EphrinA5 is involved in the regulation of the tangential migration of cortical interneurons

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EphrinA5, a ligand of EphA receptor-tyrosine kinases, is expressed in the ventricular zone of the ganglionic eminences at embryonic day 14, the zone where tangentially migrating cortical interneurons are born. To examine the effects of ephrinA5 on tangential migration of cortical interneurons we used the stripe-assay and the slice-overlay technique. On alternating stripes of ephrinA5 Fc and control protein neurons of the lateral and medial ganglionic eminence (LGE and MGE) showed a preferential growth on the control lanes, indicating that ephrinA5 has a repulsive effect on neurons of the eminences in vitro. To further investigate the effect of ephrinA5 on migrating cortical interneurons, we performed the slices assay, transplanting explants of the ventricular zone (VZ) of the MGE of green fluorescent EGFP-embryos homotopically on coronal wildtype slices. Analyzing the migration pattern in vitro revealed that MGE neurons showed a migration within the VZ of the eminences while in vivo the VZ is avoided. In situ hybridizations against lhx6, a marker for migrating cortical interneurons, on slices after 2 DIV could confirm that result showing a staining in the VZ, whereas slices after a short time in vitro showed no labelling in that region. Among different candidate molecules we found that ephrinA5 is downregulated in the VZ of the eminences after 2 DIV. This suggests that the downregulation of ephrinA5 could be responsible for the changed migration in the VZ in vitro, indicating that ephrinA5 has a repulsive effect on cortical interneurons in vivo. To test this hypothesis we used recombinant ephrinA5 Fc that was added to the medium of coronal slices. Binding assays could show that ephrinA5 Fc predominantly bound to cells in VZ which allows a reconstruction of the in vivo situation. To functionally test the locally bound ephrinA5 Fc in the VZ, we performed the slice assay with ephrinA5 Fc application that resulted in an avoidance of the VZ by migrating MGE neurons, thus simulating the in vivo situation. Further investigations revealed that this effect was dependent on the SFK activity. Blocking of SFKs or supplying monomeric ephrinA5 resulted in neutralizing this repulsive effect of ephrinA5. We therefore suggest that ephrinA5 is involved in the regulation of tangential migration of cortical interneurons stimulating new born neurons migrating out of the VZ. (Supported by the DFG).

# Temporal expression of *sequoia* defines target layer identity of *Drosophila* photoreceptor cells

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The visual system of *Drosophila* provides a well suited model to study the gene functions and developmental mechanisms underlying the establishment of synaptic specificity. Sensory neurons of the visual system, the photoreceptor cells R1-R8, can be classified in three subtypes based on their spectral sensitivity and neuronal connectivity. Whereas R1-R6 cells connect to the first optic ganglion, the lamina, R7 and R8 axons project through the lamina and terminate in two distinct layers of the second optic ganglion, the medulla. This projection is strictly topographic: R7 and R8 photoreceptors from neighboring ommatidia connect to neighboring target neurons in the medulla.

To identify genes which are presynaptically required for layer specific R7/R8 axon targeting we performed a histological mosaic screen using the eyFlp/FRT technique. Among several mutations that affect the local synaptic arrangement in the medulla, we could identify the zinc-finger transcription factor SEQUOIA as being essential for R7/R8 layer selection. During the initial ingrowth into the medulla *sequoia* mutant R-cell axons frequently miss their proper target and terminate in adjacent synaptic layers. Removing *sequoia* specifically from R7 redirects their axons into the R8 target layer. No changes in the cell type specific differentiation of R7 and R8 can be detected in sequoia mutants. Interestingly, *sequoia* displays a transient R7/R8 expression profile that starts with the postmitotic differentiation and declines rapidly after initial target layer selection. Due to the sequential R8/R7 development in the eye disc, SEQUOIA is no longer expressed in R8 by the time R7 growth cones arrive in the target area to select the adjacent medulla layer. Synchronization of the Sequoia profile in both R-cells through prolonged sequoia expression in R8 leads to their co-projection into the R7 target layer. These loss- and gain-of-function experiments together with the dynamic expression indicate a pivotal function for *sequoia* in R7/R8 target layer selection. We propose that the initial R7/R8 axon targeting in the medulla is mediated by the same type of permissive adhesion molecules but specificity is induced by a tight temporal control of the axonal competence to respond to these cues.

# Axonal guidance and cerebellar lobe formation in NrCAM-, CHL1-,and NrCAM-CHL1-double-deficient mice

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Neural cell recognition molecules are involved in neuronal migration, outgrowth, guidance and fasciculation of neurites, and synapse formation. The L1 family, a subgroup of the immunoglobulin-superfamily, includes four transmembrane members in mammals e.g. L1, CHL1, NrCAM, and Neurofascin.
In this study, we focused on the analysis of NrCAM-deficient (1), CHL1-deficient (2), and NrCAM-CHL1-double-deficient mice. The phenotypes of these mice were compared with respect to the guidance of hippocampal mossy fibers and olfactory sensory axons and the formation of specific cerebellar lobes.
Mossy fibers in the hippocampus are the axons of granule cells in the dentate gyrus and project to apical and basal proximal dendrites of pyramidal cells in the hippocampal CA3 region in a laminated fashion. Previously, hippocampal mossy fiber organization of CHL1 mutants was shown to be disturbed (2). By Timm's staining and staining for calbindin or synaptophysin, the mossy fiber organization in NrCAM mutants was analyzed and found to be indistinguishable from the wild-type, whereas the phenotype of the double mutant matched the phenotype of the CHL1 single mutant.
In the olfactory bulb, specific sensory axons are labelled by the lectin DBA. In adult wild-type mice, a particular sensory axon exclusively terminates and aborizes in a single specific glomerulus. Specificity and glomerular restriction of the axonal projections is disturbed in CHL1 mutant mice (2). Here we show, that olfactory neurons in NrCAM mutants show similar misguided axonal projections. Investigation of the double mutants revealed misguided axonal projections comparable to those found in the single mutants.
Immunohistochemical staining for CHL1 in the olfactory bulb shows the presence of CHL1 on olfactory sensory axons and its absence in all other areas of the olfactory bulb. In contrast, NrCAM is only expressed in the target region of the olfactory neuron projections but not on the axons themselves.
Furthermore, NrCAM mutant mice revealed a mild reduction in the size and a change in the shape of specific lobes of the cerebellar vermis (3). In contrast, cerebellar lobes of CHL1 mutant mice appeared not to be affected. However, in the double mutants, the size reduction and the change in the shape of these specific lobes were less pronounced, resembling more the phenotype of wild-type mice.
In summary, these results demonstrate that NrCAM and CHL1 modify overlapping cellular processes without an enhancement of phenotypes in the double mutant. With respect to the cerebellar lobe formation, the data suggest opposite effects of both molecules.

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# The histone deacetylase SIRT2 in neurite outgrowth and axon guidance

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SIRT2 is an unusual member of the histone deacetylase (HDAC) familiy acting on acetylated α-tubulin. In the neurons it is localized mainly to the soma and significantly less to the neurites. It is generally believed that acetylation of microtubules is associated with an increase and deacetylation with a decrease in their stability. As axonal outgrowth and pathfinding are dependent on dynamic rearrangements of the cytoskeleton especially the microtubules, we wanted to analyse if SIRT2 function might be crucial for these processes. Expression of wild type or a constitutively active SIRT2 in hippocampal neurons led to reduced neurite outgrowth and inhibition of ephrin-A mediated growth cone collapse. These results underline that SIRT2 plays a role in neurite outgrowth and axonal pathfinding. In pursuing experiments, the influence of SIRT2 activity on these processes will be analysed in more detail using for example a si-RNA approach and live cell imaging of neurite outgrowth of freshly plated hippocampal neurons. Another member of the histone deacetylase family, which acts on microtubules, is HDAC6. As it is also expressed in the CNS and was shown to interact with SIRT2, the influence of HDAC6 activity will also be assessed in the above experiments.

# Analysing the role of SRF in axon guidance

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The correct formation of a functional neuronal network in the brain requires processes such as neurite outgrowth and axon guidance. All of these are based on a highly dynamic cytosceleton, which can be rearranged by guidance cues such as ephrins or semaphorins. We are investigating the role of SRF (serum response factor) - a MADS box transcription factor and major regulator of the actin cytosceleton - in the hippocampal system. Srf-deleted hippocampal neurons show reduced neurite outgrowth and disturbed depolymerisation of the actin and microtubule cytosceleton after growth cone collaps induction.
Using forebrain-specific conditional Srf knock-out mice we are focusing on the identification of new SRF target genes in the brain. Further, we are analysing the modified morphological structure of cultured Srf-deleted hippocampal neurons particularly the formation of neurite branches and of filopodia in the growth cone.
We are aiming at an understanding of the underlying molecular mechanisms, leading to the observed phenotypes of hippocampal neurons after Srf-deletion.

# Poster Topic

## T3: Development III: Regeneration

**T3-1A** Electrospinning of oriented nanofibers of collagen/poly-ε-caprolactone as a matrix for cell migration and neurite outgrowth in nerve regeneration
*K. Klinkhammer, E. Schnell, S. Balzer, G. Brook, P. Dalton, D. Klee and J. Mey, Aachen*

**T3-2A** Nerve injury and inflammatory cytokines cause nuclear translocation of retinoid receptors
*J. Mey, K. Schrage and N. Zhelyaznik, Aachen*

**T3-3A** Promoters of regeneration of entorhinal fibers in mouse hippocampal slice cultures
*B. Bonnici and J. Kapfhammer, Basel (CH)*

**T3-4A** Differential promoting effects of *Clostridium botulinum* C3 proteins on axonal process growth and arborization of various neuron populations
*M. Höltje, S. Djalali, F. Hofmann, G. Große, I. Just and G. Ahnert-Hilger, Berlin and Hannover*

**T3-5A** The Cytokine/Neurotrophin Axis in Peripheral Axon Outgrowth
*S. Hendrix, G. Golz and R. Nitsch, Berlin*

**T3-6A** Expression and function of erythropoietin and its receptor in invertebrate nervous systems
*D. Gocht, D. Sargin, S. Sperling, H. Ehrenreich and R. Heinrich, Göttingen*

**T3-7A** Additive effects of CNTF and rho kinase inhibition in models of apoptosis and axonal regeneration of retinal ganglion cells *in vitro* and *in vivo*
*L. Tönges, P. Lingor, N. Pieper, C. Bermel and M. Bähr, Göttingen*

**T3-1B** Inhibition of CDK5 and rho kinase (ROCK) results in increased neurite outgrowth and regeneration in retinal ganglion cells
*CM. Bermel, L. Tönges, J. Weishaupt, M. Bähr and P. Lingor, Göttingen*

**T3-2B** Differential role of c-Jun-N-terminal kinase isoforms in regeneration and survival of primary dopaminergic neurons
*AV. Planchamp, M. Bähr and P. Lingor, Göttingen*

**T3-3B** Assessment of motor functions after sciatic nerve injury in mice using a single-frame motion analysis approach
*A. Irintchev, A. Fey and M. Schachner, Hamburg*

**T3-4B** Improved functional recovery from femoral nerve injury in mice after application of a novel cyclic HNK-1 glycomimetic
*AJ. Mehanna, A. Irintchev and M. Schachner, Hamburg*

**T3-5B** Femoral nerve repair:
impacts of lesion severity on the functional outcome in mice
*E. Sivukhina, M. Schachner and A. Irintchev, Hamburg*

**T3-6B** Nitric Oxide regulates Regeneration in the Ventral Nerve Cord of Locust Embryos
*M. Stern and G. Bicker, Hannover*

**T3-7B** The synthetic steroid mifepristone protects Purkinje cells from developmental cell death
*M. Schumacher, I. Dusart, C. Levenes, EE. Baulieu and A. Ghoumari, Kremlin Bicetre (F) and Paris (F)*

**T3-1C** The role of microtubules in retraction bulb formation and failure of axonal regeneration in the CNS
*A. Ertürk and F. Bradke, Martinsried and Munich*

**T3-2C** FIBROBLAST IMPACT ON SCHWANN CELLS AND AXONAL GROWTH
*L. Dreesmann, M. Lietz, M. Weimar and B. Schlosshauer, Reutlingen*

**T3-3C** Transplantation of mesencephalic human neural progenitor cells into the caudate putamen of Hemiparkinsonian rats
*SJP. Haas, M. Hovakimyan, O. Schmitt, B. Gerber, A. Wree and C. Andressen, Rostock*

**T3-4C** 3D scaffolds to optimize human neural progenitor cell growth and differentiation
*J. Schmich, S. Ortinau, EA. Miljan, J. Sinden and A. Rolfs, Rostock and Guildford (UK)*

**T3-5C** The role of serum response factor in axonal regeneration
*S. Stern and B. Knöll, Tübingen*

**T3-6C** Neuroprotective and growth promoting effects of a lens injury on retinal ganglion cells are not mediated by activated macrophages
*D. Fischer, T. Hauk, J. Lee and R. Schwendener, Ulm and Zürich (CH)*

**T3-7C** Characterization of LINA a new axonal regeneration promoting factor
*J. Lee, T. Hauk, L. Nonnenmacher, TM. Böckers and D. Fischer, Ulm*

# Electrospinning of oriented nanofibers of collagen/poly-ε-caprolactone as a matrix for cell migration and neurite outgrowth in nerve regeneration

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The autologous transplantation of a peripheral nerve is the best surgical treatment of nerve injury available today. Since this intervention causes sensory deficits at the donor site and has only limited applications for larger lesions, we are developing an artificial implant as a conduit for axonal regeneration. Important functional properties of the nerve bridge are (1) support and guidance of axonal regeneration and (2) the induction of Schwann cell migration from the host tissue into the implant. In this study, biodegradable, aligned poly-ε-caprolactone (PCL) and collagen/PCL (C/PCL) nanofibres designed as guidance structures were produced by electrospinning and tested in cell culture assays. We compared fibers of 100% PCL with fibers consisting of a 25%/75% C/PCL blend. To test their biocompatibility, assays of cell adhesion, survival, migration, effects on cell morphology, axonal growth and axonal guidance were performed. StarPEG and poly-L-lysine coated surfaces served as negative and positive controls.

Both types of eletrospun fibers supported oriented neurite outgrowth and glial migration from dorsal root ganglia (DRG) explants. Schwann cell migration, neurite orientation, and process formation of Schwann cells, fibroblasts and olfactory ensheathing cells were improved on C/PCL-fibers, when compared to pure PCL fibers. Cell adhesion was also stronger on C/PCL- than on PCL-fibers. While the velocity of neurite elongation from DRG explants was higher on PCL-fibers, analysis of isolated sensory neurons showed significantly better axonal guidance by the C/PCL material. In organ cultures neurites were able to grow upon the Schwann cells that had migrated out from the explants. C/PCL-fibers caused oriented elongation of Schwann cells, olfactory ensheathing cells, and fibroblasts. The data demonstrate, that electrospun fibers are a good material for artificial nerve implants.

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# Nerve injury and inflammatory cytokines cause nuclear translocation of retinoid receptors

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The transcriptional activator retinoic acid (RA) is involved in the physiological processes after nerve lesions [Zhelyaznik et al., *Eur. J. Neurosci.* 18 (2003); Mey et al., *Eur. J. Neurosci.* 21 (2005)]. Spinal cord contusion and sciatic nerve injuries affect the expression of RA receptors (RAR) and their heterodimeric partners, the retinoic X receptors (RXR). We discovered that these types of lesion also cause intracellular translocation of RAR and RXR in target cells of RA [Schrage et al., *Eur. J. Neurosci*. 23 (2006); Zhelyaznik and Mey, *Neurosci.* 141 (2006)]. In the present study these changes, which provide an additional regulatory mechanism of RA signaling, were quantified in vivo and in vitro.

Following sciatic nerve lesions the distribution of RARα, RARβ and RXRα were investigated immunohistochemically and with Western blotting. Crush injury caused an upregulation of all three receptors in Schwann cells and macrophages. RARα and RXRβ shifted from a cytosolic to a nuclear localization in the cells. We then tested whether RA itself and the pro-inflammatory cytokines IL-1β, IL-6 and TNFα influence the intracellular distribution of retinoid receptors in Schwann cell primary cultures. All three cytokines caused a shift of RARα from the cytosolic compartments into the cell nuclei. This was also observed with RA, and combining RA with IL-1β produced an additive effect. IL-1β and IL-6 affected the distribution of RARβ, although immunoreactivity of this receptor always remained stronger in the cytosol. No effect of the cytokines on RXRα or RXRβ was observed, whereas RA treatment caused a stronger nuclear signal of both receptors. Effects on the subcellular localization of retinoid receptors may provide a link in a negative feedback loop between RA/RAR and inflammation.

# Promoters of regeneration of entorhinal fibers in mouse hippocampal slice cultures

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Axonal regeneration after lesion is usually not possible in the adult central nervous system but can occur in the embryonic and young postnatal nervous system. In this study we use the model system of mouse entorhino-hippocampal slice cultures to assess regeneration of entorhinal fibers projecting to the dentate gyrus after mechanical lesions and treatment with pharmacological compounds in vitro.

We have shown previously (Prang et al. 2001) that there is a marked decrease in regenerating fibers when a lesion is made at 6-7 days in vitro or later in postnatal day 5-6 mice. We took this as a control model where there is little spontaneous axonal regeneration. On the day of lesion, we now added various treatments to our cultures, and assessed axonal regeneration by tracing entorhinal axons with biotinylated dextran amine (BDA). The amount of axonal regeneration was quantified by a score system and compared to the results from control cultures. Most of the compounds tested were modulators of signal transduction pathways. Our results show that compounds interfering with the cAMP system and the protein kinase C pathway were able to induce and promote axonal regeneration in this slice culture model.

Our findings demonstrate that slice culture models can be used to evaluate compounds for their potential to promote axonal regeneration and that the pharmacological modulation of signal transduction pathways is a promising approach for promoting axonal repair.

# Differential promoting effects of *Clostridium botulinum* C3 proteins on axonal process growth and arborization of various neuron populations

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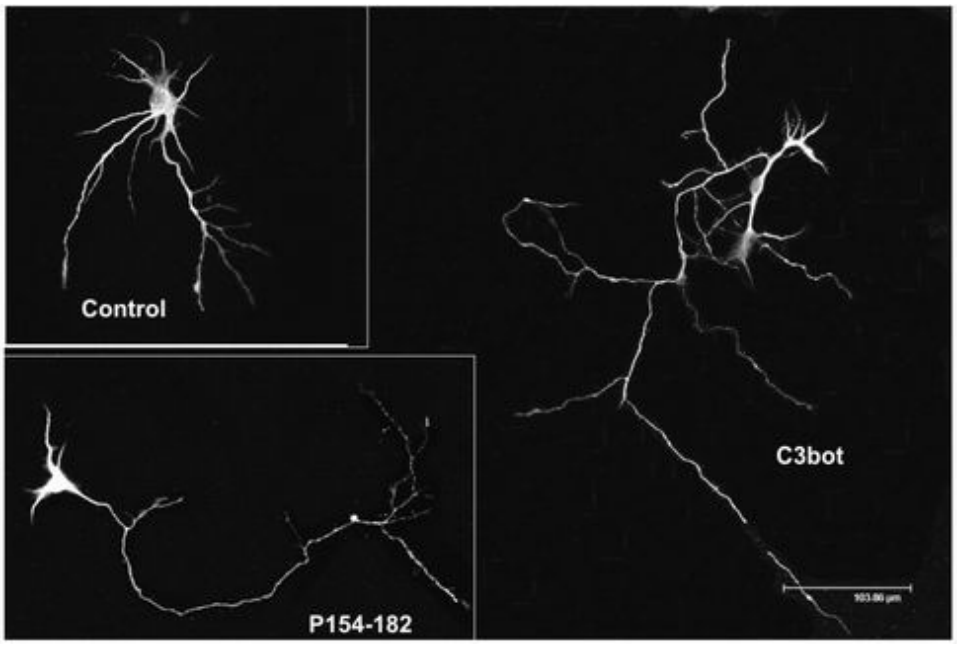
Bacterial C3 proteins are well established tools to study Rho-dependent effects in a great variety of cell types, including neurons. In addition to its canonic pathway of Rho inhibition by ADP-ribosylation C3 botulinum (C3bot) also acts as an axonotrophic factor independent of its enzymatic activity promoting process growth and arborization in certain neuronal populations. In this study we aimed to pinpoint the domain of C3bot responsible for the observed axonotrophic activity. By using overlapping C3bot-derived peptide fragments we were able to show that a region between the amino acids 154 and 182 is responsible for the observed growth-promoting effects. Additionally, we screened different neuronal subpopulations including hippocampal neurons, granule cells of the dentate gyrus, cerebellar purkinje cells, and α-motoneurons of the spinal cord for sensitivity towards full length C3 proteins and C3bot derived peptide fragment 154-182. Morphometrical measurements including axonal segment order analysis revealed promoting effects of enzymatically active C3bot on all cell types investigated whereas enzyme deficient C3bot preparations exhibited axonotrophic effects only on distinct neuronal populations like hippocampal neurons and α-motoneurons.




Hippocampal neurons stained for neurofilament protein exhibit enhanced axon growth and arborization upon incubation with C3bot or the C3bot - derived peptide P154-182

# The Cytokine/Neurotrophin Axis in Peripheral Axon Outgrowth

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Inflammation is part of the physiological wound healing response following mechanical lesion of the peripheral nervous system. However, cytokine effects on axonal regeneration are still poorly understood. Since cytokines influence the expression of neurotrophins and their receptors, which play a major role in axonal outgrowth after lesion, we have investigated the hypothesis that cytokines influence specifically neurotrophin-dependent axon elongation. Therefore, we have characterized neurotrophin-dependent neurite outgrowth of murine dorsal root ganglia (DRGs) in vitro and investigated the influence of pro- and anti-inflammatory cytokines on these outgrowth patterns.

Embryonic day 13 (E13) DRGs were cultured in Matrigel for two days and axonal morphology, density and elongation were determined using an image analysis system. Nerve growth factor (NGF), neurotrophin-3 (NT-3) and -4 (NT-4) were applied alone (50ng/ml), in double or in triple combinations. NT-3, NT-4, and NT-3+NT-4 combined induced a moderate increase in axonal outgrowth ($p<0,001$) compared to controls, while NGF and all combinations including NGF induced an even more pronounced increase in axonal outgrowth ($p<0,001$). After characterizing these outgrowth patterns, interleukin (IL)-1β, IL-4, IL-6, interferon-γ (IFNγ) and tumor necrosis factor-α(TNFα) (50 or 500ng/ml) were added to the different neurotrophin combinations. Low doses of TNFα and IL-6 influenced neurite extension induced by endogenous neurotrophins. IL-4 increased NT-4-induced outgrowth. IL-6 stimulated NT-3+NT-4-induced outgrowth. IFNγ stimulated neurite extension in the presence of NT-3+NT-4 and NT-3+NGF. TNFα inhibited NT-3-, NT-3+NGF-, NT-4+NGF- and NT-3+NT-4+NGF-induced outgrowth. Thus, cytokines primarily influence neurotrophin-dependent axonal outgrowth. These data suggest that inflammation following nerve injury modulates reinnervation via a cytokine/neurotrophin axis.

# Expression and function of erythropoietin and its receptor in invertebrate nervous systems

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There is accumulating evidence that the multitude of biological functions served by the cytokine erythropoietin (Epo) and its receptor (EpoR) originated as a primitive defense mechanism against invading pathogens and other harmful stimuli. Epo has been demonstrated to mediate an evolutionary conserved immune response to limit damage caused by injury to various tissues including the nervous system. Epo and EpoR have been detected in the nervous systems of vertebrates including humans, mice, rats, rabbits and fish.

We have extended these studies to invertebrates and detected both Epo- and EpoR-immunoreactivity (further confirmed by western blots) in the nervous systems of insects (grasshopper and fruitfly), crustaceans and annelids (leech). The Epo/EpoR-signaling system of invertebrates may (similar to its function in the mammalian nervous system) contribute to injury-induced responses that support survival and regeneration of directly and indirectly affected cells. Epo has been found to accelerate the regeneration of neurites in primary cultured neurons from grasshopper brain and we are currently investigating whether survival of the dissociation procedure in general is also promoted. Crushing a peripheral nerve and interrupting its sensory input to the central nervous system of grasshoppers induced widespread increases of EpoR expression in interneurons projecting into various neuropils of directly connected and adjacent ganglia. Whether exogenous Epo improves the regeneration of acoustic orientation behaviour in grasshoppers following transection of the auditory nerve by promoting axonal growth, pathfinding and re-establishment of synapses onto auditory interneurons is under current investigation.

Our data suggest that the Epo/EpoR-signaling system is expressed in the nervous systems of invertebrates from various taxa and has therefore evolved long before the emergence of vertebrates. The neuroprotective and neurotrophic functions of Epo and EpoR in the mammalian nervous system may therefore be mediated by ancient evolutionary conserved mechanisms whose characterization could be facilitated by studies on invertebrate organisms.

# Additive effects of CNTF and rho kinase inhibition in models of apoptosis and axonal regeneration of retinal ganglion cells *in vitro* and *in vivo*

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Ciliary neurotrophic factor (CNTF) is a known survival promoting factor for retinal ganglion cells (RGC). Pharmacological inhibition of rho kinase (ROCK) by Y-27632 has been shown to increase axonal regeneration of CNS neurons after lesion.

Here, we evaluated the role of CNTF and/or ROCK inhibition in models of retinal ganglion cell apoptosis and regeneration *in vitro* and *in vivo*. Application of CNTF or Y-27632 significantly enhanced cell survival and led to an increase in neurite outgrowth in primary and immortalized RGC cultures. Co-application of the substances resulted in an additive effect on neuronal survival, but was not more potent than each substance alone in a permissive neurite outgrowth paradigm. This effect was shown to be partly mediated by MAPK activation. *In vivo,* numbers of RGC were increased after axotomy in both CNTF- and Y-27632-treated animals, while the combination was not more beneficial than either substance alone. However, combinatorial treatment significantly increased regeneration of RGC axons into a peripheral nerve graft and in the optic nerve crush model.

In summary, we show that both CNTF and Y-27632 increase survival of RGC and enhance neurite outgrowth/axonal regeneration. A combinatorial treatment with ROCK inhibitors and growth factors may thus be a promising strategy to induce functional regeneration in the CNS.

# Inhibition of CDK5 and rho kinase (ROCK) results in increased neurite outgrowth and regeneration in retinal ganglion cells

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Cdk5 and rho kinase (ROCK) are both activated by signalling cascades employed by repulsive guidance cues and inhibitory myelin components.
We examined the role of Cdk5 and ROCK for survival and neurite outgrowth in an *in vitro* paradigm of immortalized retinal ganglion cells (RGC-5) and primary retinal ganglion cells. While indolinone D, a selective Cdk5 inhibitor, robustly rescued RGC numbers in a growth factor deprivation model, the application of ROCK inhibitor Y-27632 showed only minimal protective effects. The combinatorial treatment with both inhibitors showed only a minimal increase in RGC survival compared to indolinone D treatment alone.
On a permissive culture substrate, treatment with indolinone D resulted in an increased neurite number per cell, while application of Y-27632 increased the neurite length. The application of both inhibitors induced a combinatorial effect resulting in both longer and more neurites. Similar results were obtained when primary RGCs were grown on an inhibitory CSPG substrate. In an *ex vivo* regeneration model of retinal explant cultures the combinatorial treatment with both inhibitors equally resulted in increased number and length of regenerating processes, compared to application of each inhibitor alone or control cultures.
Our data suggest that Cdk5 and ROCK act independently on neurite formation and elongation in retinal ganglion cells. Simultaneous inhibition of both kinases thus targets different mechanisms of growth inhibition and may represent a promising approach to increase regeneration *in vivo* .

# Differential role of c-Jun-N-terminal kinase isoforms in regeneration and survival of primary dopaminergic neurons

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Members of the c-Jun N-terminal kinase (JNK) family of MAPK have emerged as important players in neurodegenerating disorders, such as Alzheimer´s and Parkinson´s disease, as well as in cellular stress responses, where they are mainly playing a pro-apoptotic role. Moreover, numerous reports attribute JNKs functions in neurite outgrowth in vitro, in the neuronal response to growth factors, and in regeneration in vivo. However, it is still unclear, which differential role each single JNK isoform plays in the mediation of a pro-apoptotic or regenerative response.

For more specific dissection of the JNK signalling pathway we generated knock-down cultures of primary midbrain dopaminergic neurons via siRNA electroporation, which enabled us to investigate the role of each single JNK isoform. Regeneration was studied in vitro by the quantification of number and length of regenerating neurites from the whole culture and dopaminergic neurons specifically at the site of mechanical transection (scratch model). We show a differential role for JNK isoforms for survival and regeneration of these neuron populations: downregulation of JNK1 and JNK2 resulted in a slight impairment of neurite regeneration, while the most pronounced effect was observed after JNK3 downregulation resulting in ~30 % decrease in neurite length. In addition JNK3 silencing significantly decreased the number of surviving dopaminergic neurons regenerating neurites (~40 %). Rescue of regeneration and survival by overexpression of JNK3 is currently investigated.
We conclude that the three JNK isoforms have differential effects on regeneration and survival in midbrain neurons in a context-dependent manner. Putative therapeutic approaches will therefore require isoform specific targeting of these kinases.

# Assessment of motor functions after sciatic nerve injury in mice using a single-frame motion analysis approach

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Assessment of motor abilities after sciatic nerve injury in rodents, the most frequently used paradigm for peripheral nerve regeneration research, relies primarily on the walking track analysis despite recognized limitations of this method. Using principles employed recently for video-based analyses of motor functions after femoral nerve and spinal cord injuries in mice (Irintchev et al., Eur. J. Neurosci. 22, 2005, 802; Apostolova et al., J. Neurosci. 26, 2006, 7849), we have designed and report here an approach for functional assessments after sciatic nerve lesions. Functional deficits are estimated by angle and distance measurements on single video frames recorded during beam walking and inclined ladder climbing performed voluntarily by the animals. Analyses of adult C57BL/6J mice after crush injury of the sciatic, tibial or peroneal nerve allowed the identification of a battery of parameters reliably detecting impairments of the plantar flexion of the foot and toe spread. Some of these parameters, as well as the sciatic and tibial functional indices of the walking track analysis revealed severe functional impairment after crush injury of the sciatic or tibial nerves, and complete recovery within 4 weeks after lesion. Other novel estimates, however, showed that functional recovery at 4 weeks is by far less complete than could be predicted on the basis of previous knowledge. While the walking track analysis did not allow detection of dysfunctions resulting from peroneal nerve crush, some of the novel measures did so and revealed incomplete restoration of function within 4 weeks after lesion. Thus, the novel video-based approach allows objective numerical assessment of motor functions after lesions of the sciatic nerve or its major branches and is more sensitive and versatile than established tests.

# Improved functional recovery from femoral nerve injury in mice after application of a novel cyclic HNK-1 glycomimetic

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*Functional recovery after peripheral nerve damage in both humans and laboratory animals is often poor, despite the ability of injured axons to reinnervate the axon-deprived targets. Previous work in our laboratory (Simova et al., 2006) has shown that the outcome of femoral nerve repair in mice can be improved by application of a linear peptide that functionally mimics the human natural killer (HNK) cell glycan known as HNK-1 epitope, a carbohydrate indicated to favor specificity of motor reinnervation in mice. Here we tested the effects of a novel cyclic HNK-1 glycomimetic [c-(RTLPFS)] shown previously to enhance more potently motoneuron neurite outgrowth in vitro than the linear mimic FLHTRLFV (Bächle et al., 2006). We applied the cyclic glycomimetic or a control cyclic peptide in polyethylene cuffs used to reconstruct the severed femoral nerves of adult mice. Recovery of quadriceps muscle function was monitored over a 3-month recovery period using a novel video-based approach (Irintchev et al., 2005). In contrast to previous observations with the linear glycomimetic, significantly better functional performance was observed already at early time-points (2 and 4 weeks) after nerve repair in mice treated with cyclic mimic compared with control peptide. Similar to the linear peptide, positive effects of the cyclic glycomimetic treatment were found at 2 and 3 months after nerve repair indicating permanently improved functional outcome. Thus, we can conclude that the novel cyclic glycomimetic is more efficient in vivo compared with the linear mimic. The difference in the efficacy of the two peptides can be attributed to better mimicry of the HNK-1 epitope by the cyclic peptide and increased metabolic stability. The potential clinical application potential of the novel cyclic peptide mimic can now be tested in larger animal models.*

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Simova O, Irintchev A, Mehanna A, Liu J, Dihne M, Bachle D, Sewald N, Loers G, Schachner M (2006) Carbohydrate mimics promote functional recovery after peripheral nerve repair. Ann Neurol (in press) DOI 10.1002/ana.20948

# Femoral nerve repair: impacts of lesion severity on the functional outcome in mice

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It is well-known that peripheral nerves are capable to regenerate after injury. However, significant functional recovery after peripheral nerve repair is rarely achieved. Researchers are intensely searching for novel approaches for nerve repair and treatments that could improve regeneration. These studies have been hampered by lack of sensitive measures for the functional outcome in well established paradigms such as the sciatic or the femoral nerve injury models in rodents. Taking advantage of a novel quantitative approach for evaluation of the quadriceps muscle function of mice, the single-frame motion analysis, developed recently in our laboratory (Irintchev et al., Eur. J. Neurosci, 22, 2005, 802), we aimed to establish a novel femoral nerve injury/repair paradigm of higher clinical relevance than those previously used. We tested the long-term functional consequences of femoral nerve repair with entubulation and a moderately large gap (4 mm inter-stump distance) in adult female C57BL/6J mice. Femoral nerve trunk transection was performed at a distance of about 3 mm proximal to the bifurcation of the quadriceps and saphenous branch and repaired using a 5-mm long polyethylene tubing (0.58 mm inner diameter) and single epineural 11-0 nylon sutures. Functional analyses were performed prior to and at different time-points after surgery over 6 months. Evaluation of two specific parameters, the heels-tail angle and the foot-base angle, and calculation of the stance recovery index, an individual measure of the degree of recovery, revealed surprisingly poor functional outcome. The stance recovery index (100% upon full functional restoration) reached an average of only 22% at 6 months after surgery in 14 animals evaluated. Microscopic examinations revealed that the structural continuity of the nerve trunk within the tube has been restored in all these cases. Retrograde labelling of quadriceps motoneurons was also performed in the same animals at the end of the observation period by applying a fluorescence tracer (Fast Blue) to the femoral nerve stump cut at a distance of about 5 mm proximal to the tube. Although area measurements revealed significant atrophy of the labelled motoneuronal cell bodies (by 25% compared to control), numbers of labelled cells were similar to those previously reported after end-to-end suture of the mouse femoral nerve. Therefore, it appeared that poor recovery was due to axon regrowth failure rather than to massive motoneuron loss. Given the possibility to combine precise functional analysis with morphological estimates in the same animals, we believe that the model described here would be valuable for future research devoted to improvement of the functional outcome of peripheral nerve injury.

# Nitric Oxide regulates Regeneration in the Ventral Nerve Cord of Locust Embryos

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In contrast to the peripheral nervous system, the central nervous system is unable to regenerate after injury in higher vertebrates. There are now well known factors that inhibit regeneration, as well as probably positive regulators that might enhance regeneration. Invertebrates lack most of the negative regulators, thus making them useful tools to study positive factors alone. One such positive regulator of neuronal regeneration might be nitric oxide.

As an unusual, gaseous, neuroactive substance, nitric oxide (NO) is in the focus of interest for several years now. It is established as a modulator of synaptic transmission both in sensory systems and central circuits. Our topic is the role of NO during development and repair of the nervous system. In our experimental animal, the locust, it has been shown that NO can play a role in axonal growth and neural migration (Bicker (2005) BioEssays 27:495-505). Here, we present a model system in which we study the effect of NO and its downstream signaling cascade (via cyclic GMP as the second messenger) during regeneration of the ventral nerve cord. We have already shown that the locust CNS is generally capable of regeneration (Pätschke et al. (2004) Dev. Brain Res. 150:73-76).

We study the role of NO in an embryo culture preparation. The late (70%) embryo already has a relatively mature central nervous system, including functional synapses, endogenous rhythms, coordinated motor activity. These embryos can be cultured as flat-fillet preparations with their nervous systems fully exposed to the culture medium and drugs. In such preparations, we crush the connectives between the free abdominal connectives on one side, leaving the other side intact as an internal control. Using whole-mount immunofluorescence, we examine the morphology of identified serotonergic neurons which send a total of 4 axons through those connectives. Immediately after the nerve crush, axons are severed and the distal segments degenerate within 2 days. Axon stumps begin to grow out again after a few hours and reach the neighbouring ganglion within 4 days in culture. We quantify the number of regenerating axons within this period and test the effect of NO and drugs that interfere with NO action. As a result, we show that application of exogenous NO (using the NO-Donor NOC-18) promotes axonal regeneration, whereas inhibition of its receptor, soluble guanylyl cyclase, delays regeneration, an effect that can be rescued by application of external membrane-permeable cGMP-analogues.

We conclude that 1. NO promotes outgrowth of axons in semi-intact embryos, and 2. that such embryo-culture systems are useful tools for the study of mechanisms that promote regeneration in the central nervous system.

# The synthetic steroid mifepristone protects Purkinje cells from developmental cell death

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Mifepristone (RU486), which binds with high affinity to both progesterone and glucocorticosteroid receptors, is well known for its use in the termination of pregnancy. However, this steroid analog has also been shown to protect hippocampal neurons from apoptosis after traumatic brain injury or during oxidative stress. We have previously demonstrated that mifepristone protects Purkinje cells from programmed cell death in organotypic slice cultures of postnatal rat and mouse cerebellum by a novel neuroprotective mechanism that involves neither classical intracellular steroid receptors nor the antioxidant properties the compound (Ghoumari et al., PNAS 100, 7953-7958, 2003). Microarray analysis revealed that mifepristone down-regulated mRNA levels of the Na+/K+ ATPase alpha 3 subunit. Consistent with the down-regulation of the Na+/K+ ATPase, mifepristone caused Purkinje cell membrane depolarization (Ghoumari et al., FASEB J. 20, 1377-1386, 2006). Other depolarizing agents including ouabain, tetraethylammonium and veratridine also protected Purkinje cells from apoptosis, suggesting a role for excitatory inputs in Purkinje cell survival during early postnatal development. Indeed, coculturing cerebellar slices with glutamatergic inferior olivary neuron preparations allowed to rescue Purkinje cells. These findings point to a novel mechanism involved in the neuroprotective effects of steroids, and they support a pivotal role for excitatory inputs in the survival of developing Purkinje neurons. Mifepristone and related steroid analogs may thus become useful in the development of novel therapeutic approaches for maintaining the resting potential of neurons at values favorable for their survival under neuropathological conditions and after injury. Work is now in progress to identify the direct molecular targets of mifepristone as well as the intracellular signaling pathways involved in its neuroprotective actions.

# The role of microtubules in retraction bulb formation and failure of axonal regeneration in the CNS

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Axons in the central nervous system (CNS) do not regrow after injury and form characteristic swellings at their tips known as retraction bulbs, which are the non-growing counterparts of growth cones. While much progress has been made in identifying intracellular and molecular mechanisms that regulate growth cone locomotion and axonal elongation, a comprehensive understanding of how retraction bulbs form and why they are unable to grow is still elusive. We found that retraction bulbs formed after spinal cord injury contain disorganized microtubules. In contrast, microtubules are parallel aligned in growth cones generated after an injury in the peripheral nervous system (PNS). Moreover, by using in vivo microscopy we could show that disrupting microtubules in growth cones transforms them into retraction bulb-like structures which consist of disorganized microtubules similar to retraction bulbs of the CNS. We furthermore demonstrated that stabilizing microtubules of injured CNS axons prevents the formation of retraction bulbs and inhibits axonal retraction. Thus, the stability and organization of microtubules define the fate of lesioned axonal stumps to become either advancing growth cones or non-growing retraction bulbs. Our data pinpoint microtubules as a target to enhance axonal elongation.

# FIBROBLAST IMPACT ON SCHWANN CELLS AND AXONAL GROWTH

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Introduction

Currently, autologous nerve transplants are used to bridge gaps of lesioned nerves after PNS injury, but this often causes side effects in the donor region. We have developed a biohybrid, resorbable nerve guide tubes in order to improve regeneration and to avoid side effects of autologous transplants. Since scarring fibroblasts might infiltrate nerve guides, it is important to understand in more detail fibroblast interactions with Schwann cells and outgrowing neurites.

Materials and Methods

In vitro tests were performed as endpoint assays with different co-culture systems of purified Schwann cells, nerve-fibroblasts and dorsal root ganglia, as well as with time-lapse video recordings. Neuregulin expression was monitored by PCR and Western blotting. Knock-down of neuregulin expression was performed via double transfection with selected shRNAs.

Results

Co-culture experiments showed that nerve-fibroblasts hamper neurite outgrowth on Schwann cells but not neurite guidance, if fibroblasts accounted for 10% or more of the cell population. Time-lapse experiments indicated that diffusible factors of fibroblasts similarly as recombinant neuregulin induced faster Schwann cell migration. PCR and Western blot analysis demonstrated that neuregulin was expressed in nerve-fibroblasts and therefore, could be involved in these processes. The comparison of different fibroblast types (from nerve, skin, neonatal, and mature) displays different neuregulin isoform expression profiles. Current experiments aim to investigate the effect of fibroblasts on Schwann cell migration after neuregulin expression has been silenced.

Conclusion

Fibroblasts in nerve guide tubes and thus in direct contact with neurites might hamper their outgrowth. However, fibroblasts situated outside nerve guides could possibly support regeneration by fostering Schwann cell migration. Neuregulin is a candidate molecule which alters Schwann cell behaviour.

Keywords: fibroblasts, neuregulin, nerve guide, neuro tissue engineering, regeneration, Schwann cell.

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# Transplantation of mesencephalic human neural progenitor cells into the caudate putamen of Hemiparkinsonian rats

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Cell transplantation to treat Parkinson's disease (PD) shows promise. However, various cell sources that could currently be an option for the use in clinical trials, have to be investigated in preclinical experiments. In our opinion, human neural progenitor cells (hNPCs), derived from the mesencephalic region of the developing human brain could be an ideal source, especially for the treatment of PD. Here, we examined if nestin-immunoreactive (ir) mesencephalic hNPCs, derived from the 8th weeks of gestation (Hovakimyan et al., J Anat, in press), grafted in the caudate putamen (CPu) of Hemiparkinsonian rats have the potential to differentiate into (dopaminergic) neurons and are able to influence motor deficits.

Adult male rats received 26µg of 6-OHDA into the right medial forebrain bundle (stereotaxic coordinates according to IA: AP +6.7; ML -1.5; V +1.5). Three months later, successful lesions were evaluated by apomorphine-induced (a-i) rotations (0.25mg/kg body weight, 40 min) and the cylinder-test. Afterwards, lesioned animals (n = 10) received grafts into the right CPu (according to bregma: AP +0.5; ML -3.5; V -5.5), consisting of about 100,000 predifferentiated hNPCs. Animals (n = 10), receiving vehicle only, served as sham operated controls. Behaviour-tests were evaluated 1, 2 and 3 months post grafting for both groups. Animals were finally perfused with 4% PFA. Cryoprotected brains were cut on a cryostat and slices were immunostained for human nuclei antigen (HN), for neural epitopes, tyrosine hydroxylase (TH) and neuronal nuclei antigen (NeuN), and for the astroglial marker GFAP, finally detected by fluorescent secondary antibodies or the ABC-method.

For statistical evaluation of the behaviour-tests only animals, with HN-ir cells positioned in the CPu, were included (n = 5). In the cylinder-test, hNPCs grafted animals showed an improvement in left-forepaw use. In the transplanted group, compared to the sham-treated group, the a-i rotations were significantly diminished over time ($p \leq 0.05$, U-Test). Three months after transplantation HN-ir cells were detectable mainly scattered around the needle-track in the CPu. Considerable amounts migrated up to 600 µm into the host parenchyma. Many grafted cells were co-localized with NeuN or, to smaller amounts, contained GFAP. However, TH-ir somata were not observed and the depletion of TH-ir fibers in the transplanted CPu was similar to that of the sham-grafted rats.

Our results indicate, that after transplantation into the adult CPu, hNPCs survive for at least three months and differentiate into astroglial cells or neurons. However, in contrast to neonatal transplantation, we never observed a dopaminergic reinnervation by these transplants. Therefore, improvement in motor behaviour could only be explained by unknown influences of these neurons in the dopamine depleted basal ganglia loops. Moreover, compared to transplantation of hNPCs in neonatal rats (Hovakimyan et al.) they now lack a dopaminergic differentiation. Knowledge about factors influencing this differentiation would be of interest for further preclinical grafting strategies.

# 3D scaffolds to optimize human neural progenitor cell growth and differentiation

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In order to find new methods for a standardisized therapy in neurodegenerative diseases such as Parkinson disease we are investigating 3D cell culture systems for neural progenitor cells and monitoring their influence on cell adhesion, proliferation, growth and final differentiation. A clearly defined microenvironment is essential to support a high level of neuronal differentiation in these cell culture systems. We are using a human progenitor cell line (ReNcell VM197, ReNeuron, Guildford UK), derived from 10 week old fetal ventral mesencephalon. The cells are immortalized by retroviral transduction of the v-myc oncogen and have a high potential to differentiate into neurons on the withdrawal of growth factors (e.g. EGF, FGF).
The cells grow either on laminin-coated culture dishes as a monolayer or on non-laminated culture dishes as neurospheres.
In a first step, the hydrogel nano-scaffolds Matrigel and PuraMatrix were used in concentrations ranging from 0.7% up to 0.15% to generate 3D matrices in combination with different protocols: 1.) Encapsulated protocol: cells are plated inside the matrices before gelation. 2.) Surface protocol: cells are distributed on top of the gel-like matrices. 3.) matrigel was also tested as a 2D-layer, comparable to laminin.
All these approaches are monitored by immunocytochemistry with proliferating cells, and after 1, 4, 7 days after the initiation of differentiation. Undifferentiated cells were characterized by nestin, differentiated cells are labelled with βIII-tubulin and GFAP. These results are compared with standard 2D cell culture. For further evaluation of the functionalization of the 3D scaffolds by bioactive compounds, such as laminin or IKVAV, a signalling peptide derived from α1-laminin, is used to improve the potential for neuronal differentiation after the withdrawal of growth factors.
The cells grown in hydrogel nano-scaffolds demonstrate a similar proliferating profile to those in 2D cultures with a 24-30h doubling rate. Compared to the 2D culture, a much higher number of cells can be cultured based on the 3D composition of the scaffolds. The comparison of the 3D cultivation methods illustrates that the encapsulated protocol supports the 3D growth much better than the surface protocol, whereas there is no difference between the laminin / matrigel 2D-layer protocols.
βIII-tubulin positive neurons show a dense network with extended neurites in the 3D PuraMatrix scaffold. Further more these cells tend to form projection-like structures.
These findings will be taken together to optimize the culture and differentiation conditions of human neural progenitor cells. Finally such studies will help to establish an optimized in vitro scaffold system in the near future, which will be used in an animal model system to test the reliability in vivo.

# The role of serum response factor in axonal regeneration

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Unlike the peripheral nervous system (PNS), neurons of the central nervous system (CNS) show almost no regeneration after injury.

One of the factors leading to impaired neuronal regeneration is the composition of the environment encountered by severed axons. It is known that the surrounding CNS myelin sheath of these neurons formed by oligodendrocytes has an inhibiting effect on axonal regeneration. To date, three components of the myelin sheath known to reduce neuronal outgrowth have been identified with Nogo, myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (OMgp).

We are investigating the role of serum response factor (SRF) in neuronal regeneration. SRF is a transcription factor of the MADS box family regulating multiple target genes, falling into two classes, the Immediate Early Genes (IEG) and cytosceletal genes. In the neuronal system, SRF plays an important role in neuronal migration and axon guidance. We assume that SRF is able to regulate cytosceletal dynamics and thereby is influencing the regeneration behaviour of neurons. To test this idea, we are employing primary hippocampal neurons of wild type as well as SRF knock-out mice grown on various inhibitory substrates. Furthermore we will investigate the underlying signalling pathways involved in these mechanisms and follow them up in an in vivo regeneration model.

# Neuroprotective and growth promoting effects of a lens injury on retinal ganglion cells are not mediated by activated macrophages

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Retinal ganglion cells (RGCs) represent typical CNS projection neurons and can not regenerate their axons after injury. Furthermore following an intraorbital optic nerve crush more than 90% of RGCs undergo apoptosis within 14 days. However, RGCs reactivate their axonal growth program when inflammatory reactions in the eye take place enabling them to survive axotomy and to regenerate lengthy axons into the distal optic nerve. This regenerative state is characterized by a dramatic change in gene expression. Beneficial inflammatory processes can be induced either by a lens injury (LI) or by injections of the proinflammatory yeast wall extract zymosan. Both provoke a strong invasion and accumulation of activated ED-1 positive macrophages in the inner eye. It was therefore concluded that these monocytes are the main source of factors causing the strong neuritogenic and neuroprotective effects observed after a LI. Oncomodulin has recently been identified as the major axon promoting protein derived from macrophages.
We show here that the invasion and intraocular accumulation of activated ED-1 positive macrophages after LI is almost completely inhibited by intravitreal and strongly reduced by intraperitoneal administration of clodronate liposoms. Surprisingly, macrophage depletion did not diminish the neuroprotective or neuritogenic effects of a LI compared to controls, suggesting that the strong neuroprotective and neuritogenic effects of an inflammatory reaction in the eye are mediated by a different mechanism than macrophage derived factors.

# Characterization of LINA a new axonal regeneration promoting factor

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The inability of neurons to regenerate injured connections within the central nervous system (CNS) severely limits the functional recovery that can occur after traumatic injury, stroke or certain neurode-generative diseases. This failure has been attributed to inhibitory signals associated with proteins of the myelin that surrounds CNS axons, the scar that forms at an injury site and to a loss of an inherent potential for axonal growth of adult neurons.

Retinal ganglion cells (RGCs) represent typical projection neurons of the CNS. Although most RGCs undergo apoptosis after axotomy and do normally not regenerate axons in the mature optic nerve, they can survive an injury and reactivate their intrinsic axonal growth program 4 days after a lens injury or zymosan injections into the vitreous body of the eye. We have recently demonstrated that this reactiva-tion of neurons' intrinsic growth program is crucial for a successful regeneration of adult neurons in vivo. Using microarrays we have shown that RGCs change their gene expression pattern once entering the regenerative state. Among the genes being specifically upregulated in the regenerative state we found and cloned a new protein possessing a ring-finger-domain. The upregulation of the gene in RGCs was confirmed by in-situ-hybridization and a specific antibody developed against the c-terminus of the corresponding protein. This protein, which was named LINA, was detected in the Trans-Golgi-Network and is transported into the growth cone of regenerating RGCs and dorsal root ganglia neurons. Overexpression of LINA in PC-12 cells enhances neurite outgrowth and suppression of LINA by RNAi attenuates neurite outgrowth in PC12 cells. A point mutation in the ring-finger-domain leads to a complete loss of function and this form decreases NGF-induced neurite outgrowth suggesting that it functions as a dominant negative variant. Using a gene therapeutic approach we found that overexpression of LINA in RGCs in vivo significantly improves the regeneration of axons from retinal explants, whereas the expression of the dominant negative variant counteracts the en-dogenous function of LINA and attenuates regeneration. Our data suggest that LINA is a new ring-finger-protein playing an important role in regulating axon regeneration in peripheral and central neurons.

# Poster Topic

## T4: Development IV: Cell cycle and cell death

**T4-1A** BAG1 function depends on its subcellular localisation
*L. Faida, J. Liman, M. Bähr and P. Kermer, Göttingen*

**T4-2A** Understanding of inclusion body formation and cell death in spinocerebellar ataxia type 3 (SCA3)
*J. Liman, K. Sroka, CP. Dohm, M. Bähr and P. Kermer, Göttingen*

**T4-3A** TGF-β2 and GDNF in the Development of the Murine Nervous System: Evidence from Double Mutant Mice
*B. Rahhal, S. Heermann, E. Roussa and K. Krieglstein, Göttingen*

**T4-1B** TGFβ-1 and ActivinA as Important Factors for Ontogenetic Cell Death
*R. Schulz, T. Vogel and K. Krieglstein, Göttingen*

**T4-2B** Advanced Glycation endproducts trigger cell cycle re-entry in Alzheimer´s disease brain
*A. Schmidt, Y. Yang, K. Herrup and T. Arendt, Leipzig and Cleveland (USA)*

**T4-1C** Activity and mechanisms of neuronal neocortical apoptosis of premature fetuses and newborns suffered from intraventricular hemorrhages
*NK. Suhak and GY. Khulup, Minsk (Belarus)*

**T4-2C** Development of tension receptors of the muscular system of *Locusta migratoria*
*C. Marinc and U. Rose, Ulm*

# BAG1 function depends on its subcellular localisation

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BAG1 is a potent neuroprotectant as well as a marker of differentiation in neuronal cells. It appears to exist an equilibrium within the cell between BAG1 binding to heat-shock protein-70 (Hsp70) and BAG1 binding to Raf-1 kinase. Recently, it was shown that neuroprotection of BAG1 is executed by binding of BAG1 to Hsp70. Furthermore, it is known that BAG1 changes its localisation within the cell during development. During proliferation BAG1 is mainly localised in the nucleus, whereas BAG1 shifts to a mainly cytosolic localisation in differentiating cells. Here, we investigated how this change of localisation may influence the qualities of BAG1 function in cells. We therefore compared neuronal cell lines over expressing a BAG mutant being exclusively found in the nucleus (BAG-NUC) with cells expressing full length BAG1 and controls. Our results show, that nuclear localisation of BAG1 abolishes the neuroprotective effects of BAG1 as well as the increase in neuronal differentiation. We therefore hypothesise, that the distinct functions of BAG1 depend on its subcellular localisation, e.g. by resticting access to its interaction partners.

# Understanding of inclusion body formation and cell death in spinocerebellar ataxia type 3 (SCA3)

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Spinocerebellar ataxia type 3 is a inherited neurodegenerative diseases caused by expansion of a polyglutamine tract within the disease-specific protein (ataxin-3), leading to aggregation of misfolded protein, formation of intranuclear and cytosolic inclusion bodies (NIs) and cell death in distinct neuronal populations.
To date, there are several theories why mutated proteins form NIs and there is also still a controversial debate if and how this aggregates are toxic to the cell. Among those theories one can find dysfunction of the ubiquitin/proteasome system and mitochondria or the relocalisation of mutated protein to the nucleus.
Our data indicates that mutant ataxin-3 partly inhibits proteasomal function resulting in aggregation. At the same time, the cyclin-dependent kinase CDK5 seems to act as neuroprotectant in SCA-3 since cells overexpressing mutated ataxin-3 form more aggregates and are less viable in comparison to WT cells, especially when CDK5 activity is blocked. This effect seems to be independent of mitochondrial fission/fusion, but might as well be related to caspase activation.

# TGF-β2 and GDNF in the Development of the Murine Nervous System: Evidence from Double Mutant Mice

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Neuronal survival, proliferation, differentiation and death are fundamental topics in neurobiology research. Among different families of growth factors, members of the TGF-β superfamily have been identified as key mediators of neuronal survival and differentiation and are therefore excellent candidates to play crucial roles in development and maintenance of systems which, when aggrieved, cause for example diseases such as Amyotrophic Lateral Sclerosis or Parkinson' s Disease.
Many recent studies have revealed that growth factors acting in synergy can regulate neuronal survival much more potently than individual factors alone. Several evidences suggest that GDNF may require cofactors for unfolding its neurotrophic potential and TGF-β was shown to act as one of them. Current work aims at elucidation of TGF-β2 and GDNF cooperation in vivo via generation of TGF-β2/GDNF double mutant mice. For that purpose, heterozygous animals (TGF-β2+/- GDNF+/-) were crossed to receive mice lacking both TGF-β2 and GDNF. The analysis focused on midbrain dopaminergic neurons, hindbrain serotonergic neurons, lumbar spinal motoneurons, enteric neurons, dorsal root ganglionic neurons, superior cervical sympathetic ganglionic neurons and cranial ganglionic neurons. Our results suggest crucial roles for TGF-β and GDNF in the development of the central and peripheral neurons.

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# TGFβ-1 and ActivinA as Important Factors for Ontogenetic Cell Death

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Transforming growth factor beta-1 (TGFβ-1) and ActivinA (ActA), two members of the TGFβ superfamily, modulate next to differentiation, cell growth and proliferation also apoptosis depending on stage of cell type and expose to other growth factors. Further, lack of TGFβ suppresses neuron death during ontogenetic cell death. Both, ActA and TGFβ-1, are shown as extrinsic regulators of neuronal cell death as well.

In our cell cell model, a murine oligodendroglial cell line named Oli-neu, ActA and TGFb-1 lead both to apoptosis on two different mechanisms. First ,TGFβ-1-induced programmed cell death involving caspase3 precede a regulation of Bcl-Xl, a member of the Bcl-2 family. But ActA induces independent of executioner caspases apoptosis resulting in activation of apoptosis inducing factor (AIF).

Thus, this study shows that ActA and TGFβ-1, two members of the TGFβ superfamily, activate independent mechanisms resulting in apoptosis in one neural cell type model.

Our findings can implicate a possible interaction of this pathways to supress defects of regulatory factors of this pathways.

# Advanced Glycation endproducts trigger cell cycle re-entry in Alzheimer´s disease brain

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Nerve cells that re-enter the cell cycle will die rather than divide, a fact that likely underlies the neurodegeneration in Alzheimer's disease (AD). Re-expression of cell-cycle related genes such as cyclin-dependent kinases (cdk), cyclins, or cdk inhibitors in differentiated neurons in AD is rooted in aberrant mitogenic signaling. Since microglia and astroglia proliferate in the vicinity of amyloid plaques, it is likely that plaque components or factors secreted from plaque-activated glia induce mitogenic signaling in neurons. Mitogenic compounds might be advanced glycation end products (AGEs), protein-bound oxidation products of sugars, which are components of plaques. To demonstrate the pathophysiological relevance of AGEs for mitogenic signaling in AD brain, we showed in immunohistochemical studies that cyclin D1 positive neurons are co-localized with AGEs or directly surrounded by extracellular AGE deposits in the Brodmann area 22 of the human cortex (layer II and IV). Direct proof of DNA replication has been missing. Thus we used fluorescent in situ hybridization to examine the chromosomal complement of the interphase of neuronal nuclei. Probes of digoxygenin labeled BACs (from the BACE1 locus on chromosome 11 and BACE2 locus on chromosome 21 and) were used in a standard hybridization protocol. We confined this part of the study to the large neurons of the locus coeruleus. Consistent with the localization of cell cycle proteins, we found co-localization between aneuploid neuronal cells and AGE staining in AD brains but not in non-demented controls. Our results support the hypotheses that AGEs may serve as a source of mitogenic signaling leading to cell cycle re-entry of neurons and finally cell death in AD.

# Activity and mechanisms of neuronal neocortical apoptosis of premature fetuses and newborns suffered from intraventricular hemorrhages

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The aim of the present study is a revelation of neocortical neuronal apoptosis contribution to a morphological substratum of intraventricular hemorrhages in premature fetuses and newborns.

Post-mortem brains of 15 fetuses which died in labor and 49 newborns with gestational age of 22-36 weeks suffered from intraventricular hemorrhages were examined. Paraffin sections of citoarchitectonical fields 4 and 10 were stained with haematoxylin and eosin, method TUNEL (Promega, USA). Immunohystochemistry was performed with antibodies to apoptosis-inducing factor (Calbiochem, USA), activated caspase-3 and βIII-tubulin (Promega, USA). All TUNEL-positive neurons displayed morphological signs of apoptosis. Activated caspase-3 was predominantly expressed in cytoplasm. Apoptosis-inducing factor was revealed in cytoplasm and in the nuclei. Activity of neuronal neocortical apoptosis in fetuses did not exceed the basal level (1%). Pathological activation of neuronal neocortical apoptosis in premature newborns was displayed. It was revealed that reduction (in comparison with postconceptional norm) in the number of neocortical neurons of premature newborns was determined by combination of necrosis and pathological apoptosis of nerve cells. The cytoplasmic expression of apoptosis-inducing factor revealed from 25 gestational weeks in the field 4, from 27 gestational weeks in the field 10 of both fetuses and newborns. The fraction of neurons which expressed of apoptosis-inducing factor in the nuclei was very tiny. It was established that neuronal apoptosis in fields 4 and 10 of premature fetuses' and newborns' neocortex was predominantly caspase-dependent.

Findings are a good reason to think that pathological activation of neocortical neuronal apoptosis plays an important role in the pathomechanism of intraventricular hemorrhages. The identification of caspase-3 activation suggests that caspase inhibitors may potentially have a therapeutic neuroprotective role in perinatal brain injury.

# Development of tension receptors of the muscular system of *Locusta migratoria*

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Sensory neurons of insects usually develop from a specific cell layer and migrate to their final targets. Muscle receptors, like stretch receptors or force monitoring cells, must in addition find their adequate position and orientation on the muscle fibres. For those muscle receptors, the individual developmental and migratory steps from the neuronal progenitor to the differentiated neuron are largely unknown.

We examined the development of already described sensory cells on the dorsal ovipositor muscle 271 of female locusts (*Locusta Migratoria*). About 200 sensory cells are located at the posterior attachment site of the muscle which monitor muscle tension (Wanischeck and Rose, 2005).

To follow their fate from their first appearance on the muscular attachment site to their final position and orientation on the muscle fibres, we stained the developing neurons with an antibody directed against HRP. This technique has been previously shown to specifically stain membranes of developing neurons (Jan and Jan, 1982).

Neuronal precursors of sensory neurons were first revealed in the fourth instar larvae. These precursors consisted of a few ensembles comprising 2-4 cells which were consistently located at a site, where the muscle fibres attach to the apodeme. The cells of a ensemble were regarded as the precursors of the tension receptor and its accessory cells. According to the already described developmental pattern of type II sensory cells, the accessory cells will not survive the differentiation process.
In the early fifth larval stage almost no cells were stained except for a few sensory neurons which lay embedded between the muscle fibres. These neurons lack accessory cells and seemed almost fully developed because their dendrites had already orientated along the axis of the muscle fibres. From the fourth day of the fifth larvae however a large number of ensembles appeared on the muscle attachment side. In addition to theses ensembles, neuronal cells were identified which extend long processes that run exactly at the border where the muscle fibres blend with their tendons.
By the end of the fifth larval stage most of the ensembles gradually disappeared and more and more differentiated neurons became visible. However staining intensity of differentiated neurons was strongly reduced in the late fifth instar larvae presumably because the epitope recognised by the HRP-antibody, was no longer expressed by the cells. In the late fifth larval stage, retrograde fills of the peripheral nerve, in which the axons of the tension receptor cells project to the central nervous system, revealed a large number of differentiated sensory cells. This finding suggested that the cells have already send their axons to the central nervous system.

From our findings we conclude that the tension receptor cells on the ovipositor muscle develop from precursors located on the anterior apodemal attachment site of the ovipositor muscle. Surprisingly, most of the cells develop and differentiate not until the last larval stage appeared. Consistent with the general developmental pattern of type II sensory cells was that the accessory cells obviously undergo apoptosis.

# Poster Topic

## T5: Neurogenetics

**T5-1A** Characterisation of a *no-on-transientA* like gene in the cricket *Gryllus bimaculatus*, with focus on behavioural relevance
*S. Knapinski, M. Hennig, H. Saumweber and B. Ronacher, Berlin*

**T5-2A** Dyskinesia induced changes in gene expression in Parkinson´s disease.
*S. Schuster, Biberach*

**T5-3A** Investigating Parkin and DJ-1
*s. deeg, göttingen*

**T5-4A** ErbB4 function in neuron-neuron interactions in the adult brain
*MN. Gummert, P. Soban, KA. Nave and MH. Schwab, Göttingen*

**T5-1B** Cortical Development and Myelination in the Absence Schizophrenia Susceptibility Gene Neuregulin1.
*A. Agarwal, MH. Schwab, K. Radyushkin, S. Boretius, A. Garratt, C. Birchmeier, J. Frahm, H. Ehrenreich and KA. Nave, Göttingen and Berlin*

**T5-2B** Nuclear factor I-A (NFI-A) antagonizes glucocorticoid induction of neural adhesion molecule L1 expression
*T. Tilling, T. Schneegans, U. Borgmeyer, M. Hentschke, RM. Gronostajski and M. Schachner, Hamburg and Buffalo, NY (USA)*

**T5-3B** Adaptive changes in gene expression patterns in the somatosensory cortex after deletion of ephrinA5
*C. Peuckert, J. Rapus and J. Bolz, Jena*

**T5-4B** Investigation of Rgs2 and Rgs13 gene variants in human panic disorder
*C. Hohoff, A. Leygraf, P. Krakowitzky and J. Deckert, Münster and Würzburg*

**T5-1C** Investigation of genetic variants in the Rgs7-gene in panic disorder
*A. Neumann, C. Hohoff, K. Domschke, P. Krakowitzky, M. Rothermundt and J. Deckert, Münster and Würzburg*

**T5-2C** Morris water maze novelty-dependent gene regulation in mouse hippocampus
*SI. Haege, D. Galetzka, U. Zechner, T. Haaf, C. Hiemke and U. Schmitt, Mainz*

**T5-3C** Familial acanthocytosis with paroxysmal exertion-induced dyskinesia and epilepsy (FAPED)
*Y. Weber, A. Storch, T. Wuttke, K. Brockmann, A. Pekrun, J. Kempfle, E. Kraft, B. Walter, F. Mottaghy, R. Roebling, K. Tatsch, G. Grön, F. Lehmann-Horn and H. Lerche, Ulm, Dresden, Göttingen, Bremen, Munich and Berlin*

# Characterisation of a *no-on-transientA* like gene in the cricket *Gryllus bimaculatus*, with focus on behavioural relevance

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The sex-linked *no-on-transientA (nonA)* gene encodes a putative RNA-binding protein. In *Drosophila*, mutations in this gene affect viability (Stanewsky et al. 1993), vision (Hotta & Benzer 1969) and the male courtship song (Kulkarni et al. 1988) of the fly. A mutant allele, called dissonance, produces abnormal courtship song with more cycles per pulse and higher amplitude in *D. melanogaster* (Kulkarni et al. 1988). The first RRM (RNA-Recognition-Motif) is necessary for all the known functions of NONA, and mutation in this region also cause defects in courtship song of *Drosophila* (Stanewsky et al. 1996).

The *nonA* gene is of a special interest in song evolution studies, since point mutations in the coding region of the gene are known to affect male courtship song characters in *D. melanogaster*. Via gene transfer experiments between *D. virilis* and *D. melanogaster* mutants, Campesan et al. (2001) showed that the gene carries species-specific information in courtship song, since some of the characteristics of *D. virilis* song were detected in *D. melanogaster*.

We have identified a gene in *G. bimaculatus* which is closest related to the nonA isoform B in the honeybee and shares the main functional structures of the nonA gene in *Drosophila*, namely two RRMs.
Two *nonA G. bimaculatus* isoforms (*nonAGb1, nonAGb2*) have been identified. The *nonAGb2* isoform lacks a 159 bases sequence of low complexity upstream of the first RRM sequence, however it is still unclear whether these isoforms are generated by alternative splicing from a single pre-mRNA.
To figure out whether *nonA* is functionally conserved between the hemimetabolous insect *Gryllus bimaculatus* and the holometabolous *Drosophila melanogaster*, we performed rescue experimets by expression of the *nonAGb* in *Drosophila nonA*-mutants.
It is still unclear why complete rescue failed within *Drosophila* species while expressing nonA cDNA under the control of the naturally occurring promoter, but was successful in case of two-fold overexpression. For this reason we constructed two groups of rescue vectors: one group to express *nonAGb* cDNA via the *Drosophila melanogaster* promoter, and the other group to achieve an overexpression of *nonAGb* by a heat shock promoter.

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Stanewsky R, Rendahl KG, Dill M & Saumweber H (1993) Genetics 135: 419-442.
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# Dyskinesia induced changes in gene expression in Parkinson´s disease.

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Parkinson's disease is a chronic, slowly progressing movement disorder. The primary cause of the motor symptoms (bradykinesia, tremor, rigidity and postural instability) is the degeneration of dopaminergic neurons in the substantia nigra.
Adaptive changes in the cortico-striato-subthalamico-palido-thalamo-cortical motor pathway compensate for the loss of dopaminergic input in early phases of the disease. However, with disease progression motor deficits become evident and at later disease stages, dyskinetic, abnormal involuntary movements eventually develop.

The subthalamic nucleus plays a central role both in the early compensatory and late-stage dyskinetic processes. Changes in the neuronal firing pattern of subthalamic nucleus neurons have been observed in Parkinson's disease patients and in animal models. However, the molecular basis of these changes is not understood.
The aim of the present study is the identification of genes, which are involved in the adaptive changes of subthalamic nucleus neurons.

Dyskinesias are a big problem for late-stage PD patients with chronic L-Dopa treatment. This condition is mimicked in a rat model where dyskinesia is induced by chronic L-Dopa treatment after severe lesion of the dopaminergic system with 6-OHDA and 21 days priming with L-Dopa and Benserazid.

In the present study we compare changes in gene expression levels in late stage PD patients and in this rat model.
Human samples and the rat samples of the subthalamic nucleus were compared with Affimetrix gene chips.

# Investigating Parkin and DJ-1

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The identification of rare monogenic forms of Parkinson`s disease (PD) has provided tremendous insight into the molecular pathogenesis of PD. Currently, we focus on two of those genes causing familial PD.
Mutant parkin, a protein believed to function as an E2-dependent E3 ubiquitin ligase, causes autosomal recessive juvenile onset PD (AR-JP) and is responsible for up to 50% of all recessive PD cases.
Mutations within the DJ-1 protein are also linked to autosomal recessive early-onset PD accounting for 1-2% of cases. DJ-1 is believed to be a mitochondrial protein with chaperone function, but its biological function still remains obscure.
Investigating possible neuroprotective effects of BAG1, a co-chaperone linking stress response with protein degradation, we detected protein interactions between Parkin, the L166P-mutant of DJ-1 and BAG1. These protein interactions line out a model to study the degradation of a mutated protein via the ubiquitin-proteasome-pathway on one hand and the refolding of misfolded protein through the chaperone system on the other hand.

# ErbB4 function in neuron-neuron interactions in the adult brain

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ErbB4 (HER4) is a member of the epidermal growth factor (EGF) receptor family of tyrosine kinases that interact with neuregulins and other EGF-domain containing growth factors. Ligand binding induces receptor dimerization, tyrosine autophosphorylation, and activation of downstream signaling cascades that control multiple cellular responses. The ErbB4 null mutation in mice is lethal at embryonic day 10.5 due to a heart defect. The phenotype of conditional null mutants lacking ErbB4 in neural precursors suggests an involvement of the receptor in the development and migration of interneurons. Little is known about the function of ErbB4 in the adult mouse brain where ErbB4 expression has been clearly identified in cortical interneurons. In contrast, data regarding the expression in pyramidal neurons have been controversial.

For the analysis of ErbB4 function in mature pyramidal cells mouse mutants harboring a floxed ErbB4 allele were bred to transgenic mice that express Cre-recombinase in pyramidal neurons under control of the α-CaMKII promotor. The resulting mouse mutants did not display obvious behavioral or histological abnormalities. Next we performed a co-expression analysis in the adult mouse brain and found that ErbB4 mRNA expressing cells frequently co-expressed Parvalbumin, but almost never Calbindin or Calretinin. Moreover nearly all Parvalbumin positive interneurons in neocortex and hippocampus expressed ErbB4 mRNA. Therefore, we are currently generating an interneuron-specific ErbB4 null mutant using a Parvalbumin-Cre mouse line. These mouse mutants will allow us to study ErbB4 function in specific subsets of neurons via histological analyses, electrophysiology and behavioral tests.

# Cortical Development and Myelination in the Absence Schizophrenia Susceptibility Gene Neuregulin1.

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The human neuregulin-1 gene (NRG1) on chromosome 8p is a confirmed susceptibility gene for schizophrenia (SZ). The gene encodes a family of widely expressed neuronal growth factors. For the central nervous system, multiple functions of NRG1 have been suggested, including neuronal migration, synaptic plasticity, oligodendrocyte development, and myelination, however functional in vivo data are lacking, largely because NRG1 mutant mice die embryonically. While the association of specific SNPs in the NRG1 gene with SZ have been well documented, the effect of the human "at risk" haplotype on the NRG1 expression level and brain development are unknown. As a first step to understand NRG1 dysregulation, we are using transgenic techniques and conditional mutagenesis to determine the effect of (i) elevated, (ii) reduced, and (iii) complete absence of NRG1 expression on CNS development and behavioural functions in mice. Heterozygous mice with a 50% reduced NRG1 gene dosage appear normally developed in the CNS, but exhibit a reduction of sensory motor gating (prepulse inhibition) similar to findings in human SZ. Surprisingly, conditional null mutants with ablation of NRG1 expression in cortical and hippocampal projection neurons, beginning between E12 (NEX-Cre) and P5 (CamKII-Cre), exhibit no obvious defect of cortical development, oligodendrocyte differentiation, and cortical and subcortical myelination. Even in the complete absence of NRG1 signaling from neural cells perinatal oligodendrocyte development is largely uneffected. In contrast, behavioral analysis of mouse mutants with a postnatal onset of projection neuron-specific inactivation of NRG1 display reduced motor activity. These observations suggests that during evolution oligodendrocytes have acquired distinct axonal signals that control myelination independently of NRG1. Normal variations of NRG1 expression appear therefore unlikely to account for myelin abnormalities in schizophrenia but might alter cortical functions, such as the control of motor behavior.

# Nuclear factor I-A (NFI-A) antagonizes glucocorticoid induction of neural adhesion molecule L1 expression

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The neural cell adhesion molecule L1 stimulates neurite outgrowth and plays a crucial role in development and plasticity of the nervous system. L1-deficient mice share phenotypic characteristics with animals lacking expression of the transcription factor nuclear factor I-A (NFI-A), namely hydrocephalus and impaired formation of the corpus callosum. We therefore looked for nuclear factor I (NFI) recognition sequences in the regulatory region of the mouse L1 gene and found a putative optimal NFI binding site. By electrophoretic mobility shift assay (EMSA), binding of two NFI-A isoforms to this site could be shown in vitro. Moreover, we were able to demonstrate this interaction in vivo using chromatin immunoprecipitation (ChIP). Reporter gene assays showed that in neuroblastoma cells, overexpression of full-length NFI-A did not affect L1 expression. In contrast, L1 expression was enhanced fivefold in the presence of an NFI-A mutant lacking the activation/repression domain. This increase in L1 expression was further potentiated when glucocorticoids were added to the neural cell cultures. By contrast, glucocorticoid treatment of mock-transfected cells or of cells overexpressing full-length NFI-A did not cause changes in L1 gene activity.
We thus conclude that NFI-A represses L1 gene expression and that induction of L1 expression by glucocorticoids is counteracted by NFI-A. These findings establish a more complex relationship between expression of L1 and NFI-A than may have been expected from the phenotypes of the respective knock-out mice.

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# Adaptive changes in gene expression patterns in the somatosensory cortex after deletion of ephrinA5

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The Eph receptor tyrosine kinases and their ligands, the ephrins, are implicated in contact-mediated axon guidance, cell migration and the establishment of segmental boundaries in the nervous system. Most of the proposed functions of Eph/ephrin signaling for axonal pathfinding and cell migration have been determined by studying spatial and temporal expression patterns in combination with functional in vitro assays as well as by analysis of mutant animals in which an individual Eph receptor or an individual ephrin ligand has been genetically manipulated. However, the phenotypes in single Eph/ephrin mutants are in most cases very subtle. In contrast, knock-outs of two or more ephrins or Eph receptors often evoked much stronger effects then the deletion of a single gene. Usually, this is explained by the redundancy of the Eph/ephrin system. Moreover, knocking out an individual gene might lead to changes in the expression of other genes that could partially compensate its function. However, as yet there is very little known about the extend and the nature of such compensatory mechanisms in gene expressions. Here we compared with DNA chip technology gene expression patterns in the somatosensory cortex of wild type and ephrinA5 deficient mice, which exhibit subtle, but highly reproducible alteration of thalamocortical projections and intrinsic cortical circuits. Using very stringent criteria, we identified 2.7% of the investigated transcripts as deregulated in the mutant. Performing a functional group analysis of the differentially expressed genes, we found that a large fraction of the annotated genes that exhibited different expression levels in the mutant are related to the development and function of the nervous system. With in situ hybridization techniques and quantitative RT-PCR we found that even 20% of the Eph/ephrin family genes expressed in the somatosensory cortex are up-regulated in the mutant. Moreover, in some cases the up-regulation of the Eph/ephrin genes only occurred in selected cortical layers, whereas expression patterns in the other layers remained unchanged. These results provide clear evidence for adaptive and compensatory mechanisms in gene expression patterns during brain development after mutation of a single gene and they also point to the complexity interpreting the phenotypes of gene knockout animals. (Supported by the IZKF Jena).

# Investigation of Rgs2 and Rgs13 gene variants in human panic disorder

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Rgs2 and Rgs13 as members of the Rgs (regulator of G protein signalling) family reduce G protein activity via their GTPase function. Rgs gene variation therefore might lead to variation in GTP mediated neurotransmission. In mice, Rgs2 knockouts show increased neurotransmission and anxiety compared to their wildtype counterparts (1). Rgs2 and Rgs13 were recently identified as positional candidate genes located in a quantitative trait locus, which quantitatively modulates anxious behaviour in mice (2). In humans, different anxiety disorders exist of which panic disorder (PD) is a common form. PD is characterized by sudden, unexpected attacks of intense fear and anticipatory anxiety between attacks, i.e. a permanent hyperarousal (3) and has a heritability of 48%. In about 50% of the cases PD is accompanied by agoraphobia (AgP), for which an even higher heritability of 67% is described (4). We therefore hypothesized that variations in the Rgs2 and the adjacent Rgs13 gene might effect the development of human PD.

In a first step Rgs2 and Rgs13 were screened in silico for single nucleotide polymorphisms (SNP) within the genes and the flanking regions. Altogether 13 SNPs, six covering the Rgs2 gene and seven covering the Rgs13 gene were genotyped by TaqMan 5' exonuclease assays for allelic discrimination. For genotyping a control sample of 173 probands (anonymous blood-donors) was used. Genotype frequencies were then used to determine validation status and conformity to Hardy-Weinberg-Equilibrium (HWE) as well as to detect linkage-disequilibrium (LD) structure.

All 13 SNPs were polymorphic and 12 of them were conform to HWE, six of the Rgs2 and six of the Rgs13 gene. One large block of overall high LD was detected for Rgs2 comprising of SNPs 1 to 5, for which four different haplotype alleles with frequencies above 1% could be assigned. For Rgs13 two blocks of high LD were detected, the first spanning SNPs 1 and 2, for which three haplotype alleles could be assigned and the second block spanning the SNPs 3 to 5, for which six different haplotype alleles could be assigned.

The investigation of a PD sample that matches the control sample in size and according to gender and age is in progress.

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# Investigation of genetic variants in the Rgs7-gene in panic disorder

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Background:
Panic disorder (PD) is a common anxiety disorder with a world-wide life-time prevalence of 2 to 3% (1). In 50% of the cases, PD occurs with agoraphobia (AgP) (2). Based on family and twin studies, its heritability is estimated to about 48% (3). Rgs7 (regulator of G-protein signaling 7) as a member of the large Rgs family reduces G protein activity via its GTPase function and thereby modulates signal transmission (4). The gene is localized near a marker, for which Gelernter and colleagues described linkage with PD with a promising LOD-score of 2.04 (5). Positive association with PD is reported for another member of the Rgs family (6). We therefore hypothesized that variation in the Rgs7 gene might influence the development of PD.

Methods:
Eight SNPs were chosen to cover the Rgs7 gene on the basis of different databases that include potentially functionally relevant genomic regions like transcription start sites (TSS), transcription factor binding sites (TFBS) and promoter regions. For these SNPs 239 Caucasian controls (anonymous blood-donors) were genotyped by TaqMan 5' exonuclease assays for allelic discrimination. Hardy-Weinberg equilibrium was examined using the program Finetti provided as an online source (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; Wienker TF, Strom TM; accessed September 2006) and linkage disequilibrium (LD) between single SNPs was assessed by Haploview version 3.2.

Results:
In the overall sample and in subgroups stratified for gender all eight SNPs conformed to Hardy-Weinberg-Equilibrium. The analysis of the LD structure of the gene showed a strong correlation value (D' = 0.92) between SNPs 1 and 2 in the overall sample as well as in the female (D' = 0.93) and male (D' = 0.83) subgroups.

Discussion:
Our results are the first step of an association study showing that all detected SNPs conformed to HWE and thus can be included in next step. Therein we will genotype a gender- and age- matched PD sample to test our hypothesis that genetic variation in Rgs7 might influence the pathogenesis of panic disorder. Statistical analysis will comprise single marker (Chi-square) and haplotype (Haploview version 3.2; explorative sliding-window-analysis) analyses.

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# Morris water maze novelty-dependent gene regulation in mouse hippocampus

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The Morris water maze (MWM) task is used to test spatial learning and memory in rodents in which the hippocampus has been shown to be involved. In this study we investigated the effect of MWM novelty on de novo gene expression in mice. Therefore, we applied mice to the MWM arrangement lacking the platform, used triple cDNA microarray, reverse Northern blot and real-time PCR technology to determine differential gene expression at post-novelty time points one, six and twenty-four hours between MWM-experienced and test-naive mice. We demonstrated a MWM novelty-dependent upregulation of different components of the three core mitogen-activated protein kinase (MAPK) pathways ERK, JNK and p38MAPK. In detail, we detected an upregulation of the genes of the terminal MAPK enzymes ERK1 and p38α, as well as the JNK scaffold protein JIP1 at the time points 6 and 24 hours, but not 1 hour after four consecutive swimming trials in the MWM. Thus, we demonstrated an impact of the MWM arrangement on de novo gene expression of the MAPK genes *Mapk3* (EKR1), *Mapk8ip* (JIP1) and *Mapk14* (p38α) in the mouse hippocampus. This delayed upregulation was independent from the associative learning task and implied the involvement of long-term memory into MWM novelty.

# Familial acanthocytosis with paroxysmal exertion-induced dyskinesia and epilepsy (FAPED)

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Neuroacanthocytoses are inherited disorders with abnormal erythrocytes and neurological symptoms. Paroxysmal dyskinesias are characterized by attacks of involuntary movements classified as kinesiogenic, non-kinesiogenic or exertion-induced. We describe a novel autosomal dominant disease in a four-generation family with four affected individuals presenting with a combination of acanthocytosis, paroxysmal exertion-induced dyskinesia and epilepsy. The syndrome was characterized by extensive clinical and laboratory work-up, neuroimaging and genetic studies.

The index patient and his mother complained about attacks of cramps and involuntary movements after heavy workload since early childhood. Interictal neurologic examination was normal. Symptoms were relieved upon a ketogenic diet whereas response to carboanhydrase inhibitors was moderate. The two sons of the index case show mild neuropsychological deficits, mild permanent motor problems and focal a epilepsy with myoclonic and atonic seizures occurring as status epilepticus in fasting state. The epilepsy responded well to a ketogenic diet. Affected individuals presented with decreased CSF glucose, moderate hemolytic anemia and increased percentage of acanthocytes. The known forms of neuroacanthocytosis as well as the paroxysmal dyskinesias were excluded by laboratory tests, clinical discrimination and genetic analysis of the chorein gene responsible for the choreoacanthocytosis.

Since the combination of familial acanthocytosis, paroxysmal exertion-induced dyskinesia and epilepsy was not observed up to now we called the syndrome FAPED.

# Poster Topic

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# Circadian dependent sorting of vesicular glutamate transporter (VGLUT 1) 1 may involve the plasma membrane

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Synaptic strength depends on the amount of neurotransmitter stored in synaptic vesicles. The vesicular transmitter content has recently been shown to be directly dependent on the expression levels of vesicular neurotransmitter transporters indicating that the transport capacity of synaptic vesicles is a critical determinant for synaptic efficacy. It has been shown that the amount of vesicular glutamate transporter (VGLUT) 1 undergoes strong diurnal cycling in synaptic vesicles prepared from whole brain at different times of the day (Yelamanchili et al). VGLUT 1 protein levels are high before the start of the light period, decline at noon, increase again before start of the dark period, and decline again at midnight. In contrast, mice lacking the period gene Period 2, a core component of the circadian clock, did not show any light-cycle-dependent changes of VGLUT 1 levels. These results indicate that synaptic vesicles containing VGLUT 1 could be sorted into a special compartment or pool that still needs to be characterized. One possibility could be that VGLUT 1 is sorted to the plasma membrane. To test for this hypothesis, we used synaptosomes from rat or mouse brain which were subjected to various protocols of stimulation. To analyze the amount of plasma membrane translocated specific protein, pronase digestion was applied, which will only cleave integral membrane protein faced at the plasma membrane. Evidence is provided that compared to other vesicular proteins more VGLUT 1 is sorted to the plasma membrane under control as well as stimulation conditions.

# Kainate receptors mediate the supression of excitatory transmission at the hippocampal AC synapse by a presynaptic, G-protein mediated mechanism

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CA3 pyramidal cells receive 3 main excitatory inputs. The so called mossy fiber terminals, originating from the granule cells of the dentate gyrus and synapsing proximal to the cell body in the stratum lucidum. Neurons from the entorhinal cortex send axons to the hippocampus via the perforant path and synapse on the granule cells of the dentate gyrus as well as on distal dendrites of CA3 pyramidal cells, forming the stratum lacunosum moleculare. An additional distal input is provided by the AC synapses, which provide an ipsi- and contra lateral feedback loop within the hippocampal CA3 region. These synapses terminate in the stratum radiatum. Since this feed-forward excitation makes the CA3 region extremely prone to seizure development, understanding the regulation of synaptic strength in this region is of crucial interest. Among the receptors found in the CA3 region the glutamate sensitive Kainate receptors (KaR) are suspected to play a role in the regulation of synaptic strength.

Since their discovery and cloning, it has been difficult to investigate the role of Kainate receptors. Much of these difficulties arose from the pharmacological overlap between Ampa-R and Ka-R. New drugs, like the GluR-5 subtype specific agonist ATPA were developed in the recent years and made it possible to asses the role of Kainate receptors in the brain. It turned out that the Ka-R can influence synaptic transmission on the postsynaptic site as well as influencing transmitter release on presynaptic terminals. The aim of this study was to investigate possible action of Kainate receptors on AC-synapse transmission in the CA3 region of the hippocampus.

In field recordings of hippocampals slices, the GluR agonist ATPA depressed the amplitude of evoked responses without affecting the size of the fiber volley. This depression was not seen in slices from animals treated Pertussis toxin, arguing for a G-Protein mediated mechanism. Furthermore, this depression of synaptic transmission was accompanied by an increase in the paired pulse ratio, indicating a presynaptic site of action. Further patch-clamp recordings of CA3 pyramidal cells revealed that ATPA decreases the size of evoked NMDA and AMPAR mediated currents, ruling out the possibility of an AMPAR specific silencing of synapses. Analysis of the coefficient of variance and of excitatory miniature currents provide further evidence for a presynaptic mechanism. Furthermore, laser stimulated uncaging of glutamate evoked EPSC like events, which were unaffected by ATPA application.

Altogether, our experiments suggest that activation of presynaptic Kainate receptors lead to a reduction of transmitter release.

# Differential protein make up in synaptic vesicle subpopulations: A biochemical study using immunoisolation.

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Synaptic vesicles (SV) are key organelles of the synaptic terminal where they convey information between neurons. All synaptic vesicles are equipped with a common set of proteins, including synaptophysin, synaptobrevin and synaptotagmin. Dependent on the character of the nerve cell SV differ in their neurotransmitter transporters necessary to concentrate the corresponding neurotransmitter in the SV. These transporters include the vesicular glutamate transporters VGLUT1 and VGLUT2 in types of glutamatergic neurons and the vesicular GABA transporter VGAT in GABAergic neurons. SV also undergo rapid exo-endocytotic cycles during which part of them may be transiently included in the early endosomal compartment characterized by Rab5. In addition to these membrane compartments the presynaptic terminal also harbours vesicles which contain ion channel proteins or transporters designed to be translocated to the plasma membrane.

The aim of the present study is to compare the set of vesicular proteins of different subpopulations by immunoisolation using antibodies against synaptophysin, VGLUT1, VGLUT2, VGAT, and Rab5. Synaptosomes from rat or mouse whole brain were lysed by hypoosmotic shock and the resulting LS0 fractions were subjected to immunoisolation using paramagnetic beads and the various antibodies. Immunoisolates were analysed by SDS-PAGE and western blotting following quantification. As expected immunoisolates by synaptophysin antibodies contained all vesicular transmitter transporters. There was no overlapping between VGAT and VGLUT1 isolates, however, VGLUT1 was also seen on immunoisolates using VGLUT2 antibody. A strong VGAT signal associated with Rab5 immunoisolates suggests a special role of early endosomes for the recycling of VGAT. Investigations at the electron microscopic level are in progress to characterize morphological properties of isolated subpopulations.

# Dynamics of synaptic signalling between graded potential presynaptic neurons and a spiking neuron in the fly motion-vision pathway

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It is a general assumption that synapses operate efficiently only if their signalling range is matched well to the spectrum of the prevailing presynaptic signals in terms of both amplitudes and dynamics. However, the requirements to optimally match the signalling range may differ considerably between spike-mediated and graded synaptic transmission. While signal transfer between spiking neurons needs to distinguish only between spikes and noise, at synapses mediating graded potentials small amplitude differences have to be discerned to ensure a sufficiently large signalling capacity. Synapses which convey both graded and spike signals at the same time have to comply with these different requirements or, at least, implement a trade off between opposing demands. To address this issue and to disentangle the role of graded and spike-mediated transmission we recorded from single neurons in the blowfly's visual-motion pathway which receive mixed signals consisting of graded potentials with superimposed spike-like depolarizations (spikelets) from presynaptic cells. We used presynaptic voltage clamp and current clamp to selectively assess the performance of individual synapses and to elicit reproducible presynaptic signals with different dynamics. Thus, we were able to investigate the transmission of graded signals of various amplitudes and dynamics and to specifically address the role of spikelets in synaptic transmission. We found that the synapses can effectively convey signals over a much larger amplitude and broader frequency range than is normally utilized during graded transmission of visual signals.

# Influence of glial-derived matrix components on electrophysiological properties of hippocampal neurons

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The presence of astrocytes is crucial for the development of synapses in the CNS, but the factors mediating their effects on the formation, maintenance and function of synapses are only partially known. In order to investigate the involvement of astrocyte-secreted extracellular matrix (ECM) molecules, we established astrocyte-neuron cocultures, in which embryonic hippocampal neurons were plated on top of astrocytes. In these cultures, several ECM components were removed by treatment with appropriate degrading enzymes. Electrophysiological characterization of enzyme-treated and control cultures showed that not only synaptic activity but also voltage-dependent currents were affected by the removal of certain matrix components. For characterizing synaptic currents in more detail, we established single-neuron microcultures that allow the determination of the number and functional properties of synapses formed by a single neuron (autapses).

# ECM and Synaptogenesis: Monitoring the Impact of Extracellular Matrix on Synapse Formation in Hippocampal Neurons

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Synapses represent specialized cell-cell contact sites between nerve cells. These structures mediate the rapid and efficient transmission of signals between neurons and are surrounded by glial cells. Former investigations have shown that astrocytes and astrocyte-derived extracellular matrix (ECM) components are important for formation, maintenance and function of synapses in the CNS. In order to study the effects of glial-derived ECM on synaptogenesis, we have established an in-vitro co-culture system for E18 rat hippocampal neurons and various glial cell types. Neurons were cultured without direct contact to glial cells. After 10 days in culture, a concordant expression of pre-and postsynaptic proteins could be documented. Moreover, the colocalization of bassoon and proSAP1 indicated the formation of structurally intact synapses. In order to quantify those effects, we have developed a technique that permits the semi-automated determination of the number of synaptic puncta per neuron. Thereby, significant differences of the efficacy of cell types for and the effects of defined treatments on synaptogenesis could be documented. Thus, primary type I astrocytes proved the most efficient cell type in fostering synaptogenesis. Our present studies focus on maturation of synapses in the presence or absence of enzymes, that are able to degrade astrocyte-released ECM components.

# Analysis of γ-protocadherin-deficient synapses in neocortical neurons

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Synaptic adhesion molecules have been proposed to regulate synapse formation and thus might function as recognition molecules controlling synaptic connectivity in the brain. The superfamiliy of cadherins, consisting of classical cadherins and protocadherins, is functionally characterized by homophilic binding properties, which make them strong candidates for a molecular code specifying synaptic connectivity.
To investigate the functional role of γ-protocadherins at synapses, we cultured neocortical neurons from embryonic day 17.5 fetuses of γ-protocadherin gene-trap mice (Wiles et al, 2000; Ebert et al., 2005), which are lethal during early postnatal development. In homozygous gene-trap mice, expression of wild type γ-protocadherin proteins is completely blocked due to the integration of the gene-trap cassette (Floss and Wurst, 2002; Ebert et al., 2005) as is confirmed by Western blotting.
To characterize the role of γ-protocadherins in formation and function of synapses, we used whole-cell patch-clamp recordings from cultured neocortical neurons obtained from homozygous γ-protocadherin gene-trap mouse fetuses. Miniature postsynaptic currents (mPSCs) were recorded in the presence of TTX at -60 mV holding potential from neurons in mass culture at 7-9 DIV and at 12-14 DIV. AMPA receptor-mediated mEPSCs were pharmacologically isolated by addition of gabazine and GABAA receptor-mediated mPSCs were isolated by addition of DNQX. No significant differences in the mean frequency and in the mean amplitudes of both AMPA mEPSCs and GABAA mPSCs were observed between homozygous γ-protocadherin-deficient and wildtype neurons. To further investigate the properties of synaptic NMDA receptors, homozygous γ-protocadherin-deficient neocortical neurons were cultured on glial microislands. AMPA- and NMDA-receptor mediated PSCs were evoked by extracellular stimulation and were recorded at -60 and +40 mV holding potential at 10-12 DIV. The absence of γ-protocadherins did not lead to any significant differences between homozygous γ-protocadherin-deficient and control neurons concerning the amplitudes, failure rates, rise times and decay times of AMPA and NMDA PSC-components. The amplitude ratio of NMDA (+40 mV) and AMPA (-60 mV) PSC-components was also not significantly different.
Our results suggest that γ-protocadherins are dispensible for the formation and basic function of glutamatergic and GABAergic synapses. However, because we used homotypic cultures, γ-protocadherins might nevertheless be important in controlling selective synapse formation. To test for this, experiments giving neurons a choice between molecularly different targets using chimeric cultures are currently performed.

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# Function of N-cadherin in developmental regulation of presynaptic vesicle accumulations

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Synapse formation is thought to be initiated and regulated by the transsynaptic interaction of synaptic adhesion molecules. Classical cadherins, e.g. neural (N)-cadherin, represent a family of synaptic adhesion molecules that are well known to be localized perisynaptically close to the active zone and the postsynaptic density. However, relatively little is known about the functional role of N-cadherin in synapse formation, stabilization and plasticity.

We have now used a live imaging approach to study presynaptic vesicle accumulations in N-cadherin deficient, ES cell-derived neurons (Moore et al., 1999, Radice et al., 1997) ES cell-derived neurons were obtained by in vitro differentiation of mouse ES cells, were purified by immunoisolation and were further cultured on glial cells (Jüngling et al., 2003, 2006). The use of ES cell-derived neurons enabled us to study N-cadherin knockout synapses, because this circumvented the early embryonic lethality of the N-cadherin knockout mice. To fluorescently label presynaptic vesicles, cultured ES cell-derived neurons were acutely transfected with a DsRed-SynaptobrevinII fusion protein using the lipofectamin technique. Control experiments using co-transfection of DsRed-SynaptobrevinII and EGFP-PSD95 revealed that almost all dendritic DsRed-SynaptobrevinII puncta were associated with a postsynaptic accumulation of PSD95 and thus represent glutamatergic synapses.

In homozygous N-cadherin-deficient, ES cell-derived neurons the density of dendritic presynaptic vesicle accumulations was similar as compared to heterozygous controls. Other synaptic adhesion molecules might compensate for the loss of N-cadherin. We further analysed the size (area) and the mean intensity of the DsRed-SynaptobrevinII puncta. In neurons after 10-16 DIV the mean intensity of the DsRed-SynaptobrevinII puncta was decreased in N-cadherin-deficient neurons, suggesting that N-cadherin controls the spatial organization of the vesicle pool.

In summary, our results indicate that N-cadherin is dispensable for synapse formation per se, while it appears to play an important role in regulating presynaptic vesicle pools thus promoting presynaptic specialization.

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# Proteinkinase CK2-dependent serine phosphorylation of MuSK regulates acetylcholine receptor aggregation at the neuromuscular junction

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The release of Agrin by motoneurons activates the muscle-specific receptor tyrosine kinase (MuSK) as the main organizer of subsynaptic specializations at the neuromuscular junction. MuSK downstream signaling is largely undefined. Here we show that protein kinase CK2 interacts and co-localizes with MuSK at postsynaptic specializations. We observed CK2-mediated phosphorylation of serine residues within the kinase insert (KI) of MuSK. Inhibition or knockdown of CK2, or exchange of phosphorylatable serines by alanines within the KI of MuSK, impaired acetylcholine receptor (AChR) clustering, whereas their substitution by residues which imitate constitutive phosphorylation led to aggregation of AChRs even in the presence of CK2 inhibitors. Impairement of AChR cluster formation after replacement of MuSK KI with KIs of other receptor tyrosine kinases correlates with potential CK2-dependent serine phosphorylation within KIs. MuSK activity was unchanged but AChR stability decreased in the presence of CK2 inhibitors. Muscle-specific CK2β knockout mice develop a myasthenic phenotype due to impaired muscle endplate structure and function. This is the first description of a regulatory crosstalk between MuSK and CK2 and of a role for the KI of the receptor tyrosine kinase MuSK for the development of subsynaptic specializations.

# Functional characterization of transporters in native synaptic vesicles using a novel electrophysiological technology.

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Synaptic vesicles and other intracellular compartments are difficult to access, thus, making functional electrophysiological analysis of vesicular transporters and ion channels tedious. Moreover, heterologous expression of intracellular transport proteins at plasma membranes often causes non-specific effects on the membrane properties possibly resulting in misinterpretation of the data.

The SURFE$^2$R technology permits the electrophysiological analysis of transporters as well as other electrogenic proteins on solid supported membranes (SSMs). Membrane vesicles containing the protein of interest are adsorbed onto a gold coated sensor surface. The resulting biosensor is placed into the integrated fluidic system of the setup. Rapid exchange between non-activating and activating solutions results in protein-specific charge movements, which can be detected as electrical currents in a highly sensitive manner.

In this study, synaptic vesicles from rat brain were purified and functionally characterized utilizing the SURFE$^2$R technology to demonstrate its suitability for electrophysiological measurements of intracellular transporters. First of all, we examined the synaptic v-type ATPase. The proton electrochemical gradient generated by this v-ATPase across the vesicular membrane serves as the driving force for the transport of the majority of neurotransmitters into synaptic vesicles by specific transport systems. Activation of the v-ATPase through ATP concentration jumps induced electrical currents that could be inhibited by the specific v-ATPase inhibitor bafilomycin A1. These currents were $Mg^{2+}$- and ATP-dependent with an apparent $K_{0.5}$ for ATP of approximately 50 µM.

Assay development and experiments allowing the functional identification and pharmacological characterization of further vesicular transporters and ion channels are ongoing. Preliminary recordings from sensors coated with synaptic vesicles indicate that electrical responses can be elicited by application of different neurotransmitters. To our knowledge, this study represents the first electrophysiological investigation of proteins from native synaptic vesicles. In summary, our data demonstrate that the SURFE$^2$R technology is a powerful tool to study native transport systems not only in plasma membranes but in intracellular compartments like synaptic vesicles as well.

# Role of Synaptic Ribbons in Hair Cell Sound Coding

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The synaptic ribbon is an electron-dense structure surrounded by synaptic vesicles found at hair cell (HC) afferent synapses. In a knockout mouse of Bassoon (synaptic scaffolding protein) ribbons are no longer tethered to the presynaptic membrane that is coincided with a strong reduction of synchronous HC exocytosis and a degraded compound action potential, suggesting that the synaptic ribbon stabilizes a large readily releasable pool (RRP) of vesicles, thereby increasing the likelihood of multi-vesicular release and reducing the jitter of postsynaptic spiking.

Here we test this hypothesis of reduced temporal precision at the level of single auditory nerve (AN) fibers of Bassoon mutants in vivo. AN tuning appeared normal in mutants except at high frequencies. AN discharge retained the normal stochastic pattern seen in interval histograms of spontaneous activity, but spontaneous rates were reduced. Sound-evoked discharge was assessed by responses to tone bursts at 30 dB threshold at CF. Although mutant fibers showed sustained responses, both steady-state and onset rates were reduced. In line with our hypothesis, distributions of first spike latencies showed increased variance in the mutants, while the mode was not different from wildtype. Presynaptic recordings of inner HC potassium currents and membrane potentials indicated that the membrane time constant was unchanged. Immunohistochemistry showed that Ca2+ channels remain clustered at the synapse. Although the recovery of spontaneous discharge rate of the ANs was reduced in mutants the recovery of the RRP IHC exocytosis was not impaired.

In conclusion, our data indicate that postsynaptic detection of coincident release of multiple vesicles contributes to the synapse's superior temporal acuity. As a result, the reduced RRP of ribbon deficient synapses lowers the rate of AN discharge and causes a degradation in the temporal precision of sound coding.

# Morphogenic Signaling in Neurons via 5-HT-Receptors

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The neurotransmitter serotonin (5-HT) plays an important role in the regulation of multiple events in the CNS. Recently we demonstrated that in mouse hippocampal neurons, activation of endogenous 5-HT7 receptors significantly increased neurite length, whereas stimulation of 5-HT4 receptors led to a decrease in the length and number of neurites. We showed that the 5-HT4 receptor is coupled not only to the heterotrimeric Gs, but also to G13 protein. Activation of this signaling pathway results in RhoA-mediated modulation of gene transcription and in reorganization of the actin cytoskeleton. We also demonstrated that serotonin receptor 5-HT7 can activate heterotrimeric G12 protein, leading to the selective activation of small GTPases RhoA and Cdc42. Agonist-dependent activation of the 5-HT7 receptor induced pronounced filopodia formation via a Cdc42-mediated pathway paralleled by RhoA-dependent cell rounding. (1,2)

This molecular model for 5-HT4 and 5-HT7 receptor mediated signalling provides a link between receptor activation and a subsequent change in morphology. We also asked whether these serotonin mediated morphological changes could be directly correlated to the modulation of synaptic plasticity.

We investigated the 5-HT7 receptor-mediated formation of presynaptic clusters (synaptophysin), filopodia, spines and the spontaneous synaptic activity in primary culture of mouse hippocampal neurons. Furthermore we looked into changes in long-term potentiation related to the 5-HT7 receptor activity in organotypic cell culture and investigated their relevance in behavioural studies in mice .

Our data suggests that serotonin plays a prominent role in regulating the neuronal cytoarchitecture and synaptic plasticity in addition to its classical role as a neurotransmitter.

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# Inhibitory synaptic transmission to hypoglossal motoneurons in glycine transporter 2 knock out mice

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The glycine transporter subtype 2 (GlyT2) is localized in synaptic terminals of glycinergic neurons. Mice deficient in GlyT2 are normal at birth but die during the second postnatal week after developing a neuromotor deficiency that resembles severe forms of human hyperekplexia (hereditary startle disease) and is characterized by spasticity, tremor, and an inability to right. We analyzed the role of GlyT2 during postnatal development (postnatal day 0-8) using whole-cell voltage-clamp recording. The amplitudes of glycinergic inhibitory currents (IPSCs) were strikingly reduced in hypoglossal motoneurons from GlyT2 deficient mice. This reduction of IPSCs was already evident early after birth (P1-2). No compensatory up-regulation of GABAergic IPSCs was observed during postnatal development. It appears that GlyT2 is crucial for efficient transmitter loading of synaptic vesicles in glycinergic nerve terminals. (Supported by DFG).

# The number of ribbon synapses in mouse inner hair cells has a maximum in the tonotopic region of best hearing and scales with exocytosis but not $Ca^{2+}$ current.

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The sensitivity of sound perception is highly dependent on the frequency - each detected at a specific tonotopic location in the cochlea. Here, we investigated whether the morphological and physiological properties of the afferent hair cell synapses could contribute to this phenomenon. We found that the number of synaptic contacts per inner hair cell had a maximum in the cochlear region that transmits sounds with highest sensitivity (10-24 kHz). Confocal microscopy of the organ of Corti following immunostaining for RIBEYE, a major component of the synaptic ribbon and for AMPA-receptor subunits GluR2 and 3 was performed to estimate the number of afferent synaptic contacts as colocalized spots of pre- and postsynaptic immunofluroescence.

We then investigated the presynaptic function of inner hair cells at different positions along the apical turn of the cochlea by perforated patch-clamp recordings. Probing exocytosis by measurements of cell capacitance increments after brief depolarizations, we found that hair cells located ~300 µm from the apex released 44% less transmitter than cells located at ~1400 µm from the apex. This functional finding corresponded to a 31% difference in the number of morphologically identified afferent synapses betwen these locations. Interestingly, size, charge and kinetics of the calcium current did not vary with the tonotopic position of the hair cells.

As the IHC $Ca^{2+}$ influx may not only depend on the synapse number but also on the active zone size we asked whether the size of presynaptic ribbons may vary tonotopically. The Ribbon size distributions at the two tonotopic positions of ~180 and ~1060 µm, as estimated by 4Pi high-resolution optical microscopy, were indistinguishable from each other.

In conclusion, the cochlea may use a maximum of neural information channels per hair cells in the range of best hearing. The comparable $Ca^{2+}$ current despite varying IHC release area might indicate a significant number of extrasynaptic $Ca^{2+}$ channels.

# Temperature Enhances Exocytosis Efficiency at the Mouse Inner Hair Cell Ribbon Synapse

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Hearing relies on fast and sustained neurotransmitter release from inner hair cells (IHCs) onto the afferent auditory nerve fibers. The temperature dependence of Ca2+ current and transmitter release at the IHCs ribbon synapse has not been investigated so far. Here we performed perforated patch-clamp recordings of voltage-gated L-type Ca2+ influx and exocytic membrane capacitance changes at room (25°C) and physiological (35-37°C) temperatures. We show that temperature increases the L-type Ca2+ current amplitude of IHCs (Q10 = 1.3). We obtained more exocytosis per Ca2+ influx at physiological temperature. The amplitude of fast exocytosis (response to 20 ms depolarization, Q10, fast = 2.1) and the rate of sustained exocytosis (exocytic rate between 20 and 100 ms of depolarization, Q10, sustained rate = 1.7) were elevated disproportional to the increase in Ca2+ influx. Therefore, we suggest that the efficiency of exocytosis is higher at physiological temperature than at room temperature. Increase in number of readily releasable vesicles available at the active zone and higher rate of their re-supply as mechanisms underlying the temperature dependence are discussed.

# Transforming growth factor-beta 2 (TGF-beta2) is required for the development of functional synapses

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Synaptogenesis is a complex process that is regulated by different factors. Recently, studies in drosophila mutants showed that different components of the transforming growth factor-beta (TGF-beta)-signalling pathway play a crucial role in the development of synapses. TGF-betas constitute a superfamily of cytokines that are involved in different aspects of development, including cell proliferation, differentiation, survival and apoptosis. Although their role in vertebrate synaptogenesis is discussed, it has not been analysed yet. In the present work, the influence of TGF-beta2 in synaptogenesis was explored in embryonic TGF-beta2-/- mice at E18.5. As these mice die perinatally due to hypoxia, we investigated a putative neuromuscular defect and possible synaptogenetic defects. Functional synaptic analysis was performed in acute slices of the pre-Boetzinger-complex in the ventral medulla which contains the respiratory rhythm-generating network using patch-clamp recordings. We found that inhibitory synaptic transmission in the acute slices was significantly impaired in TGF-beta2-/- compared to control mice. To characterize the gene-deletion-related cellular changes, we analyzed immunohistochemical stainings of the relevant brain regions. The results were compared to data from in vitro experiments where primary hippocampal neurons were treated with either TGF-beta1 or anti-TGF-beta(1,2,3) which neutralizes endogenous TGF-beta. In both cases, synapses were visualized with antibodies against synaptic proteins.
Together, these results suggest that the loss of synaptic transmission might cause the lethal phenotype of TGF-beta2-/- mice and they provide first insight into the putative role of TGF-beta in synaptogenesis.

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# The size of release quanta at the hair cell ribbon synapse

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Hair cell ribbon synapses release several vesicles within milliseconds (Fuchs et al., 2003). The mode of exocytosis underlying this behavior remains unknown. Whether it involves statistically independent synaptic vesicle fusion, compound or cumulative fusion likely has a large impact on sound coding. In order to explore the mode of exocytosis we aimed to investigate the size distribution of the exocytic quanta of mouse inner hair cells (IHCs), which depends on the specific mechanism of synaptic vesicle fusion. Usually the sizes of single vesicles are characterized by their diameter in electron micrographs of fixed tissue. Patch-clamp, on the other hand, allows the measurement of the electrical capacitance added by exocytic fusion at an operating synapse. As the capacitance is proportional to the surface of the vesicle, these measurements provide us with a geometric parameter of the quanta, which are actually released under physiological conditions.

Here, we first used patch-clamp recordings of IHC whole-cell membrane capacitance to explore trial-trial fluctuations of exocytosis during repetitive stimulation by short stimuli. The magnitude of these fluctuations is related to the amount of capacitance added by the elementary fusion event (Moser and Neher, 1997)enabling us to estimate the mean apparent size of exocytic quanta to about 80 attofarad (aF) in 15 IHCs. Bootstrapping provided an estimate of the 95% confidence interval of our quantal size estimator of 100 aF. Second, we measured the outer diameter of hair cell synaptic vesicles at and around the ribbon synapses. We observed a narrow normal distribution peaking at 43.7 nm (CV: 0.12). Converting this geometrical estimate (after correction for the bilayer thickness: -5 nm and assuming a specific capacitance of 10 $\mu F*cm^{-2}$) yielded a mean vesicle capacitance of 47 aF. Together with our functional apparent quantal size estimate this EM derived single vesicle capacitance indicates that the majority of fusion events comprises statistically independent synaptic vesicle exocytosis. In a third line of experiments we aim at direct investigation of individual fusion events using on-cell patch clamp measurements on IHCs. Towards this end we have established low-noise high resolution on-cell capacitance measurements using a software-lock-in approach.

# Maturation of ribbon synapses in hair cells is driven by thyroid hormone

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Ribbon synapses of rodent inner hair cells (IHCs) undergo developmental maturation until after the onset of hearing. Here, we studied whether IHC synaptogenesis is regulated by thyroid hormone (TH). We performed perforated patch-clamp recordings of $Ca^{2+}$ currents and exocytic membrane capacitance changes in IHCs of athyroid $Pax8^{-/-}$ mice during postnatal development. Like in wild-type (wt) IHCs, large $Ca^{2+}$ currents were present in IHCs of $Pax8^{-/-}$ mice at the end of the first postnatal week. Different from wt, their $Ca^{2+}$ currents remained elevated when tested at postnatal day 15, which then elicited robust exocytosis, albeit with reduced efficiency. Ribbon synapses were formed despite the TH-deficiency. However, different from wt, where synapse elimination takes place around the onset of hearing, the number of ribbon synapses remained elevated in 2-week-old $Pax8^{-/-}$ IHCs. Moreover, the ultrastructure of these synapses appeared immature. Using quantitative RT-PCR we found a TH-dependent developmental upregulation of the mRNAs for the neuronal SNARE proteins SNAP25 and synaptobrevin 1 in the organ of Corti. These molecular changes probably contribute to the improvement of exocytosis efficiency in mature IHCs. IHCs of 2-week-old $Pax8^{-/-}$ mice maintained the normally temporary efferent innervation further indicating an impaired postnatal IHC development. Moreover, they lacked functional large conductance $Ca^{2+}$ activated $K^{+}$ channels and $KCNQ_4$ channels and consequently fired action potentials. We conclude that TH regulates IHC differentiation and is essential for morphological and functional maturation of their ribbon synapses. We suggest that presynaptic dysfunction of IHCs is a mechanism in congenital hypothyroid deafness.

# Imaging of mRNA in dendrites: transport and interacting factors of the mRNA coding for the postsynaptic Shank1 protein

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Shank proteins are scaffolding components in the postsynaptic density of excitatory synapses in the mammalian brain. The mRNAs coding for Shank proteins were detected in dendrites of various neuronal populations by in situ hybridization. A dendritic targeting element (DTE) in the 3' untranslated region of Shank1 was recently identified by our group. The GFP/MS2 bacteriophage tagging system was used to visualize Shank1 mRNA transport to distal hippocampal dendrites in cultured neurons. Therefore a reporter mRNA was designed consisting of mRFP fused to the 3'UTR of Shank1 and the MS2 binding sites, which are recognized with a high affinity by a cotransfected GFP-MS2-NLS fusion protein. mRNA particles observed by live imaging moved unidirectional retrograde, anterograde or in the majority of cases saltatory. The average speed of translocations lies in a range of 1,3-6µm/min and agrees with published data for slow dendritic transport of other dendritic mRNAs or mRNA transporting proteins. The identity of the GFP granules was also verified by in situ hybridizations. By using various inhibitors, we show that Shank1 mRNA transport into dendrites is a microtubule dependent process.

# Regulation of the Interaction between Shank and Sharpin

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The Shank family of proteins are large scaffold proteins of the post-synaptic density (PSD), which contain many known protein interaction domains: PDZ, SH3, SAM, and ankyrin repeats. These domains interact with other post-synaptic proteins such as SAPAP/GKAP, Densin-180, Homer, IRSp53, etc., in order to provide the linkage between the different glutamate receptor complexes of the PSD and the actin cytoskeleton of the dendritic spine.

Sharpin is one of the proteins known to interact with the ankyrin repeat region of Shank. We detected a novel intramolecular interaction in the N-terminus of the Shank protein which apparently controls the this interaction, such that the interaction between Shank and Sharpin is hardly detectable in cells expressing both proteins. We have tested various pharmicological treatments for their ability to facilitate the confirmational change which allows for the subsequent interaction between Shank and Sharpin. Our data indicate that post-translational modifications are required to enable efficient interactions between these two proteins at the synapse.

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# Targeted expression of fluorescent proteins in layer 5b neurons reveals a strongly depressing corticothalamic giant synapse imparting low-pass filtering of cortical inputs

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Layer 5 (L5) and 6 (L6) corticofugal neurons give rise to direct feedback projections to at least three different thalamic nuclei. We used targeted expression of fluorescent proteins in L5B neurons to identify large presynaptic terminals (2-8µm) of these neurons synapsing onto neurons in the posterior medial thalamic nucleus (POM) (Hoogland et al., 1991). AAV particles encoding fluroescent proteins were delivered stereotaxically into layer 5B of the barrel cortex and resulted in a spatially restricted and highly efficient infection of pyramidal neurons *in vivo*. Labelled presynaptic terminals within the POM were localized in acute brain slices using 2-photon microscopy. Due to their juxtasomatic localization the postsynaptic neuron could be identified and patch-clamp recordings could be established. Because the presynaptic terminal and its axon were labelled with GFP, fluroescence-guided local stimulation could be used to elicit unitary postsynaptic currents. We recorded a robust glutamatergic postsynaptic current with fast kinetics composed of both AMPA and NMDA receptor components. Excitatory postsynaptic currents (EPSCs) showed pronounced short-term-depression (STD) during high frequency stimulation. We observed a high initial release probability, consistent with a mechanism of synaptic vesicle depletion during repetitive stimulation. Voltage recordings revealed that unitary synaptic responses were capable of eliciting action potentials (APs) in the thalamic neuron. We studied the transfer function of the L5B-POM synapse by stimulating the presynaptic terminals with *in vivo*-like firing patterns of L5B neurons, consisting of short ~100 Hz bursts with varying numbers (1-5) of APs. The cortico-thalamic synapse acted as a low pass filter in that only the first EPSP elicited an AP in the POM neuron.

During whole cell recordings we dialyzed fluorescent dyes into the POM neurons allowing us to generate 3D reconstructions of its morphology. These neurons are large in size, often exhibiting somatic diameters of 10-25 µm and having 3-6 large proximal dendrites. The 3D reconstructions of simultaneously labelled pre- and postsynaptic cells demonstrated that most of the giant presynaptic terminals were located in close proximity (10 µm) to the soma (Hoogland et al., 1991). In most cases they formed on the thick primary dendrites. Hence, these morphological features allow tight electrotonic control of the somatic membrane potential, and therefore, control of AP initiation.

In summary, the results suggest that the L5B-POM giant synapse located close to the soma of thalamic neurons provides a means to excite these neurons by a single AP in L5B neruons, while preventing relay of repetitive APs.

# Effects of glutamine in cultured and CA1 pyramidal cells.

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Glutamine is one of the most abundant amino acids in the brain extracellular space. One important physiological role of this amino acid is thought to be the generation of the neurotransmitter glutamate through the glutamine/glutamate cycle in glia cells and neurons. Recently, it was found that glutamine can play an important role for GABA-synthesis, serving as a precursor for glutamate which is subsequently converted into GABA in terminals of inhibitory interneurons (Liang et al., J Neurosci.26(33). p 8537-48,2006). Alternatively, glutamate can be accumulated in inhibitory terminals through membrane glutamate carriers. The relative importance of glutamine- versus glutamate-uptake for GABA supply is not well known and might vary between different brain regions due to the heterogeneous expression pattern of the respective transporters and enzymes specific for glutamine/glutamate cycle. Some GABAergic interneurons in hippocampal area CA1 do express SA2 glutamine transporters at high level. We therefore studied effects of glutamine on dissociated rat hippocampal cell cultures and on inhibitory transmission in CA1 in slice preparations from juvenile (p 14-16) mice.

Cells were recorded with CsCl symmetrical intracellular chloride solution. Extracellular solution contained CNQX, APV, CGP, TTX and $CdCl_2$. Following bath application of 2 mM glutamine, the majority of cultured cells (17 of 24) displayed inward currents at -70 mV membrane potential. The amplitude of those currents as well as the time to peak varied in a wide range: 50-2000 pA and 30-300s respectively. Nevertheless, independent of their amplitude, these currents were completely blocked by 4 μM gabazine. The voltage-to-current relationship for glutamine-induced currents in all recorded cells was very specific with an intermediate conductance maximum at -30 mV and was distinct from that evoked by direct application of GABA. Thus, glutamine induces a voltage-dependent conductance change in cultured hipocampal neurons which differs from $GABA_A$ receptor-mediated currents and might involve glutamine transporters (SA1 or SA2).

In order to assess the role of glutamine for GABAergic transmission, we investigated the effect of 2 mM glutamine on miniature inhibitory postsynaptic currents in CA1 pyramidal cells. Preliminary results indicate that glutamine is able to increase the amplitudes of mIPSCs without changing the interevent intervals of minis in this area. This finding supports a role for glutamine in regulating presynaptic GABA levels in inhibitory interneurons.

The extracellular space of the mammalian brain contains glutamine at high concentration (around 0.5 mM). Our data indicate that this amino acid may serve a dual role in determining excitation/inhibition balance, first by direct activation of specific membrane conductance and, secondly, by acting as a precursor for the synthesis of GABA in inhibitory interneurons.

# Development of the neuronal microcircuitry in layer 4 of rat barrel cortex

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The 'barrel field' in layer 4 (L4) of rodent somatosensory cortex is a topographical representation of the structural organisation of the whisker hairs on the rodent's muzzle. At the end of the first postnatal week, shortly after corticogenesis is completed, cortical barrels are formed. This is a time of profound developmental changes in cell morphology, connectivity and function. We have studied these changes using whole-cell voltage recordings with simultaneous biocytin fillings in acute brain slices of rats aged 4 to 21 postnatal days (P4-P21). Neurons were subsequently fixed and processed and morphologically reconstructed using NEUROLUCIDA software.

Around the period of barrel formation (around P5) virtually all L4 spiny neurons have pyramidal-like morphology: At P4-P6, 90% of L4 spiny neurons possess a prominent apical dendrite and symmetrically oriented basal dendrites. The apical dendrite is gradually lost with age until the majority of neurons shows the adult spiny stellate-type morphology. Axonal arbours of L4 spiny neurons are sparse at P4-P8 with long-range collaterals projecting over several barrel columns, in particular in deeper layers (L5 and L6). In the second and third postnatal week the axonal arbour becomes more dense and confined to the barrel column with few if any long-range collaterals.

Intensive dye coupling between L4 spiny neurons was frequently observed at P4-P8 but was confined to a cortical barrel. Dye coupling decreased with age and was no longer observed after P14. Paired recordings from P4-P10 L4 spiny neurons revealed non-rectifying electrical coupling with a coupling coefficient of 0.04±0.01. EM analysis revealed dendritic and somatic gap junctions as the structural basis of electrical coupling.

Spontaneous synaptic potentials were observed as early as P4; synaptically coupled pairs were recorded shortly after barrel formation at P6-P8. The first connections to appear are L4 interneurons projecting onto L4 spiny neurons; L4 spiny neuron pairs were formed later. While the GABAergic synapses established by L4 interneurons onto L4 spiny neurons are highly reliable excitatory synapses even at P6, the excitatory synapses established between two L4 spiny neurons were initially rather weak and immature. This is supported by EM analysis that revealed small presynaptic terminals with few vesicles around P6. With increasing age synaptic transmission becomes more reliable and the terminals obtain a more mature appearance.

The data suggest a sequence of developmental changes in the structure and function of the L4 microcircuitry that proceeds from gap junction-coupled neuronal networks to synaptic-coupled networks at low density and of weak reliability at first; during maturation synaptic coupling increases in density and becomes more reliable.

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# The morphology of excitatory central synapses: From structure to function

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Synapses are the key elements for signal transduction and plasticity in the brain. For a better understanding of the functional signal cascades underlying synaptic transmission a quantitative morphological analysis of the pre- and postsynaptic structures that represent morphological correlates for synaptic transmission is important. In particular, realistic values of the number, distribution and geometry of synaptic contacts and the organization of the pool of synaptic vesicles provide important constraints not only for realistic models but also numerical simulations of those parameters of synaptic transmission that, at present, are still not accessible to experiment. Although all synapses are composed of nearly the same structural elements it is, however, their actual composition within a given synapse and the microcircuit in which they are embedded that determines its function.

One possible way to analyse synapses in sufficient detail are computer-assisted three-dimensional reconstructions of these structures and their subsequent quantitative analysis based on ultrathin serial sections. Here, we summarize, compare and discuss the morphology of three central excitatory synapses: the so-called Calyx of Held, a giant synapse in the medial nucleus of the trapezoid body (MNTB) in the auditory brain stem, the Mossy Fiber Bouton (MFB) in the hippocampus predominantly terminating on proximal dendrites of CA3 pyramidal neurons and cortical input synapses found on basal dendrites of layer 5 pyramidal cells.

The detailed morphological description of synaptic structures beside describing their geometry may help to define morphological correlates of functional parameters of synaptic transmission such as the number, distribution and size of synaptic contacts at active zones, the readily releasable pool (RRP) of synaptic vesicles, for release and the variability of quantal size and might therefore explain existing differences in the function between individual synapses embedded in different microcircuits.

# Morphology of spiny stellate cells in ephrinA5 deficient mice

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During neuronal development, dendrites form extensive arborisations that are covered with motile filopodial protrusions. After these dendritic filopodia establish connections with axonal partners, they are replaced by dendritic spines, which represent the postsynaptic compartment for the majority of excitatory synapses. Spine morphogenesis depends on one hand on interactions between postsynaptic spine and presynaptic axon, as mediated by cell surface receptors (e.g. Eph receptors) and on the other hand the regulation of spine morphology is dependent on neuronal activity. In our previous studies with mice lacking ephrinA5 we found reduced arborisation of thalamic axons, which normally project to the somatosensory cortex and a stronger gene expression of ephrinA2 in the layer 4. In the present study we examined the consequences of the ephrin-A5 knockout and reduced thalamic input on spiny stellate cells, the target neurons of thalamocortical projections. Using ballistic delivery of particles coated with lipophilic dyes in fixed slices with a gene gun ("diolistic labeling") and a confocal laser-scanning-microscope, we were able to quantitatively analyze the morphology of these neurons. At postnatal day 8 (P8) the dendrites from mutant cells exhibit more filopodia and are more branched than the dendrites from wild-type cells. In contrast, there is no difference in the extend of the dendritic tree between knockout and wild-type animals. Later during development, at P23, dendrites of mutant cells are still more branched, but now the spine density is reduced. These results suggest that at early stages layer 4 spiny stellate cells appear to compensate the reduced thalamic input by increasing dendritic branching and the density of filopodia. However, while at adult stages the dendrites of mutant layer 4 neurons are still more branched, the spine density is now lower than in wild-type cells. Taken together, these data demonstrate, that the morphology of spiny stellate cells is shaped by the thalamic input and Eph receptor signalling. (Supported by the IZKF Jena)

# Disruption of activin receptor signaling in forebrain neurons alters GABAergic neurotransmission in a behaviorally relevant fashion

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Activins belong to the transforming growth factor β (TGFβ) superfamily of growth and differentiation factors. Originally discovered as inducers of follicle-stimulating hormone release, activins are now recognized as multifunctional regulatory proteins with a broad spectrum of effects in many tissues and organs including the CNS. To gain insight into the physiological function of activin in the mature brain, we had generated transgenic mice expressing a kinase-deficient, dominant-negative mutant of activin receptor IB (dnActRIB) in forebrain neurons under the control of the CaMKIIalpha promoter (JBC 281:29076-84, 2006). Hippocampal slices were prepared from wild-type (wt) and two lines of transgenic (tg) mice 2 - 4 months old. Whole-cell recordings from CA1 pyramidal cells showed that disruption of activin signaling did not alter amplitude and kinetics of GABA(A)-receptor-mediated IPSCs. However, two striking abnormalities of inhibitory transmission were noted in CA1 pyramidal neurons from dnActRIB mice: 1) Tonic inhibition through GABA(A) receptors was significantly increased in a TTX-sensitive fashion. 2) The characteristic enhancement by diazepam of GABA(A) receptor-mediated IPSCs was strongly diminished. To explore the behavioral significance of altered GABAergic neurotransmission on anxiety-like behavior, wt and tg mice were examined in conflict paradigms, in which the drive to explore a novel environment is opposed by its potentially threatening and aversive appearance. The conflict between approach and avoidance is widely used to explore anxiety-related behavior and is sensitive to the anxiolytic effects of benzodiazepines. In the open field, wt and tg mice did not differ in spontaneous locomotion and exploration behavior. However, tg mice visited inner fields significantly more often than wt mice. In the light-dark exploration test, tg mice spent significantly more time on a well-lit elevated bar than wt mice. As predicted by the reduced effect of diazepam on IPSCs in tg hippocampi, the anxiolytic effect of diazepam was much stronger in wt mice than in tg mice.

In conclusion, our data suggest that disruption of activin receptor signaling alters GABAergic neurotransmission in a fashion that is likely to account for the low-anxiety phenotype on the behavioral level.

# GABA spillover and asynchronous release mediate persistent inhibition in the DNLL

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When a sound is produced in a reverberant environment, the multiple reflections (echoes) from the nearby surfaces potentially compromise accurate localization. To circumvent this problem, the auditory system uses only the first (direct) wave front for sound localization and suppresses the spatial information of sounds lagging a few milliseconds. There is evidence that suppression of spatial information of lagging sounds occurs in the dorsal nucleus of the lateral lemniscus (DNLL) and is due to GABAergic inhibition which persist several milliseconds beyond the end of stimulation (Pollak et al. 2003, TINS 26 (1):33-9). The mechanisms causing the persistent inhibition (PI) in an otherwise temporally very precise binaural circuit are unknown. One possibility is that PI is due to the properties of the synaptic transmission of the GABAergic input itself and not by any network properties.

To test this hypothesis we performed whole-cell patch-clamp recordings from visually identified DNLL neurons in acute brain slices from Mongolian gerbils (P14-P17). GABAergic IPSCs (inhibitory post-synaptic currents) were evoked by stimulating the GABAergic inputs (the commissure of Probst providing inputs from the opposite DNLL) with a bipolar-electrode.

The amplitude of single evoked IPSCs depended linearly on the strength of stimulation for stimulation strengths between 0 and 100 V. The maximum evoked IPSC was -0.85 ± 0.14 nA (n=9) in average. Fitting the decay time of the IPSCs revealed an overall increase of about 10 ms over the voltage range of the fiber stimulations. The same dependency was met by analyzing the decay half time of the same IPSCs. However, the rise time and time to peak of evoked IPSCs did not change. Pairing two successive stimulation pulses at 10 or 20 Hz indicated that the phasic components of the IPSCs are mainly independent from each other, since their charge and peak amplitude added nearly in a linear manner. As for single pulse stimulation, the decay time and half width of the second IPSC of the pair of pulses depended on the strength of fiber stimulation. Furthermore, in some cells there was an apparent increase in the miniIPSC frequency after the fiber stimulation.

These results can be explained by spillover from GABAergic nerve terminals onto DNLL principal cells, as well as by a component of asynchronous release prolonging the action of synaptic currents and reducing the firing in DNLL neurons.

# The presynaptic cytomatrix protein Bassoon interacts with DLC1, a component of motor complexes.

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In neurons, transport processes are of particular importance. Proteins and membranes are transported from the cell body to outgrowing neurites and specific proteins are enriched at or depleted from sites of nascent synaptic contacts during early development. In mature neurons, tremendous membrane translocation occurs during synaptic transmission and bidirectional specific transport is required for a variety of processes including synaptic maintenance and plasticity. Here, we report the discovery of an interaction between dynein light chain 1 (DLC1) and the presynaptic protein Bassoon. Bassoon is a core component of the cytomatrix at the active zone of neurotransmitter release (CAZ). Its particular importance during synaptic transmission has been demonstrated recently in a study of the Bassoon deficient mice. Bassoon is recruited to nascent synapses very early during synaptogenesis and together with Piccolo associates with Piccolo-Bassoon transport vesicles (PTVs). PTVs carry a whole set of proteins that function in spatial organization of synaptic vesicles, their priming and fusion. The fusion of 2-3 PTVs with the neuronal plasma membrane may rapidly form a functional release site. DLC1 was found to associate with both, dynein and myosin motor complexes. Although the exact function of Bassoon-DLC1 interaction remains elusive, the direct link of Bassoon to actin- and microtubuli-based transport processes is very intriguing. Currently we examine the role of this interaction in processes like neurite outgrowth, synaptogenesis and synaptic function.

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# Specialized synaptic contacts in the polarization vision pathway of the locust brain

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For spatial orientation, especially during seasonal migrations and homing, the sun is an important compass cue for many animals. In addition to direct sunlight, insects can also detect the polarization pattern of the blue sky and use it for navigation. In the desert locust *Schistocerca gregaria*, like in many other insect species, polarization vision is mediated through a specialized dorsal rim area of the compound eye. Polarization vision pathways in the locust brain involve the anterior optic tubercle (AOTu), the lateral accessory lobe, and the central complex.

Here we report on highly unusual synaptic contacts in the lateral accessory lobe. Double immunofluorescent labelling experiments showed that neurons of the AOTu form direct synaptic contacts with tangential neurons of the central complex. Presynaptic elements from the AOTu end in a small number of large cup-shaped terminals (at an average of 9.5 µm in diameter) that enclose numerous small GABA-immunoreactive (-ir) profiles. The GABA-ir neurons project to the lower division of the central body. Electron microscopic studies show that each cup-shaped profile makes numerous (>150) dyadic output synapses with the small postsynaptic GABA-ir profiles. We conclude that neurons of the AOTu make particularly strong and possibly divergent synaptic contacts with follower neurons to the lower division of the central body. [Supported by DFG grant HO 950/16-1]

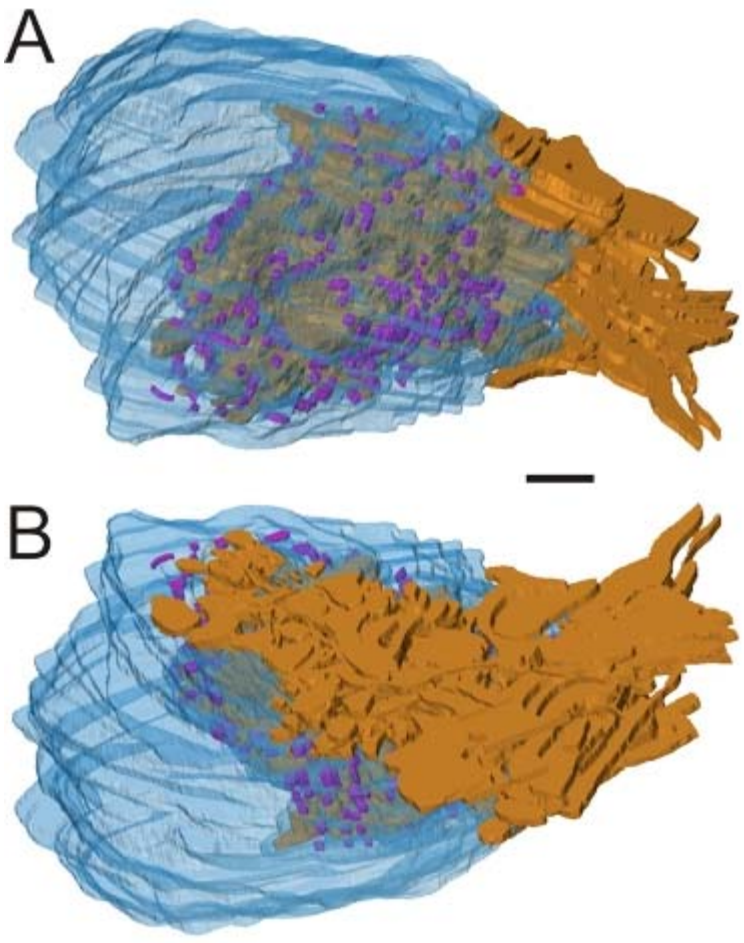


**Fig. 1** Three-dimensional reconstruction (surface views; **B** is rotated 180° to **A**) of a synaptic complex in the lateral triangle of the lateral accessory lobe. The presynaptic element (transparent-blue), originating in the anterior optic tubercle, encloses several small postsynaptic profiles (orange). More than 150 synaptic contact zones (purple) exist between the presynaptic cup and the small postsynaptic profiles. Scale bar = 500 nm.

# Protein recruitment and disappearance during constitutive endocytosis in PC12 cells

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Constitutive endocytosis is mediated by interactions between structural, enzymatic proteins and the plasma membrane. In fibroblasts the sequence of protein recruitment and disappearance to the spot of constitutive endocytosis was described (Merrifield et al. 2003) and the presence of proteins was functionally implicated (Bensch et al. 2005). Important proteins found to be present during such endocytotic events are clathrin, dynamin, actin and actin recruiting proteins. Besides the action during endocytosis actin and its meshwork are also of structural relevance. Other cells than fibroblasts, for example PC12 cells, consist of a differently organized cell cortex build of a permanent and tight actin-meshwork. Therefore, it is questioned, if structural proteins like actin are not necessarily recruited during endocytosis but nevertheless; due to their abundance interact in similar fashion as in fibroblasts.

The spatial behavior of proteins close to the plasma membrane can be visualized using total-internal-reflection-fluorescence-microscopy (TIRF-M). To determine the sequence of protein recruitment to endocytotic spots in PC12-GR5 cells, fluorescently labeled proteins were over-expressed and observed in the evenanescent field of TIRF-M. Endocytotic events were detected by the temporal profile of either marker protein clathrin-light-chain-a-DsRed (clathrin-DsRed) or Dynamin-1 in conjunction with EGFP or mCherry.

It was found using the endocytosis marker clathrin-DsRed together either with dynamin-1-EGFP, transferrin-receptor-pHluorin (TfR-pHluorin), vamp-pHluorin and EGFP-β-actin that constitutive endocytosis in PC12 cells is using a similar protein machinery than fibroblasts. However, in contrast to fibroblasts dynamin-1-EGFP seems not to be recruited shortly before endocytosis but co-localizes with clathrin-DsRed before hand. Further, the onset of decay of dynamin-1-EGFP fluorescence lags behind the onset of fluorescence decay of clathrin-DsRed. More rapid, transient changes in dynamin-1-EGFP fluorescence were also apparent in PC12 cells but did not coincide with clathrin mediated endocytosis. EGFP-β-actin appeared to be slowly recruited, over a time period of ~ 50 s and its fluorescence decayed after clathrin-DsRed fluorescence loss was apparent. To judge the sequence of fluorescence loss, comparison to dynamin-1 as endocytosis marker was performed. TfR-pHluorin, vamp-pHluorin and mCherry-β-actin showed similar recruitment and disappearance using dynamin-1 as marker compared to clathrin-DsRed. Such similar behavior was only evident in dynamin-1 spots that were in stable over 30 s and not in transient dynamin-1 fluorescence spots. In dynamin-1 detected endocytotic events β-actin was recruited slowly and its onset of fluorescence decay was before the onset of dynamin-1 fluorescence loss. This indicates first that despite their abundance structural proteins such as actin are still recruited to endocytotic regions. Further, that the sequence of protein disappearance in respect to endocytosis markers is likely to differ between PC12 cells and fibroblasts. Hence the endocytotic pathways using the same protein-tools might still differ in there protein interaction sequence.

# Synapsin, SAP47, Bruchpilot: Functional analysis of presynaptic proteins in *Drosophila*

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We are using the genetic model organism Drosophila to investigate the functional role of three conserved proteins at the presynaptic terminal.

Synapsins are proposed to modulate transmitter release by controlling the transfer of synaptic vesicles from the reserve pool to the readily releasable pool in a phosphorylation dependent manner. Synapsin null mutants display deficits in various learning paradigms (Godenschwege et al., 2004; Michels et al., 2005; Michels et al., this volume). We now find that the conserved cAMP dependent protein kinase (PKA) phosphorylation target site near the N-terminus of the protein is modified from RRFS to RGFS by pre-mRNA editing effected by the ADAR enzyme. We demonstrate that an undeca-peptide containing the genomic sequence including RRFS represents an excellent substrate for bovine PKA while the cDNA encoded peptide with the RGFS site is not significantly phosphorylated. Studies on the influence of this editing on learning and memory are under way.

Synapse associated protein of 47 kDa (SAP47) belongs to a conserved protein family of unknown function (Reichmuth et al., 1996). In Drosophila SAP47 is located at synaptic vesicles. Sap47 null mutants show no obvious impairment in viability or fertility (Funk et al., 2004) and basic synaptic transmission at larval neuromuscular junctions appears normal. However, this mutation causes a significant deficiency in larval associative learning while sensory and motor faculties required for this task are normal (Saumweber et al., this volume). We now are investigating a possible interaction between SAP47 and synapsin which could explain the similarity in the phenotypes of the two null mutants.

We recently have identified and characterized the Bruchpilot protein as an essential structural component of the presynaptic active zone of Drosophila. Suppression of this protein by RNAi blocks the formation of presynaptic T-shaped ribbons (T-bars) at photoreceptor terminals, leads to semi-lethality, and in escapers causes severe behavioural impairments including unstable flight (hence Bruchpilot = German for crash pilot) (Wagh et al., 2006). In a screen for genes which interact with the bruchpilot gene we identified a mutation that causes aggregates of BRP to form in axons of larval motor neurons. The corresponding gene codes for several isoforms of a serine/threonine protein kinase which therefore is tentatively termed Bruchpilot related protein kinase (BRPK). The Brpk gene is presently being characterized. GFP-labeled BRPK co-localizes with BRP at active zones. A direct interaction at the active zone may therefore take place. Behavioral assays provided additional evidence for a possible interaction between the two proteins. The hypomorphic mutation of BRPK leads to similar behavioral deficits in adult flies as observed for BRP-RNAi-knock-down animals. The BRPK mutant flies are also impaired in flying and in their locomotion, and in addition they display a dramatically reduced lifespan compared to wild-type flies.

# The thalamocortical synapse in cat visual cortex.

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Every neuron in area 17 of the cat sits in a cloud of hundreds of millions of synapses per mm3, only ~1%(1) of all these synapses are provided by the dorsal lateral geniculate nucleus (dLGN) axons. While many of the boutons and axons providing these synapses contact a given neuron, only a fraction of them actually forms synapses with the neuron. In this study we investigate how different types of cortical neurons lying in layer 4 and 6 of area 17 of the cat are contacted by the dLGN afferent and where in their dendritic arbour the thalamic synapses are formed.

The thalamic projection was labeled in vivo by injections of Biotinylated Dextran-amine (BDA) into layer A of the dorsal lateral geniculate nucleus (dLGN) of anaesthetized cats. After recovery of one week, the cats were again anaesthetized and single neurons were recorded in area 17 and filled intracellularly with horseradish peroxidase. After processing, the brain was cut serially and prepared for light and electron microscopy (LM & EM). The density of dLGN boutons and synapses were measured with disector methods and the locations of possible contacts between the dLGN axons and labeled cortical neurons were noted in 3-D LM reconstructions.

From the quantitative EM we find that the dLGN provides only 2% to 5% of all asymmetric synapses in layer 4 (n=3 cats). The LM reconstructions show that the dLGN axon contacts spines and dendritic shafts of spiny stellate neurons (n=4), almost exclusively the dendritic shafts of star pyramidal neurons (n=2) and the proximal dendrites of inhibitory neurons (n=1). Most of the LM contacts made by dLGN afferents into layer 6 pyramidal neurons (n=5) are in their basal dendrites, suggesting that dLGN axons do not connect to layer 6 pyramidal neurons in direct proportion of its available dendrite in the neuropil. Subsequently examination of these contacts between dLGN afferents and target neurons with EM show that only a proportion of them form synapses with each other. In the spiny stellate neuron only 35% of the investigated contacts were synapses. Sixty eight percent of the contacts investigated in the basal dendrites of layer 6 pyramidal neurons were synapses, while only 1 out of 9 formed synapses in its apical dendrite in layer 4. All of the synapses were formed with spines.

The LM reconstructions show that while many thalamic axons come close (<1µm) to the dendrites of target neurons, they do not connect. Those that do, usually form only one synapse at the point of contact. This proximity creates the opportunity for local selection and rearrangement of connections during development. Our results also suggest that dLGN axon connect to layer 6 pyramidal neurons mostly in their basal dendrites.

(1)Binzegger, T, Douglas, RJ and Martin, KAC, Journal of Neuroscience,2004, 24, 8441-8453

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# Imaging structural plasticity of hippocampal CA3-CA1 synapses

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Activity-dependent changes in the synaptic connectivity of hippocampal neurons are thought to be the key cellular substrate for learning and memory. In recent years it has become possible to examine the structural changes at the level of single synapses that accompany changes during long-term potentiation and depression (LTP and LTD). While many studies have examined plasticity-associated structural changes on the postsynaptic side of the synapse, relatively little is known about the role of presynaptic boutons in synaptic plasticity, let alone how presynaptic bouton dynamics relate to the plasticity of dendritic spines. Therefore, we set out to image the structural dynamics of pairs of presynaptic boutons and dendritic spines in the plasticity paradigm of LTD, which we have previously shown to lead to the retraction of spines on CA1 pyramidal neurons.

We used time-lapse two-photon laser scanning microscopy and extracellular field recordings to simultaneously monitor synaptic morphology and activity for up to 5 h. This approach allowed us to relate the structural plasticity we observed with the expression of LTD. CA3 and CA1 pyramidal neurons were labeled with two spectrally distinct fluorescent dyes in mouse organotypic hippocampal slice cultures. While electrically stimulating the labeled CA3 neurons, field recordings and time-lapse imaging were performed in the target region of the labeled axons overlapping with labeled CA1 dendrites. An immunohistological approach was used to confirm the existence of functional synapses at the presynaptic sites.

We found that LTD induction leads to a net loss of putative synaptic contacts, while also increasing their turnover rate compared with unstimulated control conditions. Contact loss between individual pairs of boutons and spines was much more frequently due to the bouton rather than the spine disappearing. While the loss of a bouton was not associated with a change in spine volume or motility, spine loss correlated strongly with an increase in motility as well as a decrease in volume of the associated bouton. The formation of new contacts influenced neither volume nor motility of the corresponding synaptic partner. In a separate analysis, we assessed the rates of bouton and spine turnover. The turnover rate for boutons was significantly higher than that for spines. Interestingly, the turnover rate for boutons was much higher than for contacts, while the turnover rate for spines was lower.

Our experimental approach is well suited to study structural synaptic plasticity on both sides of the synapses simultaneously and it is shedding new light on how spines and boutons interact to express synaptic plasticity.

# Poster Topic

## T7: Signal transduction cascades

**T7-1A** The first intravesicular domain of vesicular monoamine transporters serves as receptor-like structure in G-protein mediated regulation of monoamine storage
*C. Blex, I. Brunk, S. Rachakonda, S. Winter, M. Höltje, D. Walther and G. Ahnert-Hilger, Berlin*

**T7-2A** Metabolic changes in the brain of transgenic mice expressing activated Ras in neurons using multinuclear NMR spectroscopy
*S. Gottfried, C. Zwingmann, K. Kuteykin-Templyakov, D. Leibfritz and R. Heumann, Bochum and Bremen*

**T7-3A** *Is Rheb a negative regulator of the Ras-Raf-MAPK-pathway in neurons?*
*S. Karassek and R. Heumann, Bochum*

**T7-4A** Characterization of putative intracellular interactors of the cell adhesion proteins Rst and Kirre in *D. melanogaster*
*R. Braun, J. Shi, S. Völker and KF. Fischbach, Freiburg*

**T7-5A** Evaluation of TGF-β target genes in primary cortical and hippocampal neurons
*S. Ahrens, T. Vogel and K. Krieglstein, Göttingen*

**T7-1B** Detecting absolute cAMP levels in neurons: a new method of analyzing fluorescence ratio measurements of FRET based sensors
*PS. Salonikidis, A. Zeug, F. Kobe and DW. Richter, Göttingen*

**T7-2B** Toll-like receptor 4 mediates the microglial inflammatory response to thrombin-associated protein aggregates
*J. Scheffel, D. van Rossum, J. Weinstein, T. Möller, W. Brück, M. Prinz and UK. Hanisch, Göttingen and Seattle (USA)*

**T7-3B** Differential Analysis of the ErbB-Signaling Network Using *Split-TEV*
*MC. Wehr, A. Botvinnik, KA. Nave and MJ. Rossner, Göttingen*

**T7-4B** Glutamate-regulated shuttling of Foxo3a-GFP in hippocampal neurons
*O. Dick and H. Bading, Heidelberg*

**T7-5B** Signalling properties of a hormonal system that regulates various behaviours in Caenorhabditis elegans
*T. Roeder and M. Seifert, Kiel and Freiburg*

**T7-1C** Subcellular location of phosphorylated Smads is disordered in Alzheimer´s disease
*U. Ueberham, E. Ueberham, H. Gruschka and T. Arendt, Leipzig*

**T7-2C** A polymorphism linked to bipolar affective disorder does not alter the CRE activity of constitutively activated trace amine receptor 4
*J. Reiners, M. Schmidt, J. Packer, L. Unger and W. Wernet, Ludwigshafen and Abbott Park (USA)*

**T7-3C** Jacob is a cargo for synapse-to-nucleus communication via the classical importin pathway
*A. Karpova, I. Zdobnova, M. Mikhaylova, W. Zuschratter and MR. Kreutz, Magdeburg*

**T7-4C** Calneurons are a novel subfamily of neuronal Calcium sensor proteins that might regulate intracellular Ca2+-levels via the Phospholipase C-IP3 pathway
*M. Mikhaylova, T. Munsch, Y. Sharma, ED. Gundelfinger and MR. Kreutz, Magdeburg and Hyderabat (India)*

# The first intravesicular domain of vesicular monoamine transporters serves as receptor-like structure in G-protein mediated regulation of monoamine storage

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Variation of vesicular neurotransmitter content is one of the mechanisms influencing synaptic strength. Vesicular filling depends mainly on neurotransmitter transporter activity and storage capacity of the vesicle. Vesicular monoamine transport is down-regulated in neuroendocrine cells and neurons through activation of vesicle-associated α-subunits of heterotrimeric G-proteins. Our study aimed at investigating the upstream signal of activating this pathway using permeabilized CHO-cells transfected with wild-type and mutant VMAT-DNA. Transfected CHO cells express VMAT1 and VMAT2 as confirmed by western blot and immunofluorescence analysis. VMATs also co-localize with endogenously expressed Gαo2. Uptake experiments with permeabilized cells using tritiated serotonin further prove the expression of functional transporters. Serotonin-depleted thrombocytes of tryptophane hydroxylase (Tph1) lacking mice exerted no G-protein mediated down-regulation of monoamine uptake, indicating that regulation depends on vesicular filling. The same was observed for VMAT1 and VMAT2 expressing CHO cells. However, G-protein-mediated regulation can be induced after preincubating permeabilized VMAT1- and VMAT2-expressing CHO cells using several monoamines. Surprisingly, epinephrine induces inhibition in CHO-VMAT1 cells but not in CHO-VMAT2 cells. G-protein mediated regulation can also be induced after treatment with α1 and 5HT1B receptor agonists in VMAT1- or VMAT2-expressing CHO cells, respectively. Accordingly, antagonist against either α1 or 5HT1B receptors prevent G-protein mediated regulation in systems normally expressing VMAT1 (PC 12) or VMAT2 (small synaptic vesicles, platelets), respectively. In conclusion a lumenal facing structure, differing in both VMAT isoforms, senses the intravesicular monoamine content and initiates the subsequent activation of vesicle-associated Gαo2. The first intravesicular loop of the VMATs is part of this receptor-like structure as highlighted by experiments using mutant VMAT expressing CHO cells. Work is in progress to identify the cytosolic VMAT domains mediating G-protein activation.

# Metabolic changes in the brain of transgenic mice expressing activated Ras in neurons using multinuclear NMR spectroscopy

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Ras is an intracellular membrane-anchored GTP-binding protein signalling for cell proliferation and survival, whereby the activating mutation is often found in tumour tissues. The transgenic synRas mouse model expresses constitutively activated H-Ras (Val12-Ha-Ras) selectively in neurons. In the brain, this model exhibits a variety of phenotypes including prevention of lesion-induced neuronal degeneration, and neuronal hypertrophy resulting in an enlarged brain volume. Furthermore, there is an enhanced spine density which correlates with a 2-fold increased number of excitatory synapses. Our aim was to investigate if the above described Ras-mediated alterations in brain would be associated with changes in brain metabolism (Heumann et al. 2000 Journal of Cell Biology 151, 1537-1548). SynRas and wild type mice were injected with [U-13C6] glucose (500 mg/kg; i.p.) and sacrificed after 45 minutes. Whole brains were quickly snap-frozen in liquid nitrogen and subjected to a dual extraction method using perchloric acid and methanol/chloroform to obtain water-soluble metabolites and membrane components. Metabolite concentrations and metabolic pathways were investigated from high-resolution 1H-, 13C- and 31P-NMR spectra (Bruker DRX 600 MHz spectrometer). Significant changes between wild-type and transgenic mice were observed in the concentrations of lactate (+ 21 %) and taurine (+ 21 %) in 1H-NMR spectroscopy, 13C-NMR spectra revealed a 36% ± 6.0% activated de novo synthesis of glutamate through pyruvate dehydrogenase, a key enzyme of cerebral energy metabolism. Our result from 31P-NMR analysis showed that the very low concentrations in glycerophosphocholine and adenosine monophosphate (AMP) found in the brains of the wild type mice were increased by more than 20-fold in synRas mice (n = 4). The increase in concentration of phosphocreatine (+58% ± 17%) and adenosinediphosphate (+177% ± 79) were less pronounced but significant (T-Test). Interestingly, phosphoethanolamine, glycerophosphoethanolamine, and phosphocholine showed only minor changes while no significant changes were observed in lipid extracts, i.e. of fatty acids or phospholipids. In conclusion, the activation of glutamate de novo synthesis might reflect the elevated number of synapses leading to an overall enhancement of glutamate release (Arendt el. 2004, Europ. J. Neurosci. 19, 2953-2966). This is in line with higher lactate levels. In contrast to previous cell culture studies by others showing a Ras-dependent increase in phosphocholine, such changes were not found in the brain of synRas mice. The accumulation of glycerophosphocholine suggests an enhanced phospholipase A2 activity in SynRas mice. The high levels of AMP, ADP, and phosphocreatine indicate a deregulated energy homeostasis in SynRas mice which might point at previously unknown pathways in brain energy metabolism by neuronal Ras activity.

# *Is Rheb a negative regulator of the Ras-Raf-MAPK-pathway in neurons?*

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Rheb (Ras homologue enriched in brain) is a member of the Ras superfamily originally identified as an immediate early gene in brain. Like Ras, Rheb cycles between an active GTP-bound and inactive GDP-bound conformation which is regulated by tuberin/TSC1, a Rheb-GAP. In contrast to Ras, Rheb possesses higher basal activation levels which may result from an arginine residue at position 15 which is located in a homologue position to the glycine 12 of Ras. Several previous studies have shown that Rheb is involved in the mTOR/S6 pathway regulating protein translation and cell volume.

Comparison of the amino acid sequences between Rheb and H-Ras revealed a high sequence homology especially in the respective protein-effector binding regions. We have shown previously that constitutively activated Val12-Ras prevents lesion-induced neuronal degeneration in the developing or adult brain. Our aim was to investigate if Rheb is able to mimic the neurotrophin-like effect by Ras in neurons. Here, we generated various Rheb-mutants, i.e. in the effector region (N41D and T44R), in the "effector specificity" region (S34E) and in the nucleotide binding region (R15G, S16G and RS15,16G) thus transforming Rheb stepwise into Ras at critical amino acid positions. This allowed us to compare possible survival functions of Rheb and Ras in dissociated embryonic E8 chick dorsal root ganglion (DRG) neurons. In DRG neurons nerve growth factor-(NGF) mediated survival depends on the activation of the Ras-Raf-MAPK pathway. We show here that Rheb counteracts the pro-survival effect by NGF leading to neuronal degeneration. We further investigated this effect by Western Blot studies in transiently transfected mouse neuroblastoma HN10 cells. We found that Rheb significantly reduced the activation of the MAP-Kinase by 30% and the S34E Rheb-mutant decreased the MAP-Kinase activation even by 40%. This reduction was associated with a significant 2-fold activation of the caspase-3 by wt Rheb while the Rheb-S34E mutant promoted a 4-fold increase.

In summary, we demonstrate for the first time that Rheb inhibits the neurotrophin survival pathway in neurons and that this inhibition is associated with an activation of the caspase-3 suggesting a pro-apoptotic function by Rheb.

# Characterization of putative intracellular interactors of the cell adhesion proteins Rst and Kirre in *D. melanogaster*

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IrreC-rst and its paralogue Kirre belong to the immunoglobulin superfamily of cell adhesion proteins and are part of the **I**rre cell **R**ecognition **M**odule (IRM). They have manifold roles in *D. melanogaster* development such as spacing of ommatidia in the compound eye, axonal guidance in the optic lobe, development of the somatic musculature in the embryo and spacing of sensory organs on the antenna and anterior wing margin.
Although some information about the extracellular interactions mediated by the IRM proteins has accumulated, little is known about the intracellular mechanisms necessary to facilitate these development processes. While the cytoplasmic domains of IrreC-rst and Kirre are different in most parts, they share a PDZ motiv and a tyrosine phosphorylation site. We identified at least nine proteins that can bind to both intracellular domains and therefore are possibly involved in signalling processes. Here we present our data on some of these putative interaction partners with respect to their involvement in IRM mediated development.

# Evaluation of TGF-β target genes in primary cortical and hippocampal neurons

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Transforming growth factors beta (TGF-βs) are pleiotropic and multifunctional cytokines and mediate a wide range of biological activities in a context dependent manner.
It has been recently shown that TGF-βs play an important role in the developing nervous system in regulating proliferation, differentiation, survival and death of neural cells. Deregulation of the signalling pathways is also involved in several pathological conditions including neurodegenerative disorders.
Many of these TGF-β transmitted responses result from changes in the expression of key target genes.
In this study we aim to isolate and investigate downstream target genes that may mediate TGF-β activity in primary cortical and hippocampal cultures isolated from E18.5 mouse embryos. The expression levels of selected target genes are quantified by RT-PCR and real-time-PCR after treatment with exogenous TGF-β.
The identification of TGF-β regulated genes might provide us with new targets for the development of pharmacological drugs to treat different CNS pathologies.

# Detecting absolute cAMP levels in neurons: a new method of analyzing fluorescence ratio measurements of FRET based sensors

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Mainly two methods are in use to measure fast changes of intracellular cAMP-levels. One method consists in measuring time constants of ion currents through cAMP sensitive CNG- or HCN-channels using patch-clamp technique. This method allows gaining absolute values of cAMP concentration being predominant directly underneath the cell membrane of the patch or on average of the whole cell. But it is unable to detect differences in cAMP concentration over the entire cell body in order to investigate cAMP accumulation or spreading. An imaging method overcomes this deficiency by using FRET-based biosensors such as labelled PKA or EPAC proteins. FRET of these biosensors is commonly analysed by performing fluorescence ratio measurements using a single excitation wavelength. These results are dependent on the individual concentration of the sensor used. Thus only qualitative but not quantitative conclusions about changes in intracellular cAMP-levels can be obtained.

We introduce a way to analyse absolute values of cAMP concentrations on the basis of ratiometric fluorescence measurements. The fact that we use tandem constructs with a donor and acceptor stoichiometry of 1:1 as well as the fact that we perform the measurements at two instead of one excitation wavelength allows us to gain an absolute FRET-efficiency which can be calibrated towards cAMP-concentrations.

Applying this technique we are able to measure the distribution of the intracellular basal level of cAMP within a single cell and a population of intact cells. Thereby different measurements can be compared directly due to its quantitative nature. CAMP related signal processes including the intracellular cAMP dissemination can be monitored with an appropriate temporal resolution.

# Toll-like receptor 4 mediates the microglial inflammatory response to thrombin-associated protein aggregates

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The serine protease thrombin is an important blood coagulation factor and controls also diverse functions in various cell types, including neurons, astrocytes and microglia, by cleavage of protease-activated receptors (PAR). Recent studies revealed, however, that induction of proinflammatory cytokines and chemokines in microglia by thrombin preparations is not due to PAR activation or other proteolytic and non-proteolytic mechanisms involving thrombin proper, but to the presence of aggregated protein containing enzymatically inactive thrombin fragments. Based on several approaches and cells of homo- and heterozygous knockouts for crucial receptor/adapter molecules, we demonstrate that the microglial release response is triggered by activation of Toll-like receptor 4 (TLR4), requires CD14 and involves the MyD88-dependent pathway, whereas TLR2 and TRIF are not mandatory. An LPS contamination as a carrier of the effect could be ruled out. These findings may enforce a critical re-interpretation of previous studies on thrombin's cellular activities as well as assigned signaling routes and suggest a microglial mechanism of protein aggregate recognition.

# Differential Analysis of the ErbB-Signaling Network Using *Split-TEV*

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Signaling by ErbB receptor tyrosine kinases (RTKs) regulates many cellular processes including neuronal differentiation and myelination. To signal downstream, ErbB receptors must homo- or heterodimerize. Whereas the ErbB2 receptor has no functional binding domain and transmits signals without stimulus, ErbB3 and ErbB4 dimerization is dependent on neuregulin (Nrg) binding. The Nrgs comprise a gene family of four genes including various isoforms based on alternative splicing and promoter usage.

To test for differential signaling effects within the Nrg-ErbB signaling network, we applied the newly described Split-TEV system. Here, inactive fragments of the NIa proteinase from the tobacco etch virus (TEV protease) were engineered that regain proteolytic activity only when co-expressed as fusion constructs with interacting proteins. Functional reconstitution of TEV protease fragments can be monitored either with 'proteolysis-only' or with transcription-coupled' reporters, located either at the membrane or in the cytosol.

To decipher the ErbB signaling network using our approach, dimerizations of ErbB2, ErbB3 and ErbB4 and interactions of the receptors with adapter proteins, such as Grb2, Shc, the regulatory subunit of PI3K (PI3Kp85) and STATs were monitored. The Nrg1 isoforms tested showed a differentially regulatory effect on receptor dimerizations and receptor-adapter protein interactions.

# Glutamate-regulated shuttling of Foxo3a-GFP in hippocampal neurons

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In healthy cells, the forkhead transcription factor Foxo3a is bound to 14-3-3 proteins and localizes to the cytoplasm. Growth factor withdrawal for several hours leads to a translocation of Foxo3a to the nucleus where it is thought to induce the expression of pro-apoptotic genes. Using cultured rat hippocampal neurons transfected with an expression vector for Foxo3a-GFP, we investigated the role of glutamate receptor activation in the control of Foxo3a-GFP localization. Upon bath application of glutamate, Foxo3a-GFP translocated from the cytoplasm to the nucleus; the NMDA receptor antagonist, APV, blocked nuclear translocation of Foxo3a-GFP. Compared to the effects of growth factor withdrawal on Foxo3a-GFP trafficking, glutamate-induced translocation of Foxo3a-GFP was much faster and started within minutes of the stimulation. Synaptic activity and calcium flux through synaptic NMDA receptors, which is neuro-protective, can prevent the bath glutamate-induced nuclear translocation of Foxo3a-GFP. The mechanism underlying the modulation by synaptic activity of nucleo-cytoplasmic shuttling of Foxo3a-GFP in hippocampal neurons is currently being studied.

# Signalling properties of a hormonal system that regulates various behaviours in Caenorhabditis elegans

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Octopamine and tyramine, invertebrate homologues of noradrenaline, control a broad variety of behaviours in insects. We show that these compounds control a well-coordinated set of behaviours in the nematode C. elegans that allows adaptation to different physiological needs. These behavioural changes are accompanied by matched metabolic adaptations. Among the behaviours controlled by this invertebrate adrenergic system are egg laying, locomotion and the cycle frequency of the ultradian defecation rhythm. All effects are consistent with OA/TAs role as a stress hormone that triggers this progenitor of the fight or flight response. In addition, blockade of OA/TAergic neurotransmission induces obesity, pointing to an important role in the control of metabolism during starvation. The underlying intracellular pathway is parallel to the one identified for the behavioural changes, but splits downstream of the PLC. We propose a model in which OA and TA act as general stress hormones released under unfavourable conditions. Increased hormone levels trigger a set of behaviours and metabolic adaptations that enable the animal to overcome those conditions.

# Subcellular location of phosphorylated Smads is disordered in Alzheimer´s disease

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Various factors and cytokines as for example transforming growth factor beta 1 (TGF-β1) are elevated in Alzheimer´s disease (AD) giving rise to activated intracellular mitogenic signaling cascades. Activated mitogenic signaling involving the mitogen-activated protein kinases (MAPKs) and other protein kinases might alter the phosphorylation states of structural proteins such as tau resulting in hyperphosphorylated deposits. Many intracellular signaling proteins itself are potential targets of misregulated phosphorylation and dephosphorylation. Recently, a crosstalk between MAPKs and Smad proteins, both involved in mediating TGF-β1 signaling, has been reported. Although TGF-β1 has previously been shown to be involved in the pathogenesis of AD the role of Smad proteins has not been investigated. In this study we, thus, analysed the subcellular distribution of phosphorylated Smad2 and Smad3 in the hippocampus of both normal and AD brains. Here we report on strong nuclear detection of phosphorylated Smad2 and Smad3 in neurons of control brains. In AD brains these phosphorylated proteins were additionally found in cytoplasmic granules in hippocampal neurons, within amyloid plaques and attached to neurofibrillary tangles. Our data suggest a critical role of Smad proteins in the pathogenesis of AD.

# A polymorphism linked to bipolar affective disorder does not alter the CRE activity of constitutively activated trace amine receptor 4

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Altered levels of trace amines have been observed in neuropsychiatric disorders such as schizophrenia and bipolar affective disorder (BPAD) and therefore are of particular interest in this field. So far two G-protein coupled receptors (GPCR) that bind trace amines have been identified, which has led to the name "trace amine receptors" (TA) being applied to an extended receptor subfamily with multiple members, and several pseudogenes, in both humans and rodents. The endogenous ligands for the remaining TA family members are still not known, therefore they are considered orphan GPCRs. Recently a study was published linking a polymorphism in the human trace amine receptor 4 (TA4, also known as TRAR4 or TAAR6) to BPAD. More controversially, an association of TA4 to schizophrenia is discussed. These observations make TA4 a potential target for neuropsychiatric disorders.

The generation of constitutively active receptors (CAMs) by mutagenesis has been shown to enable the study of receptor function in the absence of knowledge of the physiological ligand. We applied this approach to TA4. Independent activity assays demonstrated that a CAM TA4 activates the CREB pathway. We analyzed the effect of the potential BPAD mutation on the activity of TA4. The results indicate that the BPAD mutation induces no significant difference in the overall activity of CAM TA4. Our approach shows that CAM receptors can be used to analyze the effect of potential disease mutations on orphan receptor activity without knowledge of the endogenous ligand, where otherwise inferences can only be made on basis of the sequence differences revealed by linkage studies.

# Jacob is a cargo for synapse-to-nucleus communication via the classical importin pathway

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Calcium seems to be the pivotal messenger coupling NMDA receptor-mediated synaptic activity to nuclear gene transcription and calcium-dependent signaling from the synapse to the nucleus can result in long-lasting changes of synaptic inputs and dendritic cytoarchitecture. The neuronal cytoskeleton-associated calcium sensor protein Caldendrin, which is highly enriched in the PSD and has high similarity to Calmodulin binds to a newly identified protein named Jacob. Jacob is abundant in limbic brain and cortex. It displays a similar distribution to that of Caldendrin in dendritic spines and dendrites; particularly they both are tightly associated with the PSD. In contrast to Caldendrin Jacob has also been found in neuronal nuclei. Nuclear immunoreactivity of Jacob increases upon stimulation of excitatory synapses with NMDA and glutamate/glycine (GG) which could be abolished with coincubation of AP5 indicating that activation of NMDA receptors is crucial for the recruitment of Jacob to neuronal nuclei. The nuclear localization of Jacob results in drastically reduced complexity of the dendritic tree and number of contacts, whereas its extranuclear localisation is accompanied by a formation of PSD-like structures.

We examined activity dependent nuclear translocation of Jacob and its temporal dynamic in living cells using quantitative fluorescence time-lapse confocal microscopy alone or in combination with a selective qualitative photobleaching technique on hippocampal primary neurons transfected with GFP tagged wild-type (WT)-Jacob and with deletion mutant ΔNLS-Jacob constructs. Stimulation with glutamate (50µM) WT-Jacob-GFP overexpressed cultures resulted in an increase of GFP fluorescence in nuclei that was accompanied by a reduction of fluorescent intensity in dendrites. Without stimulation no fluorescent changes in nuclei as well as in somas and dendrites was observed. It has been shown previously that upon synaptic stimulation importins translocate from the synapse to the nucleus. Jacob`s primary structure exhibits a well-conserved bipartite nuclear localization signal (NLS) and we could show that importinα binds WT-Jacob but not ΔNLS mutant. Caldendrin competes with Importinα - Jacob binding in calcium dependent manner. The overexpressed deletion mutant ΔNLS-Jacob-GFP exhibits an extranuclear localization. Application of glutamate on transfected with ΔNLS-Jacob-GFP neurons does not change the GFP fluorescence level in nuclei and in dendrites over the time suggesting that the presence of the binding site for Importinα (bipartite NLS) is a prerequisite of Jacob`s nuclear accumulation.

These data define a novel NMDA receptor dependent pathway for synapse to nucleus communication via the classical importin pathway. Moreover, Jacob is the first cargo identified for this pathway. Thus, the control of Jacob`s subcellular localisation has important consequences for synapto-dendritic cytoarchitecture.

# Calneurons are a novel subfamily of neuronal Calcium sensor proteins that might regulate intracellular Ca2+-levels via the Phospholipase C-IP3 pathway

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The binding of $Ca^{2+}$ to neuronal calcium sensor proteins leads to the transformation of discrete $Ca^{2+}$ signals into long-lasting changes in neuronal signaling which directly influence synaptic transmission and plasticity processes. Members of Caldendrin/CaBP family of neuronal Calcium binding proteins may play an important role in these processes. In this study we introduce a new subfamily EF-hand calcium sensor proteins named Calneurons that apart from Calmodulin represent the closest homologues of Caldendrin in brain. CalneuronI and II are highly homologues to each other and to 100% conserved between different mammalian species. Calneurons have a different EF-hand organization than other calcium sensor proteins (EF1 and 2 have functional calcium binding sites whereas EF3 and 4 are cryptic), are prominently expressed in neurons and bind $Ca^{2+}$ with higher affinity than Caldendrin. CalneuronI exist as a long and short isoform and is highly enriched in cerebellum but also present in cortex and hippocampus. This is in contrast to the expression of Calneuron II, which is restricted to the CA3 region of the hippocampus, the entorhinal cortex, the antero-dorsal and antero-ventral thalamus as well as the inferior and superior colliculus. Upon subcellular fractionation we found that Calneurons are largely cytosolic proteins that in contrast to Caldendrin are only loosely associated with synaptosomes and PSD. Calneurons built homo- and hetero-dimmers with each other as well as with Caldendrin. In functional terms they seem to be multivalent regulators of intracellular $Ca^{2+}$-levels via their interacting with proteins coupled to the Phospholipase C-$IP_3$ signaling pathway. Thus, CalneuronI and II can modulate release of $Ca^{2+}$ from intracellular stores not only by binding to the $InsP^3R$ but also via an interaction with the M3 type of muscarinic acetylcholine receptors upstream of this pathway.

# Poster Topic

## T8: Neurotransmitters

**T8-1A** Ancient neuroarchitectural features of the bilaterian brain
*R. Loesel and C. Heuer, Aachen*

**T8-2A** Plasticity in mouse cerebellar circuits with selectively modified GABA-A receptors
*P. Wulff, DA. Anderson, CI. De Zeeuw, M. Farrant, T. Goetz, L. Kelly, ER. Korpi, E. Leppa, AM. Linden, M. Renzi, M. Schonewille and W. Wisden, Aberdeen (UK), London (UK), Rotterdam (NL) and Helsinki (FIN)*

**T8-3A** Modulated monoamine storage in Gαo2-/- mice alters behavioural responses following cocaine but not amphetamine treatment
*I. Brunk, C. Sanchis-Segura, C. Blex, J. Sternberg, S. Perreau-Lenz, H. Hörtnagl, R. Spanagel and G. Ahnert-Hilger, Berlin and Mannheim*

**T8-4A** Brain NOS activity regulates the mating-related behavioral states of grasshoppers
*R. Heinrich, A. Wirmer and A. Weinrich, Göttingen*

**T8-5A** Hippocampal dysplasia and aberrations in cholinergic and catecholaminergic nuclei and their hippocampal projections in NCAM-deficient mice: Implications for behavior
*Y. Tereshchenko, J. Brandewiede, F. Morellini, A. Dityatev, M. Schachner and A. Irintchev, Hamburg*

**T8-1B** GABA-like immunoreactivity in the CNS of the stick insect *Carausius morosus*
*S. Marasigan, A. Büschges, T. Mentel and M. Gruhn, Cologne*

**T8-2B** The influence of ischemia/reperfusion on nNOS-immunoreactivity and NADPH-diaphorase activity in dorsal root ganglia and dorsal horns of different spinal cord lumbosacral segments in rabbits.
*A. Schreiberová, M. Lacková, D. Kolesár, N. Lukácová and J. Maršala, Košice (Slovakia)*

**T8-3B** Enrichment of the GABAergic subnetwork in cortical cultures strongly promotes higher-order activity patterns
*T. Baltz, T. Munsch, AD. de Lima and T. Voigt, Magdeburg*

**T8-4B** Electrophysiological characterization of different cell populations in embryonic neocortical cultures
*Y. Klyueva, S. Meis, A. de Lima, T. Voigt and T. Munsch, Magdeburg*

**T8-5B** Electrical responses of acinar cells in the salivary glands of *Periplaneta americana* to dopamine, serotonin and GABA.
*C. Rotte and B. Walz, Potsdam-Golm*

**T8-1C** GABAergic innervation of the cockroach salivary gland
*J. Witte, W. Blenau, B. Walz and O. Baumann, Potsdam-Golm*

**T8-2C** Increased acetylcholine release in prefrontal cortex of monkey during a cognitive task
*A. Rauch, X. Zhang, G. Rainer and N. Logothetis, Tübingen*

**T8-3C** Organization of the serotonergic system in the buccal region of pulmonate gastropods, *Lymnaea* and *Helix*
*G. Balog and K. Elekes, Tihany (H)*

**T8-4C** Nicotinic acetylcholine receptors in the honeybee brain: from molecular biology to physiology.
*V. RAYMOND DELPECH, G. BARBARA, A. JONES, L. GARREAU, S. OLIVEIRA, B. GRUNEWALD, M. Giurfa, D. SATTELLE and M. GAUTHIER, TOULOUSE (F), OXFORD (UK), and BERLIN*

**T8-5C** Functional dissection of the octopaminergic neurotransmitter system in ethanol induced behaviour in *Drosophila melanogaster*
*S. Hampel and H. Scholz, Würzburg*

# Ancient neuroarchitectural features of the bilaterian brain

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The current view of early metazoan phlyogeny suggests that the bilaterian bodyplan arose only once during evolution. This first urbilaterian animal was most likely equipped with an anterior ganglion - a brain - from which all brains of modern animals have diverged. Until recently, the ancestor of all bilaterian phyla was viewed as a very simple, maybe flatworm-like animal with an accordingly simple brain. New genetic evidence, however, demonstrated a multitude of homologous genes that are expressed in identical patterns in Drosophila and mice (e. g. Lichtneckert R, Reichert H (2005) Insights into the urbilaterian brain: conserved genetic patterning mechanisms in insect and vertebrate brain development. Heredity 94:1-13). Taken together these studies imply that the neuroarchitecture of the urbilaterian brain might have been more elaborate than previously assumed. If true, ancient architectural features might have been conserved during evolution and should be identifiable in distantly related modern animal phyla. In this study, a comparison of representatives of arthropods, onychophorans, annelids, and flatworms suggests that this is indeed the case. We present data that show that for example a well-developed mushroom body, comprising many thousand small diameter globuli (Kenyon) cells is present in arthropods as well as in annelids, two groups that represent distantly related protostome clades (Ecdysozoa and Lophotrochozoa).

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# Plasticity in mouse cerebellar circuits with selectively modified GABA-A receptors

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The brain has a rich diversity of GABA-A receptor subtypes - nearly twenty years after this discovery we still possess only a partial understanding of how this variety serves brain function [1]. Based on its well-defined circuit, clear assignations of receptor subtypes to cell types, and clear phenotypic readout, we reasoned that the cerebellar cortex offered an excellent site to genetically evaluate this receptor diversity. Fast GABAergic signaling is ubiquitous in this structure. Purkinje cells assemble synaptic alpha1/beta2/gamma2 receptors. Granule cells assemble synaptic alpha1/alpha6/beta/gamma2 and extrasynaptic alpha6/beta/delta receptors. In recent years we have generated a series of mice in which we selectively ablated (i) the extrasynaptic receptors on granule cells that confer tonic inhibition [2] (alpha6 gene knockout), (ii) synaptic GABA-A receptors on granule cells (gamma2 gene deletion with granule cell-specific Cre recombinase) ([3], unpublished); and (iii) synaptic GABA-A receptors on Purkinje cells (gamma2 gene deletion with Purkinje cell-specific Cre recombinase) (unpublished). These cell-type and adult selective gene deletions revealed an unexpected robustness and plasticity of cerebellar function when extrapolated to the whole animal level. By contrast, the cerebellar cortical circuits in these mice suffer profound disruptions. Removing gamma2 from granule cells removes the timing component of the inhibitory input from Golgi cells, predicted as essential for the cerebellum to carry out timing functions; removing GABA-A receptors from Purkinje cells destroys all feed-forward fast inhibition from molecular layer interneurons - which severely affect their firing pattern. Our studies have revealed surprising insights into the importance of fast inhibition for cerebellar cortical circuits.

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2. Brickley SG et al (2001), Nature 409, 88
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# Modulated monoamine storage in Gαo2-/- mice alters behavioural responses following cocaine but not amphetamine treatment

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Gαo subunits of heterotrimeric G-proteins belong to the most abundant proteins in mammalian brains. Mice lacking the two splice variants Gαo1 and Gαo2 suffer from severe neurological impairments and die a few weeks after birth. In contrast, Gαo2 -/- mice are in good health with a normal life span. In the past we showed that Gαo2 subunits located on synaptic vesicles inhibit the vesicular VMAT2-mediated uptake of monoamines like dopamine or serotonin. Using Gαo2 -/- mice we report here on the systemic and behavioural impact Gαo2 exerts on the monoaminergic system.

Synaptic vesicles of Gαo2-/- mice lack G-protein mediated regulation of monoamine storage. Furthermore vesicular monoamine uptake is significantly reduced. In accordance striatal dopamine levels are lower in Gαo2-/- mice compared to their wild-type littermates. Analysis of monoamine synthesizing and degrading enzymes revealed that tyrosine hydroxylase expression is decreased while tryptophan hydroxylase expression and monoaminooxidase activity are not altered. Dopamine plasma membrane transporter is equally expressed in wild-type and Gαo2 -/- mice. Surprisingly, VMAT2 expression is increased in brains of Gαo2 -/- mice compared to wild-type animals.

In order to detect behavioural differences animals were repeatedly treated with cocaine and amphetamine and subjected to sensitisation and conditioned place preference tests. While wild-type animals show sensitisation towards both drugs Gαo2 -/- mice lack sensitisation towards cocaine with no difference in sensitisation following amphetamine application. In conditioned place preference tests both genotypes behave similarly.

The absence of Gαo2 diminishes vesicular monoamine storage which probably leads to an increase of toxic cytosolic monoamines. Reduction of tyrosine hydroxylase and increase of VMAT2 expression compensate the cytosolic monoamine accumulation. That is why Gαo2 deletion mutants appear to be healthy as long as their monoaminergic system is kept in balance. Manipulations by amphetamine and cocaine treatment unravel differences between the two genotypes. The lack of sensitisation following cocaine may be explained by the slightly reduced dopamine levels. Amphetamine probably due to its various points of attack may overcome the reduced dopamine content thereby exerting the same effects in the Gαo2 -/- mice. In conclusion Gαo2 is important for the fine tuning of monoamine storage and modulates the susceptibility towards substances like amphetamine and cocaine.

# Brain NOS activity regulates the mating-related behavioral states of grasshoppers

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The gaseous neurotransmitter nitric oxide (NO) contributes to the processing of behaviorally relevant sensory information and the selection and coordinated performance of situation-specific behaviors, in both vertebrate and invertebrate species. An example suggesting that basal activity of the NO-generating enzyme NO-synthase (NOS) may modulate general thresholds to perform particular behaviors is the control of grasshopper acoustic communication mediated by neural circuits in the central body. This highly structured protocerebral neuropil appears to integrate multimodal sensory information relevant for mating-related (and other) behaviors to decide about time, pattern and intensity of sound production.
The central body of grasshoppers has been demonstrated to contain both NO-generating and NO-responsive neurons in a number of studies (e.g. Ott et al. 2004; Kurylas et al. 2005; Wenzel et al. 2005). Pharmacological studies on restrained but intact grasshoppers of the species *Chorthippus biguttulus* revealed that activation of NO/cGMP signaling pathways in the central body suppresses sound production in a reversible and dose-dependent manner (Wenzel et al. 2005). Recent cytochemical studies on grasshoppers exposed to situations that do not promote sound production revealed that a subpopulation of NADPH diaphorase-positive neurons displays intense anti-citrulline immunoreactivity, which is indicative of considerable NOS activity during the period preceding fixation of the nervous tissue. These neurons located in the pars intercerebralis might release NO into the central body neuropil to inhibit sound production in unfavorable situations. Very similar neurons have recently been described with great anatomical detail in the locust *Schistocerca gregaria* (Kurylas et al. 2005).
Basal activity of NOS in the central brain was also suggested by pharmacological studies. Blocking NOS activity within the central body by injection of aminoguanidine was sufficient to elicit species specific sound production, indicating that tonic NO-mediated inhibition may suppress singing in the restraint situation. In addition, systemic application of aminoguanidine to female grasshoppers increased the amount of spontaneous and male song-stimulated sound production. In contrast to male grasshoppers, sound production in females depends on the reproductive state, and spontaneous sound production is strictly correlated with high receptivity after prolonged periods without copulation and periodic oviposition (Loher and Huber 1964). Since experimental reduction of NOS activity induces sound production under those physiological conditions that normally suppress singing in females, NO/cGMP signaling may couple the reproductive state with neuronal functions that regulate the intrinsic threshold for this mating related behavior.

Kurylas et al. 2005, J Comp Neurol 484:206-223; Loher & Huber 1964, J Ins Physiol 10:13-36; Ott et al. 2004, Eur J Neurosci 20:1231-1244; Wenzel et al. 2005, J Comp Neurol 488:129-139

# Hippocampal dysplasia and aberrations in cholinergic and catecholaminergic nuclei and their hippocampal projections in NCAM-deficient mice: Implications for behavior

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The neural cell adhesion molecule (NCAM) is implicated in nervous system development and plasticity and its ablation in mice causes a range of functional abnormalities. We performed quantitative immunohistochemical analyses of catecholaminergic and cholinergic nuclei and their hippocampal projections in NCAM deficient (NCAM-/-) mice and wild-type (NCAM+/+) littermates with the aim to identify structural aberrations underlying abnormal functions. We found that total numbers of cholinergic neurons in the medial septa / diagonal band nuclear complex and dopaminergic neurons in the A9-A10 nuclei were by 18-27% lower in both young and adult (2- and 13-month-old, respectively) NCAM-/- mice versus NCAM+/+ littermates. Noradrenergic neurons in the locus coeruleus were not affected by the mutation. Despite deficient numbers of projecting neurons, length densities and total length of catecholaminergic and cholinergic axons in the hippocampus were normal in young NCAM-/- mice. In adult NCAM -/- mice, however, the abnormally small dentate gyrus was deficiently innervated by catecholaminergic fibers (by 27% lower fiber length compared to NCAM-/- littermates), while the cornu ammonis received abnormally higher cholinergic input (by 27% and 47% in CA1 and CA3, respectively). These results indicate early developmental loss of cholinergic and dopaminergic cells in NCAM-/- mice and differential age-related effects of the mutation on the two types of hippocampal projections. Analysis of behavioral data from morphologically analyzed adult mice revealed physiologically plausible correlations between morphological variables and parameters related to novelty-induced behavior and anxiety indicating the importance of developmentally regulated cell interactions in shaping behavioral phenotypes.

# GABA-like immunoreactivity in the CNS of the stick insect *Carausius morosus*

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The stick insect *Carausius morosus* has been successfully used to study walking in hexapod animals and it is known that walking is generated by the coordinated activity of at least three central pattern generators per leg controlling the different leg joints. Much is known on the location and function of motor neurons innervating the muscles of the stick insect leg. Three of these motor neurons are so called common inhibitors innervating multiple muscles. Central inhibitory neurons are an integral part of networks controlling walking pattern generation in insects, for example by providing synaptic drive to terminate activity at phase transitions (1). The identity of these neurons is as yet unknown. The inhibitory transmitter in insects is γ-amino-butyric acid (GABA). Therefore, we set out to describe the populations of neurons in the CNS of the stick insect that show α-GABA immunoreactivity in order to gain insight in the location of putative inhibitory neurons that may be involved in the control of walking pattern generation.

Within the three thoracic ganglia of the stick insect there are at least four paired populations and one centrally located population of GABA-immunoreactive neurons. They consist of a lateral anterior group (LAG) and a lateral ventral group (LVG), which are both located anteriorly in each hemiganglion. In addition there is a single medial ventral group (MVG), located centrally. In the posterior half of each hemiganglion there are two additional groups of neurons, a medial posterior group (MPG) and a lateral dorsal group (LDG). Single neurons are rarely observed. These populations of neurons resemble those found in the locust (2). Backfilling of the leg nerves, nervus cruris and nl3 was used to identify the common inhibitor neurons C2 and C3 (ncr) as well as C1 (nl3).

The map of GABA-immunoreactive neurons presented here should facilitate the search for more inhibitory neurons involved in the walking pattern generating circuits of the stick insect.

# The influence of ischemia/reperfusion on nNOS-immunoreactivity and NADPH-diaphorase activity in dorsal root ganglia and dorsal horns of different spinal cord lumbosacral segments in rabbits.

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Nitric oxid (NO), diffusible and highly reactive gaseous molecule is constitutively expressed by Ca2+-dependent neuronal isoform of nitric oxide synthase (nNOS) and acts as an important non-conventional neurotransmitter in the central and peripheral nervous system. However, under pathologic conditions the activation of Ca2+-dependent nNOS isoform results in overproduction of NO, which might have neuroprotective or neurotoxic effects on surrounding tissue.

This study was designed to investigate changes in nNOS expression using nNOS immunohistochemical and/or NADPH-d histochemical visualization in dorsal horn neurons of laminae I, II, III and dorsal root ganglia (DRGs) neurons after ischemia/reperfusion injury to lumbar and sacral spinal cord. We used rabbits (n = 12) of both sexes weighing 2.5-3.5 kg in the experiment. Spinal cord ischemia was induced by abdominal aorta occlusion using Fogarty catheter introduced into the left femoral artery for a period of 15 min followed by 7 and 14 days of reperfusion. We found that the number of nNOS-immunoreactivity (IR) and/or NADPH-d positive neurons and the density of neuropil were markedly increased after 15 min ischemia followed by both 7 and 14 days of reperfusion at corresponding dorsal horn laminae compared to control animals. We evaluated the intensity of nNOS-IR neurons in DRGs sections of control and ischemic animals, using software UTHSCSA Image Tool densitometry analysis of the immunopositivity of the neurons. The increased nNOS-IR was also detected by densitometrical analysis in ischemia lumbar and sacral spinal cord DRG cells.

Based on our findings we tentatively assumed that higher amount of NO synthesized during ischemia-reperfusion injury to the lumbar and sacral spinal cord contributes to the genesis of neurological impairment observed in experimental animals, however, further experiments are needed to definitely confirm this suggestion.

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# Enrichment of the GABAergic subnetwork in cortical cultures strongly promotes higher-order activity patterns

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Spontaneous synchronous activity, i.e. brief periods (~1s) during which the firing rate of numerous cells exceeds many times the baseline firing rate, dominates neuronal activity in dissociated cortical cultures. Intracellular recordings showed that rhythmic bursting are slow depolarizing oscillations with superimposed trains of action potentials (Opitz et al. 2002, J Neurophysiol), thereby resembling hallmark features of slow rhythmic activity observed in cortical slices and *in vivo*. Earlier experiments showed a crucial role of a distinct subtype of GABA (γ-aminobutyric acid) immunoreactive neurons in the formation of synchronous burst activity in cortical cultures (Voigt and de Lima, 2001, J Neurosci). This subtype shares many features of the neocortical basket cells *in vivo*, including the widespread axonal arborization that targets proximal sites of postsynaptic neurons and the high density of excitatory inputs on their own soma and dendritic shafts (de Lima and Voigt, 1997, J Comp Neurol). By manipulating dissociation protocols we created two classes of cortical cultures with same total neuronal density, containing either high (+) or low (-) density of the basket-like GABA immunoreactive neurons. We investigated the spontaneous and, by electrical stimulation, evoked activity patterns with substrate integrated multi electrode arrays (MEAs). Both classes of networks developed synchronous rhythmic burst activity during the first week *in vitro*. Until the end of the second week *in vitro* spontaneous activity patterns consisted of stereotyped bursts with comparable burst duration and intra-burst frequency in both network types. In contrast, inter-burst frequency in GABA+ networks always exceeded the inter-burst frequency in GABA- networks. This difference increased markedly with further network maturation and GABA+ networks eventually developed a complex pattern of activity with various inter-bursts intervals and intra-burst frequencies, which were never observed in GABA- networks. Application of the competitive $GABA_A$-receptor antagonist bicucullin, switched GABA+ complex network behaviour to stereotyped bursting, but had little effect on GABA- networks. Low frequency (0.07 Hz) electrical stimulation evoked short direct responses in GABA+ networks, arising few milliseconds post stimulus, which were followed by bursts of action potentials. In GABA- networks evoked bursts had much greater latencies and were rarely observed. Preliminary whole-cell voltage clamp recordings of mature networks (> 5 wks.old) showed a complete lack of phasic inhibitory post synaptic currents (IPSCs) in GABA- networks, in contrast to large, long lasting bicuculline sensitive IPSCs in GABA+ networks. In conclusion, we showed marked differences in the development of activity patterns between, but little differences within the two classes of cortical cultures. These differences of network behaviour were strongly dependent on the presence of basket-like GABAergic neurons, suggesting that the control of GABAergic subnetwork development is crucial to enhance the reproducibility of physiological maturation in cultured network experiments.

# Electrophysiological characterization of different cell populations in embryonic neocortical cultures

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Embryonic cortical neurons in culture develop spontaneous synchronous activity at the beginning of the second week in vitro (8 DIV). Maturation of cultures is accompanied by an increase in the frequency of synchronous activity and in the number of cells involved. Different subpopulations of GABAergic interneurons (INs) seem to play a pivotal role in the generation and development of oscillatory activity (Voigt et al., J Neurosci, 2001). In order to elucidate their possible role in development of oscillatory activity, we prepared embryonic cultures from E16 knock-in mice expressing green fluorescent protein (GFP) under GAD67 promoter (Tamamaki et al., J Comp Neurol, 2003). In these cultures INs could be easily identified by wide-field fluorescence microscopy. Subsequent immunocytochemistry confirmed that all GFP-fluorescent cells were GABAergic. Identified INs and Glutamatergic principal neurones (PNs) were further characterized by whole cell voltage- and current-clamp recordings. INs (n=12) showed more positive resting potentials and fired action potentials in response to depolarising current steps with max frequency of 59±8 Hz without prominent adaptation. Recordings from PNs (n=26) revealed a much lower firing rate of 26±3 Hz. INs demonstrated smaller spike amplitude reduction and shorter spike width than PNs. Development of synaptic mechanisms recruiting INs and PNs into the recurrent activity were investigated by measuring spontaneous excitatory and inhibitory post synaptic currents (PSC). In INs both amplitude and frequency of PSC were higher than in PNs and increased with the age of culture, whereas amplitude of PSC in PNs stayed unchanged during development. The distinct firing properties and maturation of synapses in INs and PNs might be decisive for their physiological role in development of synchronous oscillations in neocortex.

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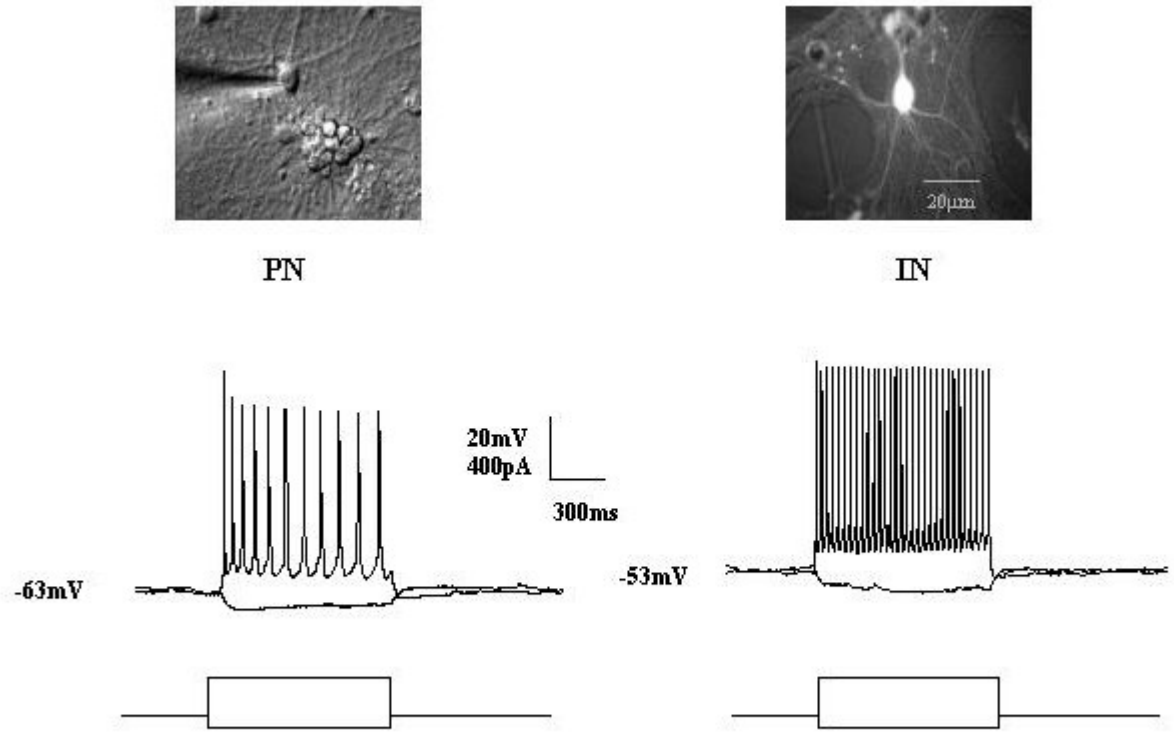

# Electrical responses of acinar cells in the salivary glands of *Periplaneta americana* to dopamine, serotonin and GABA.

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The cockroach *Periplaneta americana* has acinar salivary glands. The secretory acini consist of c-cells that are responsible for electrolyte and water secretion and p-cells that secrete protein into the saliva. The glands are innervated by dopaminergic (DA), serotonergic (5-HT) and GABAergic nerve fibres. These surround the acini and the ducts. DA stimulates the secretion of a protein-free saliva and 5-HT the secretion of protein-containing saliva. It is poorly understood in which way above neurotransmitters stimulate the secretory activity of the two acinar cell types. We started to study the electrical responses of acinar cells in isolated glands to bath application of DA, 5-HT and GABA. We found: DA and 5-HT produced changes in membrane potential of the acinar cells in *Periplaneta* salivary glands that were more complex than the changes described previously for *Nauphoeta* salivary glands. *Periplaneta* acinar cells had a resting potential of -48 + 4 mV (n=43). A 2-min pulse of 1 µM DA produced a brief hyperpolarization followed, after DA washout, by a prolonged afterdepolarization of 21 + 4 mV (EC50 = 16 nM DA). 5-HT produced similar changes in membrane potential, and bath application of GABA had no effect. We are currently studying whether electrical stimulation via the salivary nerve produces similar changes in membrane potential of the acinar cells and the ionic basis of the electrical responses described above.

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# GABAergic innervation of the cockroach salivary gland

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The salivary glands in the cockroach Periplaneta americana consist of an extensive duct system and grape-like acini formed by two main cell types, peripheral cells (P-cells) and central cells (C-cells). Salivation is controlled by a pair of large dopaminergic neurons (SN1) with the cell body in the suboesophageal ganglion, and by several smaller serotonergic neurons with their somata in the suboesophageal ganglion and the stomatogastric nervous system (1,2). In addition, there is another paired neuron (SN2) in the suboesophageal ganglion that projects towards the salivary gland and that is neither dopaminergic nor serotonergic. By immunofluorescence labelling with anti-GABA antibodies, we demonstrate that SN2 has GABA-like immunoreactivity. The axon of SN2 extends through the salivary duct nerve towards the acinar tissue of the salivary gland. There, it ramifies over the acinar lobules, forming a sparse network on the outer surface of the acini. In a minor number of acinar lobules, anti-GABA-positive nerve fibers extend into the tissue, terminating among C-cells. GABA-positive nerve endings do not abut serotonergic or dopaminergic release sites, suggesting that anti-GABA-immunoreactive fibers do not form presynaptic contacts with other nerve fibers on the salivary gland. Co-staining with anti-synapsin indicates that anti-GABA-immunoreactive release sites reside close to P- and C-cells, and in nerves that link acinar lobules. The duct system of the salivary gland is not associated with GABA-positive fibers. These findings suggest that cockroach salivary glands are innervated by GABAergic SN2. The functional significance of this GABAergic innervation is still elusive.
(Supported by Deutsche Forschungsgemeinschaft grant BL 469/4)

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2) Baumann O, Kühnel D, Dames P, Walz B (2004) J Exp Biol 207, 2565-2575

# Increased acetylcholine release in prefrontal cortex of monkey during a cognitive task

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Acetylcholine (Ach) is believed to play a major role in attentional processing in cortex, thereby contributing to memory formation and maintenance. To understand its role in these neuronal processes, we set out to monitor ACh level-changes in prefrontal cortex of a monkey performing a delayed match to sample task. The changes were monitored during a phase of rest and during the task to compare the baseline level of Ach with the level during task. We used a push and pull method to sample extracellular brain fluid (EBF) from the prefrontal cortex (PFC) at very low flow rates, low nanol/min range. These flow rates were chosen to reduce the depletion of the neuronal environment in contrast to the conventionally used microdialysis. After the sampling of the EBF, the capillary, where the EBF samples were stored, was directly coupled to capillary high performance liquid chromatography-mass spectrometry (HPLC-MS) for the chemical analysis to detect and quantify ACh. The combination of the sampling and the analysis system proofed possible to determine amounts of Ach in the attomole range from the EBF samples. In the experiments a 7-min interval was chosen to monitor the Ach changes during rest and the task. We could reliably detect and quantify Ach concentrations during rest and the delayed match to sample task, showing a significant increase in Ach concentration during the task. Our results show clearly the involvement of acetylcholine in behaving monkeys involved in a delayed match to sample task, where memory formation and attention are mandatory for the appropriate performance during the task.

# Organization of the serotonergic system in the buccal region of pulmonate gastropods, *Lymnaea* and *Helix*

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Serotonin (5-HT) is a general modulator in the snail nervous system, proven by series of physiological and pharmacological data. It was also demonstrated that 5-HT plays a principal role in regulating feeding behavior of gastropods. Key member of the central pattern generator system of feeding network is a pair of giant 5-HTergic neurons located in the cerebral ganglia, which was shown to affect inter- and efferent neurons in the buccal ganglia, and also to project to the buccal mass and lip musculature, as well the salivary gland. Although the distribution of 5-HT immunoreactive (IR) neurons was described in details in the CNS of *Lymnaea stagnalis* and *Helix pomatia*, two prominent gastropod model animals, little attention has been paid to the organization of the 5-HTergic system in the buccal ganglia and the buccal mass, responsible for the execution of active feeding. Therefore, in this study, we analyzed the chemical-neuroanatomy of a particular aspect of the 5-HT-IR innervation of the buccal ganglion: the possible form(s) of axo-somatic connection. In addition, the 5-HT-IR innervation pattern of the buccal mass musculature was also investigated. Two-step fluorescence or three-step PAP immunocytochemistry was applied on cryostat or Vibratome sections.

According to our results, the nerve cell body layer of the buccal ganglia was richly supplied by 5-HT-immunoreactive (IR) processes. Neural perikarya were surrounded individually mostly by a single immunolabeled process, displaying a number of varicosities. This type of 5-HT-IR axo-somatic innervation was quite prominent in the case of nerve cell bodies of larger size, including the identified giant B1-4 efferent neurons. In a part of the preparations or at certain section levels, the entire cell body population appeared to be innervated by 5-HT-IR fibers. These fibers originated from the buccal ganglion neuropil containing a dense arrangement of 5-HT-IR axon processes as a result of the profuse branching of the primary axon of the identified giant 5-HTergic neuron (CGC-*Lymnaea*/MGC-*Helix*). Part of the 5-HT-IR processes, meanwhile surrounding the neural perikarya, projected also towards the surface of ganglion. In the buccal mass, 5-HT-IR bundles entering arborized first into smaller axon branches or individual axon processes running between the muscle bundles, which thereafter sent thin varicose elements to the muscle cells. "End-plate"- or "spider"-like forms of terminal branching were also observed. Both the longitudinal and circular muscle bundles displayed the same 5-HT-IR innervation pattern. At ultrastructural level, labeled varicosities were found to form unspecialized, close (16-20 nm) contacts with muscle fibers.

Our findings suggest that the 5-HT-IR axo-somatic innervation of neural perikarya in the buccal ganglia represent an additional, yet unknown, level of 5-HTergic regulation of feeding, in addition to the central axo-axonic (neuropil) and peripheral neuromuscular contacts. At neuromuscular contacts, 5-HT may act as a "fast" neuromodulator.

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# Nicotinic acetylcholine receptors in the honeybee brain: from molecular biology to physiology.

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In invertebrates, acetylcholine (ACh) is the main neurotransmitter and nicotinic acetylcholine receptors (nAChRs) mediate fast cholinergic synaptic transmission. Honeybee's nAChRs are targets of the neonicotinoid insecticides although the honeybee is one of the most beneficial insects for its role in crop pollination. It is also a valuable model system for studies on social interaction, sensory processing, learning and memory.

In the honeybee, ACh is the main neurotransmitter of the olfactory circuit and nAChRs are expressed in the olfactory centres of the brain, the antennal lobes and the mushroom bodies (Thany et al., 2003, 2005). Acetylcholine is therefore involved in olfactory learning. Olfactory stimulation leads to acetylcholine release and to the activation of nAChRs. In vertebrates, such receptors are formed of an assembly of so-called αand β subunits but in insects, the molecular composition and the pharmacological properties of these receptors are unknown.

Voltage-clamp recordings in whole-cell configuration have been performed on isolated adult antennal lobe cells in culture. In most of the recorded cells, applications of ACh elicited fast inward currents. Nicotinic agonists induced responses typical for classical nAChRs. Nicotine (NIC) and imidacloprid (IMI) elicited 45% and 43%, respectively, of the maximum ACh-induced currents. The ACh-elicited currents were blocked by well-known nicotinic antagonists methyllycaconitine (MLA), dihydroxy-β-erythroidine (DHE) and α-bungarotoxin (BGT). We also demonstrated that these nAChRs are non selective cationic permeable channels. All these data demonstrated the existence of functional nAChRs on adult antennal lobe cells.

The bee genome information allowed us to characterize the complete honeybee nAChR gene family. Nine α and 2 β subunits have been identified, each of which could potentially combine to the others to form functional nAChRs.

For a better understanding of the role of the nAChRs in olfactory learning and memory, we induced silencing of the Amelα8 subunit using the technique of RNA interference (RNAi). RNA was injected into the brain and the protein expression was evaluated at different times after injection. A significant decrease of the Amelα8 protein was observed 6 hours after injection. The effect of the protein suppression on olfactory learning was evaluated using 3 trial olfactory conditioning of the proboscis extension reflex using a discriminative procedure. RNA injection was performed 6 h before the learning session or the memory test. Alpha8 RNAi induced an impairment of olfactory discrimination and a transient memory loss followed by memory recovery at longer delays. We conclude that α8 subunit enters in the composition of nicotinic receptors involved in olfactory discrimination and retrieval processes.

Thany et al. (2003) Insect Mol. Biol., 12:255-262.
Thany et al. (2005) Gene, 344:125-132

# Functional dissection of the octopaminergic neurotransmitter system in ethanol induced behaviour in *Drosophila melanogaster*

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In invertebrates octopamine is a widely used neurotransmitter, neurohormone and neuromodulator that plays an important role in a wide variety of behaviours. It is thought that octopamine functions in similar ways as noradrenaline in vertebrates. For Drosophila it has been shown to function in ethanol tolerance, sugar reward learning and egg laying. Our aim is to identify the neurons in the brain that specifically mediate ethanol tolerance. Octopamine is synthesized from tyramine by the enzyme Tyramine β-hydroxylase (TβH). Mutants in the *TβH* gene - $T\beta H^{nM18}$ - lack octopamine and show a reduced ethanol tolerance phenotype. To restore octopamine function in different subsets of neurons in the brain with the UAS/GAL4 system, we generated five different *TβH* -GAL4 driver lines. To generate these lines we used five different *TβH* promoter fragments. The different expression patterns of these GAL4 driver lines are analyzed and characterized with immunohistochemistry. In addition we inhibited neurotransmitter release in different subsets of octopaminergic neurons in the brain and tested these flies for ethanol tolerance and egg laying. We identified one subset of neurons that mediates ethanol tolerance. To test whether these neurons are required for ethanol tolerance we are currently restoring the *TβH* function in different subsets of octopaminergic neurons in the $T\beta H^{nM18}$ mutant background. Our results indicate that a specific subset of octopaminergic neurons mediates to ethanol tolerance.

# Poster Topic

## T9: Neuropeptides and Neuromodulation

**T9-1A** Effects of SDF-1α on the electrophysiology of rat cortex culture networks measured with Multielectrode Arrays (MEA)
*T. Wiegand, P. Küry and HW. Müller, Düsseldorf*

**T9-2A** The neuropeptide NAP provides neuroprotection after optic nerve crush and retinal ischemia
*C. Dimitriu, T. Jehle, S. Auer, R. Knoth, M. Vidal-Sanz, I. Gozes and WA. Lagrèze, Freiburg, Murcia (E) and Tel Aviv (Israel)*

**T9-3A** Neuromodulation: A modulated neural reactive microcircuit and a modulated bioluminescence
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*V. Tsintsadze, S. Melnik, M. Wright, J. Tanner, T. Tsintsadze, A. Miller and N. Lozovaya, Kyiv (Ukraine), London (UK) and Kyiv (UK)*

**T9-2B** Postsynaptic mechanisms underlying responsiveness of amygdaloid neurons to cholecystokinin are mediated by a transient receptor potential-like current
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*MJ. Szulc, PL. Mikolajczak, I. Okulicz-Kozaryn, E. Kaminska and T. Bobkiewicz-Kozlowska, Poznan (PL)*

**T9-3C** Characterization of pituitary adenylyl cyclase activating polypeptide (PACAP)-induced pial arteriolar dilation in piglets.
*L. Lenti, D. Kis, F. Domoki, G. Toth, DW. Busija and F. Bari, Szeged (H) and Winston-Salem (USA)*

# Effects of SDF-1α on the electrophysiology of rat cortex culture networks measured with Multielectrode Arrays (MEA)

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CXCR4 is a member of the G-protein-coupled receptor family and is expressed in neurons, astrocytes and microglia. Stromal cell-derived factor-1 (SDF-1α, CXCL12) is the only known ligand of CXCR4 and is expressed by astrocytes and neurons. SDF-1α dependent activation of CXCR4 was shown to affect cellular migration, regeneration and survival but can also modulate neuronal activity through regulation of intracellular calcium levels. In addition, CXCR4 can also serve as coreceptor for the entry of HIV-1 by enabling the major coat protein gp120 to attach to the target cell.

The aim of our work is to understand how SDF-1α affects the electrophysiology of a neuronal network cell culture with a high proportion of glial cells.

Applying immunocytochemistry we could demonstrate that the CXCR4 receptor is expressed in mixed cortical cell cultures (cryopreserved primary cortex cells from E18/19 rats) which generate functional cortical networks. We applied different concentrations of recombinant SDF-1α to such neuronal networks grown on multielectrode arrays and monitored their electrophysiological properties as a consequence of chemokine addition. The electrophysiological response of the cultures revealed to be complex and has therefore to be analysed with a defined and elaborated parameter set. Every channel has been classified individually with the following parameters: number of spikes, number of bursts, length of bursts, number of spikes in bursts, inter-burst-interval and frequency.

In addition we have also applied compounds known to block SDF-1 dependent effects and substances known to modulate the second messenger cascade.

Our results clearly demonstrate that apart from a general concentration dependent activity change (the increase of network acivity), a certain percentage of electrophysiologically active channels show non-synchronous variations in the electrophysiological pattern. We therefore conclude that the SDF1α/CXCR4 interaction results in an alteration of the activity of the neuron. In the next steps we will identify if these effects are direct effects on the neuronal cells or indirect effects mediated through the glial cells.

# The neuropeptide NAP provides neuroprotection after optic nerve crush and retinal ischemia

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Aims: NAP, an 8-amino acid peptide (NAPVSIPQ=Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln) derived from activity-dependent neuroprotective protein, plays an important role for neuronal differentiation and the surviving of neurons in different pathological situations. The axons of retinal ganglion cells (RGC) are directly affected by optic nerve trauma, stroke and glaucoma. We previously found that NAP increases the survival of RGCs in vitro and support neurite outgrowth in retinal explants at femtomolar concentrations. The aim of this pilot study was to investigate in adult rats the effects of NAP on RGC survival after optic nerve crush and transient retinal ischemia.

Methods: RGCs of male wistar rats (n = 9-16 per group) were labelled retrogradelly with 6 µl Fluorogold injected stereotactically in both superior colliculi. Seven days later the optic nerve is crushed at a distance of approximately 2 mm from the eye for 10 s using a custom made forceps after a partial orbitotomy or retinal ischemia was induced by elevation of intraocular pressure to 120 mm Hg for 60 minutes. NAP (100µg/kg) was injected intraperitoneally one day before, direct after and the first and the second day after the damage. The other group received the same concentration of a scrambled peptide respectively. Densities of surviving RGCs and activated glia cells (AGC) were counted masked ten days after damage by counting Fluorogold-labelled cells.

Results: After optic nerve crush the intraperitoneal administration of 100 µg/kg of NAP the number of surviving RGCs increased by 30.6% ($p=0.07$), whereas the number of AGCs was decreased by 29.1% ($p<0.0001$). After retinal ischemia the number of surviving RGCs was 40.2% ($p<0.005$) more than in the control group, and AGCs decreased by 45.5% ($p<0.0005$).

Conclusions: Treatment with the neuropeptide NAP supports neuronal protection after optic nerve crush and retinal ischemia in vivo.

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# Neuromodulation: A modulated neural reactive microcircuit and a modulated bioluminescence

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*Textbook chapters on neuromodulation are rare and often inconclusive, probably due to the fact that the vertebrate and mammalian nervous system is too complex in this context. For that reason our lab investigates on the one hand the recurrent inhibitory microcircuit of lateral inhibition in the auditory pathway of the cricket which is positive-modulated by octopamine and on the other hand the bioluminescence activated by serotonine in the light organs of the oceanic crustacean shrimp Meganyctiphanes norvegica.*

*The auditory pathway of the cricket has evolved to process the species specific sound signal, a pattern of sound syllables and intermissions. The resulting patterned neural activity is what neural reactive microcircuits, e.g. the excitatory reactive canonical microcircuit of neocortex, are able to process. In the inhibitory version of the reactive microcircuit as found e.g. in the recurrent lateral inhibition of the auditory pathway in the CNS of crickets, the modulator boosts activity of the feedbackloop in the circuit and thereby introduces a frequency dependency of lateral inhibition, resulting in improved localization of such sound sources which produce the pattern of sound syllables and neural activity which matches the inherent time constant of the specific activated recurrency.*

*Calibration of the changes in cellular parameters evoked by the action of the modulator reveal that transmembrane resistance Rm increases by a factor of 2, that the level of response to a standard stimulus in AP/stimulus is doubled, IPSP and PIRD also increase in amplitude by an approximate factor of 2. The synaptic coupling factor b is boosted from 0.35 in controle to 0.7 in modulated condition.*

*Bioluminescence in light organs of shrimps of krill is activated by serotonine released from nerve endings in the proximity of the photocytes containing the luciferin (coelenterazin). The release is triggered by strong changes of environmental light condition (from lighted to dark condition) and release is also triggered by signals indicating the presence of conspecifics, as there are 1)light signals resembling the quality of those produced by photophores and a signaling behavior and 2)especially the 6 Hz-modulated water flow produced by the swimmerets of conspecifics passing by in the dark. The observations confirm that neuromodulation is triggered by signal evaluators detecting vitally important signal components.*

*Equipment provided by DFG Wi 363-17-21*

# Allatostatin in the olfactory system of the honey bee

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In most animal species, complex and permanently changing olfactory signals play a major role in the shaping of behaviours. The way by which the function of the nervous system is modified to allow an animal the behavioural plasticity needed to adapt to the changing demands of its environment is one of the fundamental questions in neurobiology.

In insects, odours are detected peripherally by numerous olfactory receptors neurons (ORNs) housed in the antennae. The ORNs relay odour information to a first olfactory centre, the antennal lobe (AL). The AL is organized in a central neuropil and glomerular subunits in the periphery of the AL. The glomeruli are a site of intensive synaptic contacts between several neuron types. A subset OSNs, tuned more or less broadly to a class of odour molecules, provide the input to a particular glomerulus. Local interneurons allow computation between glomeruli but lack axonal projections to other brain areas. A small number of centrifugal neurons with projections into the AL convey information from or to other areas of the brain. Second-order projection neurons innervate single or several glomeruli and respond with characteristic odour-dependent temporal patterns of excitation and inhibition and oscillatory synchronization of activity across neurons. They relay processed information to multimodal brain centres, namely the mushroom bodies and the lateral protocerebrum.

In honeybees, some of the local interneurons in the AL contain histamine and many are GABAergic and physiological and pharmacological experiments proved that GABAa receptors are responsible for part of the inhibitory synaptic inputs to projection neurons. The prominent presence of putative peptidergic dense vesicles in local AL neurons led to the suggestion that peptides act as cotransmitters with GABA and contribute to the shaping of olfactory information.

One of the peptides present in the honey bee brain is Allatostatin (AST), a member of a large family of related neuropeptides with the conserved c-terminal sequence YXFGL. We assessed the question of colocalisation of allatostatin and GABA in the antennal lobe of honey bees and analysed the localisation of Allatostatin in the higher olfactory system. Immunhistochemical staining revealed that AST is present in about 20 homogeneous local interneurones of the AL. The staining is confined to the core regions of the glomeruli with almost no overlap with sensory projections. In only a subset of these neurons AST is colocalized with GABA. All projection neurons are devoid of AST. In the mushroom bodies, distinct AST staining is present in the alpha lobes and in the pedunculi. The distribution in protocerebral neuropiles, namely the lateral protocerebrum, suggests that Allatostatin contribute to the shaping of olfactory responses.

Despite the prominent and largely conserved cellular distribution of neuropeptides in the olfactory system of insects, only one study has so far addressed the physiological role of a peptide in the AL of Drosophila. The way that Allatostatin and other neuropeptides contribute to shaping the olfactory profiles of projection neurons and the processing of olfactory information in higher brain centres of honey bees will be an exciting subject for future research.

# Diadenosine polyphosphate analogue controls postsynaptic excitation in CA3/CA1 synapses via a nitric oxide (NO)-dependent mechanism.

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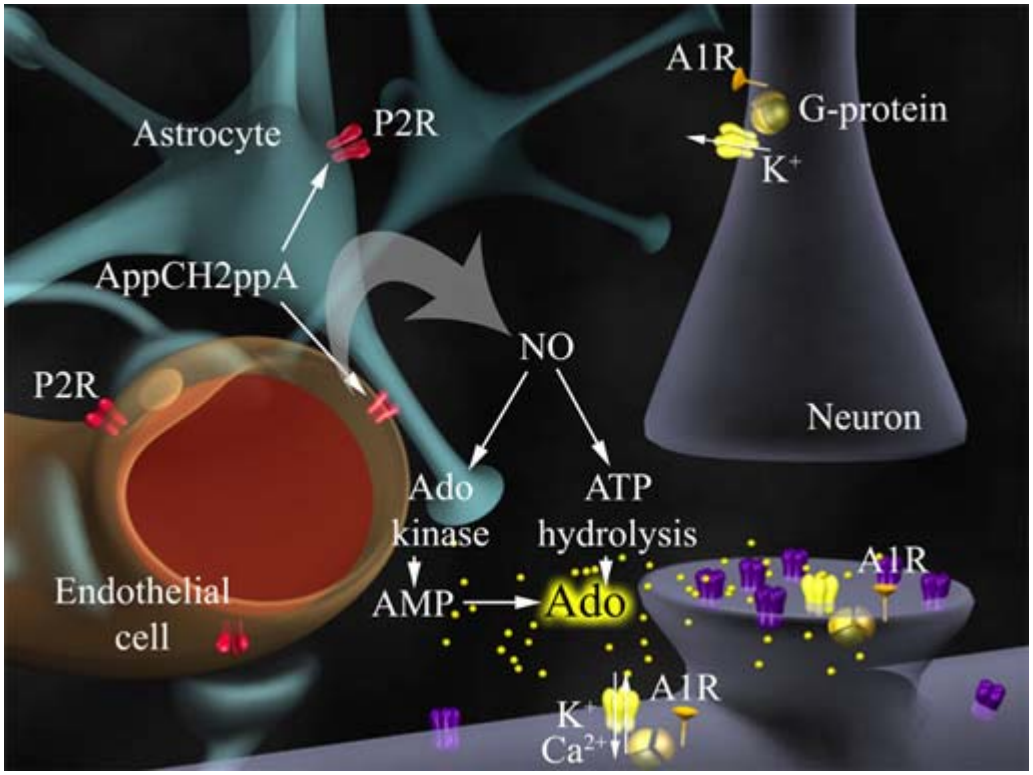
Diadenosine polyphosphates are ubiquitous in Nature and found in both intracellular and extracellular locations. However in spite of being well known for many years, pure functions of diadenosine polyphosphates have been difficult to define due to specific enzymic and non-specific hydrolysis in the presence of biological fluids and tissue samples. We have investigated the modulatory effect of a non-hydrolysable Ap4A analogue AppCH2ppA on synaptic transmission in the rat hippocampal slices. AppCH2ppA at low micromolar concentrations exerted a pure postsynaptic effect giving strong non-desensitizing inhibition of orthodromically evoked population spikes, without affecting the amplitude of excitatory postsynaptic currents and antidromic spikes (OFP). The effects of AppCH2ppA on OFP were eliminated by purine receptor antagonist PPADS but not mimicked by purinoceptors agonists, indicating that a P2-like receptor was involved but not one belonging to the conventional P2X/P2Y receptor classes. Furthermore, in contrast to AppCH2ppA, ATP inhibited the excitatory postsynaptic currents. Diadenosine polyphosphate receptor (P4) antagonist, Ip4I, was unable to modulate AppCH2ppA effects. Thus, P2-like atypical receptor for the diadenosine polyphosphates appears to selectively control dendritic excitation of the CA1 neurons. The specific nitric oxide - scavenger PTIO is shown to attenuate significantly AppCH2ppA mediated inhibitory effects, indicating that NO is involved in the cascade of events initiated by the AppCH2ppA. Further downstream mediation by adenosine A1 receptor is also demonstrated. Such a spatially selective postsynaptic dendritic inhibition may influence dendritic electrogenesis in pyramidal neurons and consequently mediate control of neuronal network activity.



# Postsynaptic mechanisms underlying responsiveness of amygdaloid neurons to cholecystokinin are mediated by a transient receptor potential-like current

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Effects of cholecystokinin (CCK) were investigated in projection neurons of mouse basolateral amygdala *in vitro*. Approximately 98 % of encountered neurons responded to CCK with an inward current at the holding potential of -70 mV. This response was mediated by $CCK_2$ receptors as indicated by agonist and antagonist effectiveness, and conveyed via G-proteins of the $G_{q/11}$ family as it was abolished in gene knockout mice. Half-maximal effects were obtained at 23.9 nM with a Hill coefficient of 0.8. Maximal current amplitude was insensitive to extracellular potassium, cesium, and calcium ions, respectively, whereas amplitude and reversal potential critically depended upon extracellular sodium concentration. The current reversed near -20 mV consistent with activation of a mixed cationic channel with relative permeability for sodium and potassium of 0.8. Abolition of calcium responses with intracellular heparin did not affect current responses after addition of CCK, indicating that they were not calcium activated. The induced current resulted from activation of a voltage-dependent, mixed cationic current reminiscent of transient receptor potential (TRP) channels. Extracellular application of the non-selective TRP channel blockers 2-APB, flufenamic acid, $Gd^{3+}$, and ruthenium red, respectively, inhibited CCK induced inward currents. Single cell PCR confirmed the expression of TRPC1,4,5 and coexpression of TRPC1 with TRPC4 or TRPC5 in some cells. CCK responses were associated with depolarization as well as decrease of input resistance in the subthreshold range leading to an increase in cell excitability.
This novel neuropeptide action may contribute to the role of CCK in the enhancement of fear and anxiety responsiveness as evidenced in particular in the amygdala.

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# Contraction measurements in body wall muscles of *Drosophila* larvae reveal differences in modulatory effects of octopamine and its precursor tyramine

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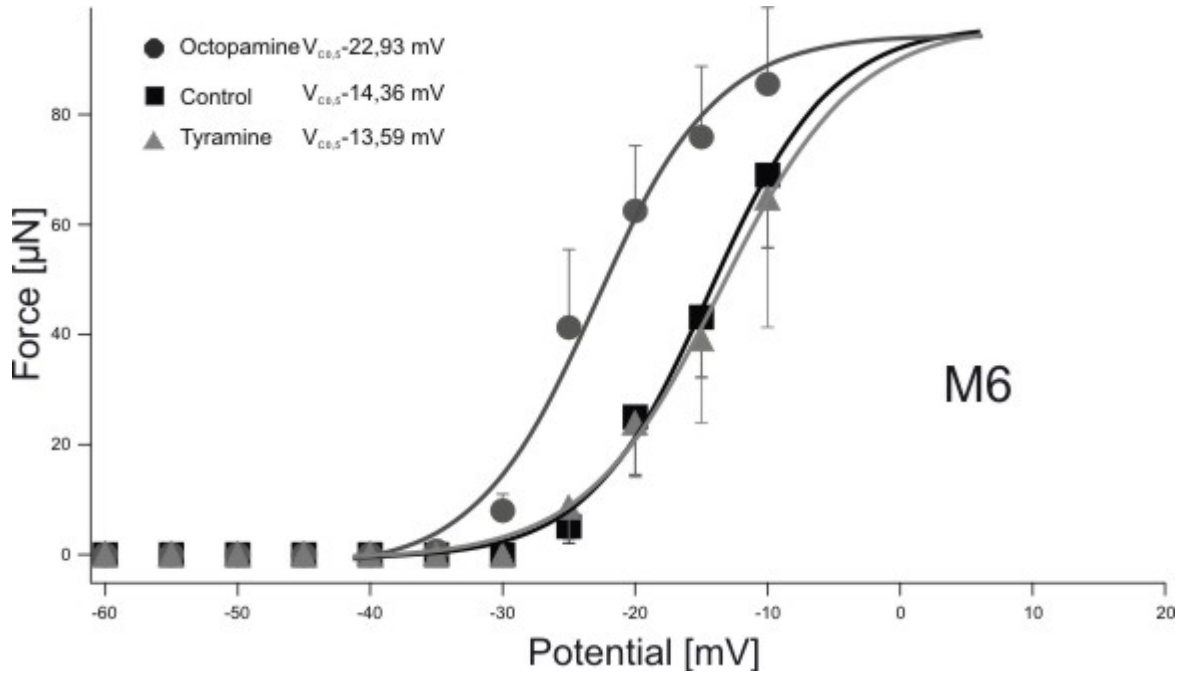
In the past decades *Drosophila* has become a powerful model system for neurobiologists. The genetic malleability and amenability to electrophysiological methods make *Drosophila* an attractive model to study the structure, function and plasticity of the neuromuscular system.
However, the limited size of the larval and adult fly has made it difficult to analyse muscular contractions of individual muscles which are, however, important parameters in studies of muscular modulation, plasticity or excitation- contraction (EC) coupling.
To make contraction parameters available for analysis, we developed a technique by which individual contractions of *Drosophila* larvae muscle fibers can be measured. Furthermore, the threshold of contractions can be determined by voltage clamping of single fibers at defined potentials while the resulting isometric contractions are being measured.
To test the technique, we compared the contractions of larval muscle 6 (M6) and larval muscle 12 (M12) and their modulation by the biogenic amine octopamine and its precursor tyramine. M12 has been reported to be innervated by octopaminergic type II boutons (Monastirioi et al., 1995; Hoang and Chiba, 2001). By contrast, muscle 6 receives glutamatergic innervation only.
Although Nagayama et al. (2002) found no effect of either tyramine or octopamine on neurally-evoked ejp's in muscle 6, we could measure pronounced differences in both muscles.
The evoked muscle force in M6 and M12 are increased about two-fold by octopamine application. The contraction threshold is only slightly shifted from -25 mV to -35 mV. Tyramine the precursor of octopamine has no effect. Therefore we conclude that (i)octopamine acts either on $Ca^{2+}$ channels or directly on the EC - machinery and (ii) that the well known effects of tyramine (Saraswati et al., 2004) are restricted to the level of synaptic transmission and do not affect the excitation - contraction complex.
With our novel technique we are able to distinguish between synaptic effects or those established on the level of the EC-coupling machinery.



# Calcium imaging in primary cell culture and in situ suggests a differential transmitter-mediated regulation of CCAP neuron subsets in Drosophila

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Insect development is interrupted by periodic molting of the exoskeleton to accommodate growth and changes in morphology. While molting is regulated by the titer of the steroid hormone 20-hydroxyecdyson, the timing and execution of ecdysis behavior (the shedding of the old cuticle) is controlled by the action of peptide hormones. In Drosophila melanogaster, at least three interacting peptide hormones regulate ecdysis behavior [see 1,2]: Ecdysis triggering hormone (ETH), eclosion hormone (EH) and crustacean cardioactive peptide (CCAP). Centrally released EH, together with ETH, activates CCAP expressing neurons (NCCAP) and the ensuing release of CCAP then initiates the ecdysis motor program. Targeted ablation of NCCAP demonstrated that these neurons are essential for the normal execution and circadian timing of ecdysis behavior. Thus, CCAP seems to be a key regulator of ecdysis behavior.
Besides the peptidergic control, it seems that descending central excitatory and inhibitory influences play an important role in the determination of the onset of ecdysis. For example, CCAP neurons in the ventral nerve cord of Manduca sexta appear to receive inhibitory input from interneurons located in the suboesophageal ganglion and the thoracic ganglia [3]. It is, however, unclear which neurotransmitters mediate this central regulation. Genetic approaches to reveal the neurotransmitter identity are strongly hampered by their ubiquity, the multitude of neurotransmitter receptor subunits and the uncertainty about the subunit composition of insect neurotransmitter receptors. Moreover, afferent neurons have not been identified.

We used a live cell imaging approach to identify putative excitatory and inhibitory input transmitters of the NCAAP. Nearly all NCCAP responded to cholinergic agonists in primary cell culture, whereas only some NCCAP showed responses to glutamatergic and/or GABAergic input. By monitoring transmitter-induced Ca2+-responses with genetically encoded Ca2+-sensors in situ, we found that only a small fraction of NCCAP in the larval as well as in the adult ventral ganglion showed [Ca2+]i-increases after application of cholinergic agonists. In the larval ventral ganglion, NCCAP that responded to cholinergic agonists belonged to a subset of morphologically distinct NCCAP. Their neurites projected to the periphery where they formed putative neurohemal zones on larval body wall muscles.
Our results provide support for the hypothesis that the population of NCCAP consists of distinct NCCAP subsets which are differentially activated and subserve various functions.
Since neuropeptide release is induced by a rise of the free intracellular Ca2+-concentration ([Ca2+]i), the identified putative neurotransmitters might participate in the control of peptide release from the NCCAP.

[1] Ewer J (2005) Horm Behav 48:418 [2] Zitnan D Adams ME (2004) In " Comprehensive Molecular Insect Science" [3] Zitnan D Adams ME (2000) J Exp Biol 203:1329.

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# Tri-peptide complexes and sleep in rabbits

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According to a recent hypothesis, not single but namely complex administration of natural peptides and their analogs should be the most effective because in the entire body the control processes are realized on a base of the peptide functional continuum. Preliminary theoretical search using mathematical vector analysis revealed several peptide complexes possible to exert a pronounced psychotropic (anxiolytic) effect, among them: **DSIP** (delta sleep-inducing peptide) + **NPY** (neuropeptide-tyrosin); **DSIP+NPY+ANP** (atrial natriuretic peptide); **DSIP+NPY+CGRP** (calcitonin gene-related peptide) (*Koroleva, Ashmarin. J.Theor.Biol. 216(3): 257-271, 2002*). As antiphobic effects usually associate to stress-protective and sleep-promoting ones, we decided to verify the above-mentioned hypothesis. Peptides were administrated directly to the lateral ventricle of adult male chinchilla rabbits preliminary implanted (under local procain anesthesia) with conventional intracranial electrodes for the EEG (neocortex, hippocampus, olfactory bulb) and EMG of neck muscles as well as a single cannula to a lateral ventricle. Otherwise the same animals treated with a saline were used as controls. Peptides (7-25 nmol per rabbit) dissolved in a saline were injected through the cannula at a volume 60-80 µl just before the 12-hr dark period in experimental chambers. Continuous digital polygraphic recording in free-moving animals started immediately since administration and lasted for 24 hr. Hypnogenic activity was found in **NPY** and **CGRP**; it was absent in **DSIP** (that conforms our previous data, see: *Kovalzon, Strekalova. J.Neurochem. 97(2):303-309, 2006*) and **ANP**. However, di-peptide complex **DSIP+NPY** was more effective than **NPY** alone. Both tri-peptide complexes were even more effective than the di-pepide one. In all cases the effect possesses an increase in slow wave sleep percentage which delayed for 1-3 hrs from the time of injection and lasted for 3-6 hrs. Despite complete structural difference, all the peptides used in this study could be united to the same functional group of inhibitory peptides (*Wiggins et al. Neurosciences 118:715-726, 2003*). This is due to their anxiolitic properties and the ability to suppress stress hormonal axis; also important, that **NPY** and **ANP** could activate anabolic and neuroprotective processes in the brain. As it is known that stress hormones induce behavioral activation and suppress sleep, unspecific influence of these peptide complexes upon sleep could be proposed probably reflecting their secondary participation in sleep-waking control which follows their involvement into endocrine processes. "Hidden" properties of neuropeptides could be proposed which are expressed only after **complex** administration or endogenous secretion.

# Acute systemic ghrelin administration induces anxiolytic behaviour and memory enhancement in rats

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Ghrelin is a natural ligand of the growth hormone secretagogue receptor (Smith et al. 2005 for review). A more recently recognized effect of ghrelin is the stimulation of appetite via the secretion of the hypothalamic orexigenic hormones, neuropeptide Y (NPY) and orexin, and inhibition of pro-opiomelanocortin (POMC)/a-melanocyte-stimulating hormone (aMSH) (Tschop et al. 2000, Cowley et al. 2003, Toshinai et al. 2003). It is possible that agonists and antagonists of ghrelin receptors could be of value in treatment of anorexia and obesity, but the exact central behavioural effects of ghrelin are still unexplained. For example, after central administration of ghrelin both increase and decrease of locomotor activity could be observed in animals (Tang-Christensen et al. 2004, Jerlhag et al. 2006). Moreover, it was found out that the peptide showed anxiogenic activities in some experimental circumstances (Carlini et al. 2002, Carlini et al. 2004). Therefore, the aim of this study was to assess the influence of ghrelin after acute systemic administration on anxiolytic and cognitive functions in rats. The study was performed using male Wistar adult rats after 12 h of fasting. The range of the doses of ghrelin was chosen according as recommended by Tebbe (Tebbe et al. 2005). The peptide was given to rats as a single injection (0.3, 3.0 and 0.0 nmol/kg, i.p.) and 100 min later sedative activity, anxiety-related reactions and cognitive function of animals were assessed using actinometer (Mikolajczak et al. 2002), elevated-plus maze (Pellow and File 1986) and passive avoidance test (Ader et al. 1972), respectively. It was found out that after acute systemic injection in all doses ghrelin did not affect locomotor activity of rats when compared to control animals. However, the peptide in the dose of 3.0 nmol/kg induced an increase in the parameters inversely related to anxiety (number of entries onto open arms, time and distance spent in open arms), whereas in the doses of 0.3 and 10.0 nmol/kg such effects were not observed. Moreover, ghrelin administration in the dose of 3.0 nmol/kg increased latency time showing the improvement of memory retention of rats, but after the injection of the peptide in doses of 0.3 and 10.0 nmol/kg the latency of animals did not differ significantly when compared to the control group. We believe that the cognitive effects produced by ghrelin in the dose of 3.0 nmol/kg were specific since the peptide affected neither the sedative nor anxiety-related reactions. Concluding, it seems that in the experimental procedure there is a pharmacodynamic window for anxiolytic and procognitive activities of ghrelin.

This work was supported by grant 501-01-3310421-04201 from Poznan University of Medical Sciences, Poland.

# Characterization of pituitary adenylyl cyclase activating polypeptide (PACAP)-induced pial arteriolar dilation in piglets.

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Pituitary adenylyl cyclase activating polypeptide (PACAP) has been found neuroprotective in numerous in vivo and in vitro experimental models of cerebral ischemia. PACAP shows molecular heterogeneity: in humans 27 and 38 amino acid forms occur (PACAP-27 and PACAP-38). PACAP-induced neuroprotection has been suggested to involve anti-apoptotic, anti-inflammatory and vasorelaxant mechanisms. Cerebral hypoxia/ischemia-induced neuronal damage appears to be -at least in part -secondary to impairment of cerebrovascular reactivity. Little is known, however, on the mechanism of PACAP-induced cerebrovascular changes in newborns often affected by asphyxia and hypoxia.

Therefore, we sought to characterize the vascular reactivity to PACAP-27 and PACAP-38, as well as shorter PACAP segments in a piglet model that shows good correlation with the vascular physiology of newborn babies. Using the non-selective cyclooxygenase (COX) inhibitor indomethacin (5 mg/kg, iv), the selective COX-1 inhibitor SC-560 (1 mg/kg, iv) and the selective COX-2 inhibitor NS-398 (1 mg/kg, iv), we investigated if COX-derived metabolites play a role in the cerebrovascular effects of the PACAP. Further, we determined the effect of the inhibition of nitric oxide synthase (NOS) activity on the PACAP-induced pial arteriolar reactions (by N-nitro-l-arginine methyl ester [L-NAME, 15 mg/kg, iv]).

Anesthetized (Na-thiopenthal 40 mg/kg, ip, followed by α-chloralose 40 mg/kg, iv), ventilated piglets (1 day old, 1-2 kg, n=54) were equipped with closed cranial windows. Pial arteriolar diameters (baseline ~100 μm) were determined via intravital microscopy.

Topical PACAP-27 and PACAP-38 elicited similar, repeatable, dose-dependent pial arteriolar dilations. Percent changes in diameters to 10-8, 10-7, and 10-6 M PACAP-38 and to PACAP-27 were 6±1, 16±2, and 40±4, and 9±2, 19±3, 36±4 (mean±SEM), respectively. In contrast, the shorter segments of the peptides (6-27, 6-38, 6-15, 20-31, 1-15) did not display any vasoactivity. Arteriolar dilations to PACAP-38 were abolished 20 min after indomethacin and SC-560 (to 10-6 M from 35±4% to -1±1%*, and from 36±6% to 1±1*, *$p<0.05$), but the response was not significantly affected by NS-398 (to 10-6 M 39±4% and 45±8%, before and after the treatment, respectively). L-NAME proved to be ineffective too (to 10-6 M 34±5% and 26±5%, before and after the treatment).

In summary, PACAP is a potent vasodilator in the neonatal cerebral circulation, and this vascular reaction appears to depend critically on COX-1 derived prostanoids, whereas the role of NO seems to be ancillary.

# Poster Topic

## T10: Receptors

**T10-1A** The role of the NR3 subunits in NMDA receptors
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*YY. Huang and SCF. Neuhauss, Zürich (CH)*

# The role of the NR3 subunits in NMDA receptors

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Ionotropic glutamate receptors (iGluRs) mediate most of the excitatory signal transduction in the vertebrate central nervous system. The NMDA receptors (NMDARs) assemble from NR1, NR2 and NR3 subunits. Depending on the subunit composition, NMDARs can be blocked by a variety of substances, including $Mg^{2+}$ ions and protons. While NMDARs are involved in physiologically important mechanisms such as long-term potentiation, memory and learning, overstimulation of these receptors is associated with pathophysiological events and neurodegenerative diseases.

The role of the NR3A and NR3B subunits in NMDARs has yet to be thoroughly elucidated. Recent studies suggest a dominant-negative effect of both subunits on current responses and $Ca^{2+}$ permeability, but those studies solely focused on combinations with the NR1 splice variant NR1-1a. Additionally, NR3 subunits have been reported to assemble independently with NR1-1a to form excitatory glycine receptors.

In this study we used the *Xenopus laevis* expression system to examine the interaction of NR3 subunits with every functional NR1 splice variant. For the first time we could show that excitatory glycine receptors are formed independently of the NR1 variant, but require the presence of NR3B. Contrary to published data, no functional influence of NR3A-2 could be detected.

Typically, NR3B-containing receptors are insensitive to NMDAR antagonists. This could be corroborated for the first time for all NR1 splice variants. Pharmacological studies in this context showed the unexpected influence of an endogenous, $Mg^{2+}$-sensitive subunit activated by glutamate. mRNA for a *Xenopus* NR2B subunit (*Xen*NR2B) has already been shown to exist in oocytes. Here, we report for the first time a functional influence of *Xen*NR2B at the protein level.

The postulated dominant-negative influence of NR3B on NR1-1a-containing heteromers did not hold up when other NR1 splice variants were present. Instead, we found a decrease in pH sensitivity when NR3B was present. These findings suggest a considerably different physiological role of NR3B than its postulated down-regulating influence.

# Modulation of nicotinic acetycholine receptor by (-)menthol in mammalian sensory neurons

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In mammals, part of the common chemical sense is made up by free endings of the trigeminal nerve which are distributed throughout the nasal cavity and respond to a variety of substances. The cell bodies of trigeminal primary afferents are located in the trigeminal ganglion and in the brain. Nicotine, a potent stimulus of trigeminal chemoreceptors, is sensed by nicotinic acetylcholine receptors (nAChR) expressed in trigeminal sensory neurons. In the present study, the effects of (-)menthol on nAChR agonist responses were examined. Whole-cell recordings from acutely isolated rat trigeminal neurons (P20 ± 3d) revealed that the nicotinic receptor agonists acetylcholine, (±)epibatidine and (-)nicotine induce robust currents in a dose dependent manner. The IC50 values for acetylcholine, (±)epibatidine and (-)nicotine were 72 µM, 36 nM, 38 µM, respectively. It has been shown that (-)menthol induces a sensation of cold mediated by the cold receptor TRPM8. Bath application of menthol (10-750 µM) at 25°C induced reversible inward currents between 20 and 400 pA in 64 % of the cells (n=66). Currents induced by 75 µM nicotine were significantly reduced in amplitude in the presence of menthol. Inhibition of the nicotinic response by menthol was observed in all cases and was independent of the current induced by menthol itself. The IC50 value for inhibition by (-)menthol was 132 µM. These findings suggest that menthol modulates nAChR function independent of cold receptor activation.

# NRA-1 and NRA-2, two novel proteins associated with the levamisole-sensitive nicotinic Acetylcholine Receptor in *C. elegans*

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To date little is known about proteins that regulate the activity or cell surface expression of nicotinic Acetylcholine Receptors (nAChRs). We identified such proteins by tandem-affinity-purification with the levamisole-sensitive nAChR (LevR) in *C. elegans*. Depletion of these **n**icotinic **r**eceptor **a**ssociated proteins using RNAi confers resistance to cholinergic agonists, and affects nAChR cell surface expression. Two of those proteins are NRA-2 and NRA-1.

NRA-1 is a Copine, which comprises two N-terminal C2-domains that can bind to phospholipids in a $Ca^{2+}$-dependent manner, and a C-terminal protein-protein interaction domain. NRA-2 is a homolog of vertebrate Nicalin (nicastrin-like protein), a single-pass transmembrane protein. Genomic deletion of nra-1 and nra-2 verified the nicotine resistance phenotype.

The nicotine-resistance phenotype of the *nra-1* and *nra-2* mutants is specific to impaired cholinergic neurotransmission, since the animals also show resistance to paralysis induced by levamisole but not to the $GABA^A$ receptor agonist muscimol. We could show that synaptic expression of the levamisole receptor is reduced in *nra-1* and *nra-2* mutants, providing a likely explanation for the observed resistance to cholinergic agonists (1). In addition, *nra-2 (tm1453)* animals also show slightly uncoordinated movement, indicative of a defect at the neuromuscular junction (1).

A NRA-1::GFP fusion is expressed in muscles and neurons, where it is preferentially found at the plasma membrane, and co-localizes with the LevR, although not in a clustered fashion. NRA-1 may recruit proteins to the plasma membrane that assist in nAChR cell surface insertion or clustering.

The vertebrate homologue of NRA-2, Nicalin, in complex with Nomo (nodal modulator), was shown to antagonize a TGF beta pathway, putatively acting in the endoplasmic reticulum (ER) (2). Also the *C. elegans* homologue of Nomo was co-purified with the LevR, and RNAi of CeNomo causes a levamisole resistance phenotype similar to RNAi of nra-2 which was not further enhanced by double knock-down of both genes, indicating that both proteins function in the same pathway. We found that NRA-2::GFP localises to the ER of muscle cells, where it influences LevR activity. Thus, NRA-2 may have a function in nAChR biogenesis or maturation.

To examine direct or indirect effects of NRA-1 and NRA-2 on nAChR function, we will perform structure-function analysis using truncated forms for domain-dependent activity- and localization-studies. Furthermore we aim to find interactors of NRA-1 and NRA-2 using tandem affinity purification in order to get more insights into the protein network involving the LevR, NRA-1 and NRA-2/CeNomo.

References:

1) Gottschalk *et al.* (2005) EMBO J 24, 2566

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# Oligomerisation of serotonin receptors: structural and functional implications

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G-protein-coupled receptors (GPCRs) represent one of the largest protein superfamilies in the human genome. They mediate the body´s reaction to a variety of stimuli like light, odour or neurotransmitters. GPCRs have been classically assumed to function as monomers that activate one heterotrimeric G-protein in order to transduce the signal. This view has to be reevaluated since evidence indicating that GPCRs can dimerise or even assemble as high molecular weight complexes emerged. Oligomerisation could influence pharmacological and signalling properties and therefore provide a novel mechanism to modulate signal transduction. We used co-immunoprecipitation and Förster energy transfer approaches to study 5-HT1A receptor oligomerisation. We found that 5-HT1A forms not only homooligomers but also heterooligomers with the related 5-HT7 receptor as well as with other GPCRs which do not belong to the family of serotonin receptors. We also analysed whether the oligomerisation of 5-HT1A is influenced by posttranslational modifications.

# Role of palmitoylation in the 5-Hydroxytryptamine 4a receptor functioning.

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Serotonin receptors belong to a super family of the G-protein coupled receptors (GPCRs), and are responsible for many important functions in the organism such as memory, respiratory control and sexual behavior. Some of GPCRs are modified with a palmityc acid on their C-terminus. For some receptors, it is an irreversible attachment, but for others is dynamic. The mouse serotonin receptor 5-$HT_{4a}$ is an unusual member of the serotonin receptors because it possesses two separate carboxyl-terminal sites for palmitoylation. This allows the receptor to adopt different conformations in an agonist-dependent manner. By mutating of these sites, we generated receptor variants with distinct functional properties. In present study we showed that the receptor undergoes rapid and dose-dependent phosphorylation on serine residues upon stimulation. The mutant, which is constitutively active in the absence of ligand, exhibited enhanced receptor phosphorylation under basal and agonist-stimulated conditions and was more effectively desensitized and internalized by a beta-arrestin2 mediated pathway as compared to the wild-type receptor. To analyze the molecular kinetic underlying receptor desensitization and internalization in more details, we also established bioluminescent resonance energy transfer assay ($BRET^2$) to study receptor/β-$arrestin^2$ interaction with a second resolution. The half times of receptor ß-$arrestin^2$ interaction ($\tau_{50}$, where 50% of maximal effect was observed) were estimated for wild type and mutants. The mutant has smaller $\tau_{50}$ in comparison with the wild type, but the overall beta-$arrestin^2$ recruitment kinetic was similar. Our data suggest that by modulating receptor conformation, palmitoylation plays an important role in regulation of 5-$HT_{4a}$ receptor activities, including G-protein activation, phosphorylation, and β-$arrestin^2$ recruitment.

# MOLECULAR CLONING AND CHARACTERIZATION OF *PERIPLANETA AMERICANA* SEROTONIN RECEPTORS

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Biogenic amines play a crucial role in regulating and modulating various physiological and behavioral processes in both vertebrates and invertebrates. In invertebrates, the group of classical biogenic amines consists of five members: dopamine, tyramine, octopamine, serotonin (5-HT), and histamine. 5-HT functions as a neurotransmitter, neuromodulator and neurohormone affecting behaviors as diverse as aggression, locomotion, circadian rhythms, and learning. These functions are mediated through the binding of 5-HT to multiple receptor subtypes. These subtypes are divided into seven distinct classes largely on the basis of their structural and operational characteristics. Except for the 5-$HT_3$ receptor ion channel, all receptors belong to the superfamily of rhodopsin-like G protein-coupled receptors. The identification of these receptors is decisive for the understanding of the cellular pathways influenced by biogenic amines. Here, we focus on cDNA sequences encoding 5-HT-receptors of the cockroach *Periplaneta americana*. Cockroaches are not only important pest species but also established model organisms for neurobiological and physiological research.
In order to molecularly clone *Periplaneta* 5-HT-receptor cDNAs, degenerate primers were designed corresponding to highly conserved sequences within certain transmembrane domains of known insect biogenic amine receptors. Polymerase chain reaction (PCR) amplification from oligo dT-primed *Periplaneta* brain cDNA resulted in products of the expected length. One fragment shares high sequence similarity with 5-$HT_1$ receptors from other insect species. The full length cDNA (Pea*5-ht1*) was cloned by RACE (rapid amplification of cDNA ends) PCR and comprises 2,564 base pairs. The open reading frame of Pea*5-ht1* encodes a protein of 683 amino acids (Pea5-$HT_1$) that displays the characteristic heptahelical structure of G protein-coupled receptors. We identified seven consensus sites for N-linked glycosylation located in the amino terminus and four consensus sites for phosphorylation by protein kinase C located within the third intracellular loop. Furthermore, the deduced amino-acid sequence contains one cystein residue ($Cys_{664}$) that is a potential target for posttranslational palmitoylation. In addition, Pea5-$HT_1$ contains amino-acid residues that are highly conserved between members of the 5-HT-receptor family. The tissue-specific expression pattern of the gene has been investigated by RT-PCR (reverse transcription PCR). This analysis revealed a high amount of the receptor mRNA in RNA samples isolated from brain, salivary glands and midgut. The spatial expression pattern in the brain was investigated in more detail by *in situ*-hybridization of digoxigenin-labeled riboprobes to cryosections of cockroach brains. Hybridization signals were observed in many neurons including mushroom body intrinsic neurons, neuronal cell clusters associated with the antennal lobes and neurons scattered around the optic lobes. We are currently generating a cell line stably expressing the receptor protein in order to investigate its intracellular signalling pathways as well as its pharmacological properties.

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# Molecular mechanisms of interaction between the neuroprotective substance Riluzole and GABAA receptors

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The antiepileptic drug Riluzole is used as a therapeutic agent in Amyotrophic Lateral Sclerosis due to its neuroprotective properties. Besides presynaptic inhibition of GABAergic and preferentially glutamatergic transmission it also potentiates postsynaptic GABAA receptor function. We investigated the postsynaptic effects of Riluzole on GABAA receptors by use of the patch clamp technique. Recombinant α1β2γ2s- and α1β1γ1- GABAA receptors were transiently expressed on HEK 293 cells by co-transfection. 200 ms and 1 ms pulses of a saturating GABA concentration (1mM) were applied in combination with different concentrations of Riluzole to outside out patches containing either α1β2γ2s- or α1β1γ1- GABAA receptors. Application of Riluzole led to lower absolute peak current amplitudes and steady state currents. Furthermore, it accelerated desensitisation time constants, pointing to an open- channel block. Both receptor types were affected similarly, revealing that Riluzole does not interact with the Benzodiazepine binding side of GABAA receptors.

# Cloning and functional characterization of the G protein-coupled receptors GPR7 and GPR8 in the guinea pig

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GPR7 and GPR8 are two structurally related GPCRs that are activated by the neuropeptides NPB and NPW. The current knowledge of GPR7 function is mostly derived from rodents. Targeted deletion in mice suggests a role of GPR7 in the regulation of food intake, energy expenditure and body weight. Similarly, GPR7 may be involved in anxiety as the central administration of NPB and NPW activates stress hormone secretion in rats. The relevance of these observations to man, however, is not known as rodents only express GPR7 while humans express both GPR7 and GPR8.

Here we report that the guinea pig may be the more appropriate species to study the role of GPR7 and GPR8. We have cloned both receptors from guinea pig and show that gpGPR7 and gpGPR8 share 88% and 81% identity, respectively, with their human orthologues. In addition, both receptors displayed pharmacological properties similar to their human counterparts as assessed in a FLIPR-based assay using NPW23 as the ligand. In cells expressing human or guinea pig receptors, NPW23 dose-dependently stimulated intracellular calcium release with EC50 values of 29nM for GPR7 and 23 and 65nM for GPR8.

Thus, in order to study GPR7/GPR8 function and pharmacology with relevance to humans, the guinea pig appears a more appropriate species than rats or mice.

# Identification of the CB1 cannabinoid receptor in serotonergic cells of raphe nuclei in mice

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The endocannabinoid system (ECS) possesses neuromodulatory functions by influencing the release of various neurotransmitters, including α-aminobutyric acid (GABA), noradrenaline, dopamine, glutamate and acetylcholine. Even though there are studies indicating similar interactions between the ECS and the serotonergic system, there are no results showing clear evidence for cannabinoid receptor type 1 (CB1) location on serotonergic neurons. In this study, we show by in situ hybridization that a low but significant fraction of serotonergic neurons in the raphe nuclei contains CB1 mRNA as illustrated by the coexpression with the serotonergic marker gene tryptophane hydroxylase 2, the rate limiting enzyme for the serotonin synthesis. Furthermore, by double immunohistochemistry and confocal microscopy, we were able to detect CB1 protein on serotonergic fibers and synapses expressing the serotonin uptake transporter in the hippocampus and the amygdala. Our findings indicate that the CB1-mediated regulation of serotonin release can depend in part on a direct cross-talk between the two systems at single cell level, which might lead to functional implications in the modulation of emotional states.

# Regional and Cellular Distribution of the Receptor-Interacting Protein PIST in Rat Brain

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The protein interacting specifically with Tc10 (PIST; Neudauer et al., BBRC, 2001) is associated with the Golgi apparatus. It appears to be involved in membrane vesicle trafficking and targeting of a large variety of proteins. Recently, neuronal PIST in concert with stargazin was shown to play a key role in the targeting of AMPA- receptor subunits to synapses (Cuadra et al., J Neurosci, 2004). Moreover, we demonstrated that PIST interacts via its PDZ domain with the C-terminus of the somatostatin receptor subtype 5 (sst5) and retains the bulk of sst5 in the Golgi apparatus (Wente et al., JBC, 2005). However, relatively little is known of its regional and cellular distribution in the brain. Here we use a specific antibody to map the distribution of PIST in rat brain by immunohistochemistry. At the cellular level, PIST was detected in neurons only. In the telencephalon, PIST-like immunoreactivity (PIST-l IR) was highly expressed in olfactory areas such as the olfactory bulb (mitral and periglomerular cells) and the piriform cortex. In the neocortex, pyramidal cells exhibited moderate to intense Golgi-like staining delineating their dendritic arborizations. Many PIST-positive pyramidal cells were present throughout layer V. Immunolabeling varied in layers II/III with high numbers of cells in the motor and insular cortices and relatively few, lightly stained neurons in frontal and occipital cortices. In the hippocampus, CA2 contained densely packed, intensely labeled neurons, whereas PIST-positive cells were sparse in CA1, CA3, and the dentate gyrus. All divisions of the basal forebrain exhibited moderate to high numbers of intensely stained neurons. In the thalamus, anterodorsal, paraventricular, ventrolateral and laterodorsal nuclei contained moderate to high numbers of PIST-positive neuronal cell bodies. Intralaminar nuclei were almost devoid of labeling. The subthalamus was intensely stained, while the habenula was virtually devoid of PIST-l IR. In the hypothalamus, the supraoptic nucleus exhibited very dense staining. Moderate numbers of moderately labeled cells were also detected in the lateral hypothalamus, tuber cinereum, para- and periventricular nuclei. PIST-l IR was very widespread in the brainstem. Substantia nigra pars compacta, central gray, interpeduncular and pontine nuclei, and several monoaminergic cell groups such as A1, A5, and C1 stood out by high numbers of intensely immunolabeled cells. All cranial nerve nuclei also displayed at least low to moderate numbers of PIST-positive neurons. In the cerebellar cortex, PIST-l IR was restricted to Golgi-like staining of Purkinje cells and interneurons. Taken together, PIST appears to be associated with virtually all motor systems as well as most ascending sensory systems. Its association with the areas known to express the sst5 receptor in the basal forebrain (Stroh et al., JCN, 1999) suggests that its receptor trafficking chaperone function may play a role in vivo in the regulation of neuronal functions such as cortical activation and sleep.

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# Chronic mild stress induces depressive behaviours: role for AMPA receptors and brain-derived neurotrophic factor

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Exposure to chronic mild stress (CMS) induces anhedonia in adult animals, and is associated with the development of depressive neurosis in humans. Several studies suggested that acute stress in young animals induce behavioral abnormalities but the effects of chronic stress on motivational behaviors have not been yet characterized and little is known about the long-term neurochemical effects of CMS in either young or adult animals.

In this study we compared the effect of 4 weeks of chronic mild environmental stressors in young (30 days) versus adult (60 days) male rats on various behaviors. Spontaneous locomotor activity in the home cage, explorative behavior in an open field, the forced swim test, preference for sucrose solutions and the sexual behavior were measured. Expression of AMPA and brain-derived neurotrophic factor (BDNF) levels within specific reward-related brain regions (prefrontal cortex, anterior and posterior nucleus accumbens, anterior and posterior ventral tegmental area) as well as the dorsal and ventral hippocampus were measured using immunohistochemistry.

The present study demonstrated that CMS in adult animals induced anhedonic symptoms, as observed in the sucrose preference ($t(14) = 3.275$, $P = 0.006$) and the sexual activity ($t(12) = 2.468$, $P = 0.03$), as well as anxiety, as observed in the exploration test ($t(29) = 3.362$, $P < 0.002$). On the other hand, CMS in young animals did not induce such behavioral changes. Interestingly, the neurochemical effects of CMS in young animals were different and sometimes opposite from those observe in the adult groups. While exposure of adult animals to CMS caused a decrease in BDNF levels in the dorsal hippocampus ($t(10) = 2.715$, $P = 0.022$), the effect of CMS in young animals on BDNF levels was exactly opposite ($t(13) = 2.994$, $P = 0.01$). Similarly, BDNF levels in the anterior nucleus accumbens (NAc) were decreased in the adults only ($t(12) = 2.32$, $P = 0.039$). In addition, CMS induced a decrease and an increase in GluR1 subunit in the dorsal hippocampus ($t(10) = 4.775$, $P < 0.0001$) and the anterior NAc ($t(14) = 2.186$, $P = 0.046$), respectively, only in the adults.

In conclusion the present results strongly point to a differentiation between young and adult rats after experiencing chronic mild stress. One possible explanation for the increase in BDNF levels in the young CMS group is the capability of neuronal adaptations in younger ages which allows coping strategies with environmental changes, such as chronic unpredictable stressors. Further research on the link between BDNF, AMPA and their role in the pathophysiology of depression may help in establishing novel therapeutics for the treatment of major depressive disorders. This study have allowed us to gain better insight into the neurochemical basis of depressive behavior and indicate that adolescent depression is different in its pathophysiology and therefore should differ in treatment strategy.

# Molecular characterization of metabotropic glutamate receptor type 6 (GRM6) in zebrafish's eye

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Metabotropic glutamate receptors (mGluRs) have been identified at synapses of both photoreceptors and bipolar cells where they likely serve as autoreceptors to regulate neurotransmitter release. However, the expression of mGluR6 on ON bipolar cell dendrites is unique to the retina where they play a direct role in the signal transmission pathway from photoreceptors to ON bipolar cells. It is the only case of non-modulatory direct synaptic transmission of an mGluR in the nervous system. This synaptic transmission can be blocked by glutamate agonist 2-amino-4-phosphonobutric acid (APB), which specifically binds to mGluR6. Administering APB to the retina blocks the ERG b-wave, a component associated with ON bipolar cell activity. Similarly, transgenic knockout mice lacking mGluR6 also exhibit a b-wave loss. The specificity of mGluR6 expression makes it a good target gene for studying postsynaptic specialization, using anti-sense nucleotide gene knockdown technology as a reverse genetic approach. Since zebrafish larvae have cone dominant vision analogous to humans, it is an excellent model to study the role of mGluR6 in cone vision. The goal of this study was to clone the zebrafish mGluR6 orthologue and to further identify the function of mGluR6 in ON bipolar cells in the visual pathway of cone vision. We successfully cloned the zebrafish mGluR6 orthologue. The unique expression pattern of this receptor was confirmed by RNA in situ hybridization. The morpholino gene knockdown technique will be applied to examine its molecular and physiological role in the retina network. It will be interesting to test the larvae's visual behavior without functional mGluR6, in assays testing for contrast sensitivity, visual acuity, and light/dark adaptation.

# Poster Topic

## T11: Ion channels

**T11-1A** Calcium-permeable ionotropic glutamate receptor subunits promote dendritogenesis but not spinogenesis of early postnatal neocortical pyramidal cells.
*M. Hamad, T. Veitinger, T. Habijan, J. Grabert, MJ. Wirth, ZL. Ma, M. Mollmann and P. Wahle, Bochum*

**T11-2A** *Xenopus laevis* oocytes express the NMDA receptor subunit *Xen*NR2B endogenously
*C. Schmidt and M. Hollmann, Bochum*

**T11-3A** Interplay of hot- and cold-gated ion channels: thermo-TRPs as emerging novel therapeutic targets of future
*AM. Sherkheli, G. Gisselman, VE. Angela and H. Hanns, Bochum*

**T11-4A** $Na_v1.6$ subunits and intrinsic membrane properties in CA1 pyramidal neurons
*M. Bauer, S. Remy, M. Reitze, Y. Yaari and H. Beck, Bonn, Chicago (USA) and Jerusalem (Israel)*

**T11-5A** Altered persistent sodium currents in hippocampal CA1 neurons of chronically epileptic rats
*D. Sochivko, M. Bauer, Y. Yaari and H. Beck, Bonn and Jerusalem (Israel)*

**T11-6A** Impaired carbamazepine block of persistent $Na^+$ currents at subthreshold voltages in mice lacking accessory beta2 subunits
*M. Uebachs, L. Isom and H. Beck, Bonn and Ann Arbor, MI (USA)*

**T11-7A** $Ca_V2.3$ E-/R-type voltage-gated $Ca^{2+}$ channel and its functional associates
*K. Radhakrishnan, A. Krieger, MA. Kamp, SA. Siapich, M. Henry, J. Hescheler, M. Weiergräber and T. Schneider, Cologne*

**T11-8A** The $Ca_v2.3$ voltage-gated calcium channel - unmasking an enigmatic player in epileptogenesis
*M. Weiergräber, M. Henry, N. Matar, A. Krieger, J. Hescheler and T. Schneider, Cologne*

**T11-9A** $BK_{Ca}$-Cav Channel Complexes as the Mechanism for Rapid and Localized $Ca^{2+}$-activated $K^+$ Signalling
*H. Berkefeld, C. Sailer, W. Bildl, JO. Thumfart, V. Rohde, S. Eble, N. Klugbauer, E. Reisinger, J. Bischofberger, D. Oliver, HG. Knaus, U. Schulte and B. Fakler, Freiburg and Innsbruck (A)*

**T11-10A** Coenrichment of Kir4.1 and AQP4 channels in spinal cord astrocytes suggests coupling of $K^+$ flux and water transport: swelling experiments using transgenic mouse technology and 2-Photon microscopy
*P. Dibaj, M. Kaiser, J. Hirrlinger, F. Kirchhoff and C. Neusch, Göttingen and Leipzig*

**T11-11A** Heterologous expression of glial Kir channel in motorneuron-like cells: a novel candidate for neuronal silencing.
*J. Zschuentzsch, S. Ebert, M. Bähr and C. Neusch, Göttingen*

**T11-12A** Erg $K^+$ currents in mouse neonatal mitral/tufted neurons modulate excitability
*W. Hirdes, N. Napp, I. Wulfsen, M. Schweizer, JR. Schwarz and CK. Bauer, Hamburg*

**T11-1B** Hippocampal network patterns in KCNQ/M-channel-deficient mice
*D. Isbrandt, Q. Le, O. Pongs and G. Buzsaki, Hamburg and Newark (USA)*

**T11-2B** Behavioral consequences of age-dependent transgenic suppression of HCN/H channels in mouse brain
*S. Sandke, J. Roeper, M. Kruse, F. Morellini and D. Isbrandt, Hamburg and Marburg*

**T11-3B** Subunit composition of *Drosophila* microvillar TRPC channels: New answers to an old question
*DO. Richter, T. Oberacker, J. Kern and A. Huber, Hohenheim*

**T11-4B** Effects of BACE1 on Sodium Channel Gating
*T. Huth, K. Neuenfeldt, A. Rittger, M. Schwake, N. Nukina, B. De Strooper, P. Saftig and C. Alzheimer, Kiel, Wako City (J) and Leuven (B)*

**T11-5B** TRP channels in microglia: Pharmacological and biophysical properties of TRPM2 and TRPC6
*R. Kraft and J. Eilers, Leipzig*

**T11-6B** Mechanisms underlying diverging T-type Ca2+ currents of thalamic relay- and interneurons in epileptic and non-epileptic rats
*T. Broicher, T. Kanyshkova, P. Meuth, HC. Pape and T. Budde, Münster*

**T11-7B** Altered expression of HCN channels in developing rat visual thalamus
*T. Kanyshkova, P. Ehling, T. Broicher, HC. Pape and T. Budde, Münster*

**T11-8B** Menthol effects on hypothalamic neurons
*BT. Wollweber, S. Wegner, A. Randolf, MKH. Schäfer, H. Schneider, K. Voigt and HA. Braun, Marburg*

**T11-9B** The Impact of Network Activity on Layer 5 Neocortical Pyramidal Neurons from the Rat
*D. Bar-Yehuda and A. Korngreen, Ramat-Gan (Israel)*

**T11-10B** A Numerical Solution to the Ion Channel Inverse Problem using Full-Trace Analysis and a Genetic Algorithm
*M. Gurkiewicz and A. Korngreen, Ramat-Gan (Israel)*

**T11-11B** Endogenous polyamines regulate cortical neuronal excitability via activity-dependant blockade of voltage-gated Na+ channels
*L. Libman, MJ. Gutnick and IA. Fleidervish, Rehovot (Israel)*

**T11-12B** Characterization of *Drosophila* mutants with defects in the subcellular translocation of the ion channel TRPL
*NE. Meyer and A. Huber, Stuttgart*

**T11-1C** Effect of hypothyroidism and lack of TH receptors alpha and beta on the Expression of BK channels
*N. Brandt, S. Münkner, C. Braig, H. Winter, M. Knipper and J. Engel, Tübingen*

**T11-2C** *Endocytosis of ion channels in the stria vascularis: Of any importance for hearing?*
*M. Knipper, C. Claussen, L. Rüttiger, P. Saftig, O. Koenig, TE. Willnow, M. Gross and U. Zimmermann, Tübingen, Kiel and Berlin*

**T11-3C** Effect of thyroid hormone deficiency on $Ca^{2+}$ currents and exocytosis in cochlear inner hair cells
*S. Kuhn, C. Braig, S. Münkner, H. Winter, M. Knipper and J. Engel, Tübingen*

**T11-4C** Ionic currents through $Ca^{2+}$ channels in mature mouse Inner Hair Cells under mobil phone field exposure
*S. Münkner, A. El Ouardi, J. Streckert, V. Hansen and J. Engel, Tübingen and Wuppertal*

**T11-5C** Analysing the $Ca^{2+}$ currents in inner and outer hair cells of mice lacking the beta3- or beta4 auxiliary $Ca^{2+}$ channel beta subunit
*M. Knirsch, S. Kuhn, L. Rüttiger, S. Kasperek, S. Münkner, M. Freichel, V. Flockerzi, M. Knipper and J. Engel, Tuebingen and Homburg*

**T11-6C** Functional analysis of mutations in CNGA3: altered channel function or impaired trafficking?
*K. Koeppen, P. Reuter, T. Ladewig and B. Wissinger, Tuebingen*

**T11-7C** An increase in persistent Na current induced by a mutation in the $Na_v1.2$ channel is associated with neonatal-infantile seizures
*Y. Liao, L. Deprez, L. Claes, MO. Popa, S. Bail, S. Petrou, P. De Jonghe and H. Lerche, Ulm, Antwerp (B) and Melbourne (AUS)*

**T11-8C** A HYPERPOLARISATION-ACTIVATED CHLORIDE CURRENT IN THE DROSOPHILA MUSCLE
*U. Rose, M. Wanischeck, C. Derst and C. Walther, Ulm, Berlin and Marburg*

**T11-9C** A novel mutation in the voltage sensor of the Kv7.2 channel causes myokymia
*TV. Wuttke, F. Lehmann-Horn, W. Paulus, K. Jurkat-Rott and H. Lerche, Ulm and Göttingen*

**T11-10C** TRESK tandem-pore potassium channels are the major component of background potassium currents in murine DRG neurons
*TM. Dobler, S. Tovornik, J. Freitag, A. Karschin, F. Döring and E. Wischmeyer, Würzburg and Martinsried*

**T11-11C** TASK forces stroke: the functional impact of the TWIK-related acid-sensing potassium channels 1 and TASK3 for cerebral ischemia

*SG. Meuth, C. Kleinschnitz, T. Budde, G. Stoll and H. Wiendl, Würzburg and Münster*

# Calcium-permeable ionotropic glutamate receptor subunits promote dendritogenesis but not spinogenesis of early postnatal neocortical pyramidal cells.

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Dendrites are the major compartment for signal input and integration, and their morphology determines the amount and the pattern of afferent synapses. Developmental growth of dendrites is regulated by environmental factors like neurotrophins and neuronal activity which evoke intracellular signalling cascades converging on structural protein expression and dynamics. We have analysed the roles of the ionotropic glutamate (AMPA) receptor subunits GluR1(Q)flop, GluR2(R)flop, and GluR3(Q)flop, and the kainate receptor subunit GluR6(Q) to assess their impact on elongation, branching and spine formation, of early postnatal rat visual cortical pyramidal neurons of layers II/III and VI in organotypic cultures. We hypothesized that calcium-permeable subunits containing a glutamine (Q) at the editing site promote dendritic differentiation . We selected the fast-desensitisation flop-variants to prevent cell death by overexcitation. Expression plasmids (pcDNA3) encoding the receptor subunits were overexpressed in individual neurons by biolistic transfection together with EGFP as reporter from 5-10 days in vitro. Quantitative morphometric analysis was performed after EGFP immunoperoxidase staining, and with confocal microscopy. EGFP transfectants served as controls. We report that GluR1(Q)flop, GluR3(Q)flop and GluR6(Q) promoted dendritic elongation and branching, albeit in a layer- and subunit-specific manner. GluR1(Q)flop most efficiently promoted apical and basal dendritic growth in layers II/III and VI pyramidal cells likely because it is highly calcium-permeable. Surprisingly, GluR1(Q)flop failed to increase the spine density. GluR6(Q) promoted apical and basal dendritic growth only in layers II/III. GluR3(Q)flop only promoted apical dendritic growth in layers II/III; however, it failed to alter spine densities. As expected, the calcium-impermeable GluR2(R)flop failed to promote dendritic growth, unexpectedly, however, it was most effective in increasing the spine density, which was 50% higher on apical and basal dendrites after 5 days of overexpression. The results suggest that glutamatergic signalling via calcium-permeable GluR subunits has a profound and subunit-specific influences on the development of dendritic complexity and the formation or maintenance of spines.

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# *Xenopus laevis* oocytes express the NMDA receptor subunit *Xen*NR2B endogenously

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The South African clawed frog *Xenopus laevis* is one of the most important model systems in neuroscience, not only for the investigation of the development of the central nervous system, but also as supplier of oocytes which can be used as a heterologous expression system. As these oocytes do not express functional glutamate-gated ion channels endogenously this system became a very important tool for the characterization of ionotropic glutamate receptors. However, *Xenopus* oocytes show a major difference compared to other heterologous expression systems when N-methyl-D-aspartate (NMDA) receptors are expressed: The injection of cRNA encoding for the NMDA receptor subunit NR1 into oocytes leads to functional NMDA-gated ion channels although these receptors are thought to be functional only as heteromeric receptor complexes with either an NR2 or an NR3 subunit. In contrast, there are no functional ion channels present in HEK293 cells expressing NR1 alone. These findings suggested early on that *Xenopus* oocytes might express a receptor subunit endogenously which is unable to form functional homomeric glutamate-activated ion channels but which needs an NR1 subunit to do so. Therefore, it was not surprising when in 1997 a report was published which showed that a glutamate receptor subunit, the kainate binding protein *Xen*U1, is indeed expressed in oocytes endogenously [1]. As this subunit has also been reported to be able to interact with NR1 forming a so-called unitary glutamate receptor the mystery about the oocyte-specific homomeric NMDA receptor seemed to have been solved [2].

However, the unitary glutamate receptor always has been a controversial entity, and in a recent study our group refuted this concept [3]. Consequently, a new explanation for the homomeric NMDA receptors in oocytes was required. We therefore screened *Xenopus* oocytes for NR2 subunits and actually found the mRNA of the *Xenopus* NR2B homolog (*Xen*NR2B) to be present. We then cloned the full-length *Xen*NR2B cDNA from oocyte total RNA and electrophysiologically characterized this subunit in coexpression with NR1, comparing its pharmacology with that of the NMDA receptors found in oocytes after injection of NR1 cRNA alone. Additionally, we investigated by quantitative PCR if the expression level of the "homomeric" NMDA receptor is linked to the amount of the mRNA present in *Xenopus* oocytes.

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# Interplay of hot- and cold-gated ion channels: thermo-TRPs as emerging novel therapeutic targets of future

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Transient receptor potential (TRP) super-family of ion channels constitutes (except for two members) calcium permeable non-selective cation channels which are gated by a variety of stimuli such as phospholipids, pheromones, cell volume changes, acidity and cellular metabolic status, chemicals associated with hot and cold sensations and changes in ambient temperature. Because of this poly-modularity and their broad distribution from brain to peripheral organs and tissues, TRP channels are now thought to be involved in a multitude of physiological processes including pain and taste perception, thermo- and mechano-sensations, regulation of mineral absorption/re-absorption, blood pressure, gut motility, airway responsiveness and cell proliferation. Thermo-TRPs are a subset of TRP super-family and are capable of transducing thermal information over entire physiological range. TRPV1-V2 and TRPA1 are also nociceptors mediating noxious hot and noxious cold pain. In the present study individual thermo-TRPs were investigated by discovering highly specific agonists/antagonists for ion channel in question. Recombinant TRP ion channels were expressed in Xenopus oocytes or HEK-293 cells and functional characterization was carried out by voltage clamp techniques. Structural analogs of natural compounds like menthol, thymol, eugenol, camphor and cineol were tested for improved affinity for the receptors. Highly active candidates have been identified for TRPM8 & TRPV3 activation along with TRPV3 specific blockers. TRPV3 is activated by substances acting on two distinct ligand binding sites, one for 2-APB and the other for camphor. Interestingly, menthol, a cooling substance was shown to activate the warmth sensing receptor (TRPV3) elucidating molecular target involved in burning pain sensation associated with high doses of menthol. In addition camphor has been shown to desensitize TRPV3 after chronic exposure, hence providing rational basis to topical analgesic effect of this natural substance. Present study provides substantial contribution to the concept of TRP ion channels as novel therapeutic targets of future.

# $Na_V1.6$ subunits and intrinsic membrane properties in CA1 pyramidal neurons

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Neurons convert graded synaptic inputs to an output signal in which information is encoded as a pattern of all or nothing action potentials. The threshold for generation of action potentials is a key factor in this process, and the underlying mechanisms have been subject of intense study.

We show here that $Na_V1.6$ subunits are concentrated at the axon initial segment of hippocampal neurons. Experiments in mice lacking functional $Na_V1.6$ α-subunits ($Na_V1.6^{MED}$ mice) revealed a depolarizing shift in the voltage-dependence of activation of the transient $Na^+$ current, without changes in other properties. This finding suggests that $Na_V1.6$ subunits, aggregated at the axon initial segment, display a more hyperpolarized threshold for activation compared to other cellular $Na^+$ channels. Loss of functional $Na_V1.6$ channels in $Na_V1.6^{MED}$ mice caused a pronounced depolarizing shift in the threshold for the generation of action potentials.

Our results suggest that $Na_V1.6$ subunits, by virtue of their hyperpolarized voltage-dependence of activation, constitute a key player in setting the action potential threshold in CA1 pyramidal cells.

# Altered persistent sodium currents in hippocampal CA1 neurons of chronically epileptic rats

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Epilepsy is one of the most common neurological disorders affecting 0.5 to 0.7% of the population worldwide. The hallmark of epilepsy is recurrent epileptic seizures, which, on the cellular level, consist of synchronized discharges of large groups of neurons that interrupt normal function. One of the most frequent and devastating forms of epilepsy involves the development of an epileptic focus in temporal lobe structures.

In our previous studies, we have demonstrated a dramatic change in the intrinsic properties of hippocampal CA1 neurons in the pilocarpine model of temporal lobe epilepsy (TLE). Hippocampal CA1 neurons normally fire regular, independent action potentials during prolonged depolarization and a single action potential upon brief depolarization. This is dramatically changed in the pilocarpine model of epilepsy, in which most neurons respond to depolarization with high-frequency burst discharges. We have previously shown that the depolarizing drive underlying an increased propensity to burst is supplied in part by an up-regulation of T-type $Ca^{2+}$ currents.

Because persistent Na+ currents ($I_{NaP}$) can also underlie burst discharges under certain conditions such as altered osmolarity, reduced extracellular $Ca^{2+}$ concentration, or pharmacological reduction of certain $K^+$ currents, we have examined whether these currents are changed in chronic epilepsy. We find that $I_{NaP}$ is significantly augmented at early stages following pilocarpine-induced status epilepticus (68% up-regulation, 10-20 days after SE, $p<0.01$), but not at late stages (60-120 days after status epilepticus). The up-regulation was not accompanied by changes in $I_{NaP}$ voltage-dependence.

We next attempted to determine the molecular mechanisms of this up-regulation. $I_{NaP}$ proved to be strongly up-modulated by oxidation. Because increased production of reactive oxygen species is a feature of chronically epileptic CA1 neurons, we tested whether the epilepsy-associated up-regulation of $I_{NaP}$ is due to oxidative modulation. Recordings of $I_{NaP}$ with inclusion of the reducing agent GSH at high concentrations (5 mM) into the recording pipette, however, did not alter the epilepsy-associated up-regulation of $I_{NaP}$. Thus, oxidative modulation is unlikely to account for up-regulation of this current.

Our results suggest that $I_{NaP}$ is up-regulated in chronic epilepsy, and that this up-regulation may contribute to the increased intrinsic excitability of CA1 pyramidal cells. The cellular mechanism for the increased $I_{NaP}$ remains to be elucidated.

# Impaired carbamazepine block of persistent $Na^+$ currents at subthreshold voltages in mice lacking accessory beta2 subunits

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Despite carefully monitored drug treatment, about 30% of epilepsy patients remain refractory to antiepileptic drugs (AEDs). The underlying cellular mechanisms are diverse, and may include changes in the cellular targets of AEDs that occur in chronic epilepsy, and render them insensitive to these compounds. Such a reduced sensitivity to AEDs has been demonstrated for voltage gated $Na^+$ channels, which are an important target for many AEDs including carbamazepine (CBZ). We had previously found that a loss of accessory beta subunits is associated with a decrease in CBZ sensitivity, leading us to hypothesize that $Na^+$ channel complexes lacking beta subunits might be CBZ-insensitive.

To test this hypothesis, we used mice lacking the beta 2 subunit of the voltage gated $Na^+$ channel and examined the effect of CBZ on $Na^+$ currents in patch-clamp experiments. Intriguingly, we found a slight but significant shift of the voltage dependence of activation of the fast transient $Na^+$ current towards more hyperpolarized potentials following application of 100 µM CBZ that was significantly larger in the beta2(-/-) mice. With respect to other effects of CBZ, no differences were observed between beta2(-/-) and (+/+) mice. We also tested the effects of CBZ on the persistent $Na^+$ current $I_{NaP}$, a low-threshold, noninactivating $Na^+$ current. CBZ reduced $I_{NaP}$ peak amplitudes. At the same time, however, a hyperpolarizing shift in the voltage-dependence of activation was also observed for $I_{NaP}$. This CBZ-induced shift was larger in magnitude when comparing beta2(-/-) mice to littermate controls.

Taken together, these data demonstrate that Na+ channel complexes lacking beta2 subunits give rise to $I_{NaP}$ which is still blocked by CBZ. However, in a subthreshold voltage range, the enhanced hyperpolarizing shift in voltage-dependence of activation counteracts this effect, causing CBZ to be ineffective in blocking subthreshold persistent inward currents. Thus, loss of beta2 subunits represents an interesting candidate mechanism for further consideration with respect to CBZ resistance in chronic epilepsy.

# $Ca_V2.3$ E-/R-type voltage-gated $Ca^{2+}$ channel and its functional associates

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Voltage-gated $Ca^{2+}$ (CaV) channels are ubiquitously expressed in various cell types in living system. In principle, the molecular identity, biophysical profile, and pharmacological properties of CaV channels are independent of the cell type where they are expressed, whereas these channels execute unique functions in different cell types, such as muscle contraction, neurotransmitter release, and hormone secretion. Ten CaVα1 subunits have been identified as pore-forming subunits, forming complex with certain auxiliary subunits to conduct L-, P/Q-, N-, R-, and T-type CaV currents, respectively.

Among these, the function of $Ca_V2.3$ remains relatively elusive, perhaps due to it's resistance to various antagonists. To understand the physiological functions of this channel better, we pursued two approaches. As the first, we performed a yeast two-hybrid (Y2H) screening with human brain cDNA library using the C-terminal cytoplasmic tail of $Ca_V2.3$ as bait. Secondly, we did a FLAG-tag immunoprecipitaion in HEK 293 cells over-expressing the II-III linker region of $Ca_V2.3$ channel with a FLAG-tag. We identified, among other candidates, V-ATPase & Hsp70 with the above two methods respectively. We further confirmed the interaction either by co-localization studies or co-immunoprecipitation. We also bring about a functional relevance for the interaction of Hsp70 with the II-III loop using whole-cell patch clamp technique and electroretinography in the presence or absence of Hsp70 blocker, deoxyspergualin.

# The $Ca_V2.3$ voltage-gated calcium channel - unmasking an enigmatic player in epileptogenesis

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Voltage- and ligand-gated ion channels are known to be aetiologically related to various forms of epilepsies in humans contributing to the newly defined channelopathies (1). Voltage-gated calcium channels (VGCCs) are important players in that field, however, their pathophysiogical capacity to some extent still remains unsolved. In particular, the functional implications of the $Ca_V2.3$ E/R-type $Ca^{2+}$-channel in terms of epilepsy have been large underestimated for a long time (2). In the CNS, the $Ca_V2.3$ E/R-type $Ca^{2+}$-channel is involved in triggering epileptiform discharges by contributing to plateau potentials and afterdepolarisations. Furthermore, pharmacological analysis revealed that various antiepileptic drugs (e.g. lamotrigine, topiramate) target $Ca_V2.3$ VGCCs capable of blocking epileptiform burst activity (3,4). Various $Ca_V2.3$ splice variants have been characterized (5) being subject to complex regulation via internal $Ca^{2+}$ and PKC mediated phosphorylation (5-8). Whereas electrocorticographic (ECoG) and deep intrahippocampal long-term EEG recordings in $Ca_V2.3^{-/-}$ mice did not reveal any ictal-like discharges indicative of convulsive or non-convulsive seizure activity, PTZ seizure susceptibility was dramatically reduced in $Ca_V2.3^{-/-}$ animals compared to controls (9), supporting the observation that $Ca_V2.3$ is an important factor in triggering epileptiform activity in neuronal populations. As no compensatory changes in other VGCCs could be detected, this effect seems to be exclusively attributable to $Ca_V2.3$. Although some aspects of its relationship to epilepsy have been uncovered, further functional characterisation of $Ca_V2.3$ in etiology and pathogenesis of human epileptic syndromes as well as development of new antiepileptic drugs specifically targeting $Ca_V2.3$ turns out to become indispensable.

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# $BK_{Ca}$-Cav Channel Complexes as the Mechanism for Rapid and Localized $Ca^{2+}$-activated $K^+$ Signalling

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$BK_{Ca}$ channels are unique modulators of neuronal excitability that are activated by both membrane depolarization and elevation in intracellular $Ca^{2+}$ concentration. Under physiological conditions, channel activation requires intracellular $Ca^{2+}$ concentrations in the micromolar range as known to be present in close proximity of Cav channels.

Using affinity-purification and LC-MS/MS spectrometry on plasma membrane fractions from rat brain we found $BK_{Ca}$ channels to be complexed with the voltage-gated calcium (Cav) channels Cav1.2 (L-type), Cav2.1 (P/Q-type) and Cav2.2 (N-type). Patch-clamp recordings of heterologously coexpressed (in Xenopus Oocytes and CHO cells) $BK_{Ca}$ channels and either of these Cav channels yielded a functional coupling between Cav and $BK_{Ca}$ channels identical to the situation described in native tissues: Depolarisation to around 0mV lead to $Ca^{2+}$ inward current which immediately activated a $K^+$ outward current through $BK_{Ca}$ channels with millisecond kinetics. This coupling persisted under 5 and 10mM intracellular EGTA. Only the presence of equally high concentrations of the faster $Ca^{2+}$-chelator BAPTA strongly reduced the outward current. Simulations of the $Ca^{2+}$-domain around a Cav channel show that a distance between Cav and $BK_{Ca}$ channels of around 10nm must be given to explain these results.

Cav2.3 (R-type) that was not found in the purification failed to activate $BK_{Ca}$ channels in the coexpression. This means that $BK_{Ca}$-Cav coupling is specific for only a subset of Cav channels.

These results demonstrate that the 'nano-domain' organization suspected for activation of $BK_{Ca}$ channels under physiological conditions is realized by the bimolecular assembly of $BK_{Ca}$ channels with a distinct subset of Cav channel subtypes. This allows for a rapid $BK_{Ca}$ mediated hyperpolarization of excitable cells in response to intracellular $Ca^{2+}$ elevation.

# Coenrichment of Kir4.1 and AQP4 channels in spinal cord astrocytes suggests coupling of $K^+$ flux and water transport: swelling experiments using transgenic mouse technology and 2-Photon microscopy

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Introduction: Edema in the spinal cord is a serious complication of spinal cord injury, inflammatory spinal cord diseases or spinal cord ischemia. Inwardly-rectifying K+ (Kir) channels are believed to have an impact on edematous swelling by increasing water transport across cellular membranes in pathological conditions. In the retina, Kir4.1 channels are expressed on astrocytic membranes and coenrich with aquaporin4 (AQP4) channels at distinct subcellular compartments. Also in the spinal cord, co-expression of Kir4.1 and AQP4 was detected on astroglial endfeet and a role in coupling K+ flux to water transport has consequently been proposed. Methods: We investigated the expression of Kir4.1 and AQP4 in the spinal cord using immunocytochemistry and Western blot techniques. Functional implications of Kir channels in cell swelling was studied on mouse spinal cord slices from P6-P11 by means of confocal and 2-photon microscopy. As a model for acute edema, hyposmolaric solutions were applied in combination with pharmacological Kir channel blockade. Kir4.1-/- mice were used to understand the specific role of Kir4.1 in astroglial cell swelling. For the purpose of visualizing astrocytes, Kir4.1 mice were crossbred with transgenic mice expressing EGFP under the human GFAP promoter. Results: In spinal cord sections, Kir4.1 labeling was determined on astroglial cell from the second postnatal week onwards. These results were mirrored by immunoblot experiments using spinal cord homogenates. Co-labeling with AQP4 antibodies showed a marked overlap of Kir4.1 and AQP4 in spinal cord astrocytes, predominantly on astroglial endfeet structures. In spinal cord slices from wildtype mice, 30% hyposmolaric solution induced swelling of astrocytic cell bodies whereas no significant swelling was observed on astroglial endfeet structures. In contrast, co-application of hyposmolaric solution and Ba2+, a Kir channel blocker, also induced prominent swelling of astroglial endfeet. In Kir4.1-/- mice, a substantial increase of endfeet swelling was already observed upon application of 30% hyposmolaric solutions solely. Co-application with Ba2+ did not induce further astroglial endfeet swelling. Both, cell body swelling as well as astroglial endfeet swelling was completely reversible upon application of normosmolaric solutions. Conclusions: Our data show that (a) Kir4.1 coenrich with AQP4 channels in subcellular domains of astrocytes, predominantly in the astroglial network and on astroglial endfeet in the spinal cord. Furthermore, we (b) determine that Kir channels are functionally implicated in astroglial endfeet swelling by compensating for extracellular hyposmotic changes. Finally, (c) our results using a Kir4.1-/- mouse demonstrate that the Kir4.1 channel subunit is the major K+ channel that regulates endfeet swelling under normal and hyposmolaric solutions. Kir4.1 channels may represent a new target to treat cytotoxic edema that is observed, e. g. as a consequence of spinal cord trauma, inflammation or ischemia.

# Heterologous expression of glial Kir channel in motorneuron-like cells: a novel candidate for neuronal silencing.

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Introduction: Reducing the excitability of specific neurons will provide insight into global neuronal functions as well as offer novel approaches to protect neurons from excess neuronal activity. Selectively silencing subsets of neurons may provide a neuroprotective strategy to treat neurodegenerative and paroxysmal neurological diseases, such as amyotrophic lateral sclerosis or epilepsy. Inwardly-rectifying potassium (Kir) channels contribute to the resting membrane potential (RMP) of neurons and glial cells and thereby regulate firing activity, transmembrane gradients of transmitter molecules and energy state of the cell. The Kir4.1 subunit (KCNJ10) is expressed predominantly in glial cells of the CNS. Genetic inactivation of Kir4.1 in mice has revealed a prominent role in establishing and maintaining the RMP of astrocytes and oligodendrocytes. Methods: We investigated a putative role of Kir4.1 in reducing neuronal excitability by plasmid transfection of motoneurons with green-fluorescent protein (EGFP)-tagged Kir4.1. The NSC34 cell line, a motoneuron-like cell line was transiently transfected with EGFP/Kir4.1. Original NSC34 cells, EGFP-transfected control cells and Kir4.1-transfected cells were voltage-clamped in whole-cell mode using voltage steps from -150 mV to +50 mV in 10 mV steps. K+ current, K+ conductance and Na+ inward currents were studied. RMP was analysed in the current clamp mode at zero-current potentials. Immunocytochemistry was used to confirm expression of Kir4.1 in transfected cells. Cell death was determined by counting DAPI-labeled apoptotic nuclei under control and experimental conditions. Results: Transient overexpression of Kir4.1 channels in motoneurons did neither induce obvious morphological changes nor increased cell death. Immunolabeling of cells with a Kir4.1 antibody showed a marked overlap of Kir4.1 with the EGFP fluorescence of the EGFP-Kir4.1 fusion protein. Patch clamp experiments revealed that NSC34 motoneurons retain immature characteristics lacking spontaneous action potentials (Cashmann et al., 1995). In Kir4.1 transfected motoneurons (n=13), RMP was significantly hyperpolarized by ~30 mV compared to non-transfected (n=12) and EGFP-transfected controls (n=12). Furthermore, upon change of extracellular K+ to 50 mmol, Ba2+-blockable K+ currents were significantly higher than in controls. Na+ inward currents that were observed in all NSC34 control cells (n=24) upon depolarization, were not detected in Kir4.1 transfected motoneurons (0/13 cells). Conclusion: Our results demonstrate that transfection of a predominantly glial Kir channel in motoneurons leads to functional Kir channel expression and modifies neuronal electrophysiological characteristics. Kir4.1 channels significantly hyperpolarize motoneurons, increase K+ current density and reduce Na+ influx upon depolarization without affecting neuronal survival. This study demonstrates the feasibility of using glia-specific Kir channels to modulate neuronal activity in an in vitro model. Channel-based silencing strategies using weakly-inwardly rectifying Kir channels may form the basis for novel treatments for neurological conditions arising from excess neuronal activity.

# Erg $K^+$ currents in mouse neonatal mitral/tufted neurons modulate excitability

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Different erg (ether-à-go-go-related gene; Kv11) $K^+$ channel subunits are expressed throughout the mouse brain, and especially the mitral cell layer of the olfactory bulb is stained intensely by erg1a, erg1b and erg3 antibodies (Guasti et al, J Comp Neurol, 2005). In the present study we have characterized the erg current of mitral/tufted (M/T) neurones from the olfactory bulb of neonatal mice in primary culture. M/T neurones were identified by their morphology, the presence of functional mGluR1 receptors and their electrical activity. Erg currents were isolated with the help of the selective blocker of erg channels, E-4031. A relatively uniform erg current was regularly found in M/T neurones, but rarely in other neurones. RT-PCR revealed the expression of merg1a, merg1b and merg3 mRNA in M/T neurones. A similar erg subunit expression pattern has recently been found in embryonic rat raphe neurones (Hirdes et al, J Physiol, 2005). Compared to the raphe data, half maximal erg current activation occurred at about 10 mV more negative potentials and the M/T cell erg current exhibited slower deactivation kinetics. In addition, the erg current density was higher in M/T neurones (31 ± 3 pA/pF; n = 37) than in raphe neurones (8 ± 0.6 pA/pF; n = 66). Application of the specific mGluR1 agonist DHPG accelerated the deactivation kinetics and slightly reduced the erg tail current amplitude. In an external solution with 8 mM $K^+$ and 1 mM $Ca^{2+}$, pharmacological block of erg channels by E-4031 depolarized the membrane potential by about 5 mV. The results demonstrate that erg currents are functionally expressed in mitral/tufted neurones and that the biophysical properties differ e.g. from those of erg currents in raphe neurones.

# Hippocampal network patterns in KCNQ/M-channel-deficient mice

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We addressed the physiological roles of M-channels by suppressing KCNQ/M-channel activity in the brains of transgenic mice expressing a KCNQ2 subunit with a dominant-negative pore mutation that suppressed M-channel activity by co-assembling with other KCNQ subunits. In addition, we restricted transgene expression to the nervous system and gained temporal and spatial control over transgene expression by using the Tet-Off system. Thus, we obtained viable transgenic mice deficient in functional KCNQ/M-channels. Restriction of transgene expression to defined developmental periods revealed that M-channel activity plays a critical role in the development of normal hippocampal morphology during the first postnatal weeks. Suppression of the M-current after this critical period resulted in mice with normal brain morphology but with signs of increased neuronal excitability and deficits in hippocampus-dependent spatial memory. Hippocampal CA1 pyramidal neurons in M current-deficient mice exhibited increased discharge frequency, reduced spike frequency adaptation, attenuated mAHP, and reduced subthreshold resonance properties. In order to test the consequence of M-channel suppression for hippocampal network patterns and unit activities in the intact brain, we recorded multiple single neurons with silicon probes obtained in freely moving control and mutant mice. In addition, chronically implanted 16-site linear silicone probes were implanted in the CA1-dentate-CA3 axis to record the depth profiles of various network patterns in the hippocampus. Stable recordings were obtained from each animal up to two months during slow wave sleep (SWS), rapid eye movement (REM) sleep, exploration in the home cage, and during voluntary wheel or linear track running. The experiments revealed hippocampal network patterns that were comparable to those previously reported (Buzsaki et al. 2003), including the presence of theta oscillations in CA1 during wheel and linear track running and REM sleep with maximal amplitudes in the CA1 stratum lacunosum-moleculare, and sharp-wave ripple fast oscillations during SWS. The long-term recording of population patterns and unit activity will allow us to study the same mutant animal in the presence and (doxycycline-mediated) absence of the dominant-negative KCNQ2 transgene.

# Behavioral consequences of age-dependent transgenic suppression of HCN/H channels in mouse brain

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Hyperpolarization-activated and cyclic nucleotide-gated (HCN) cation channels mediating neuronal I(h) are homo- or heterotetrameric channel complexes composed of HCN1-4 subunits. HCN subunits are distributed widely throughout the central nervous system and contribute to the control of resting membrane potential, integrative properties and rhythmic ('pacemaker') activity of neurons. Mouse mutants with targeted deletion of the HCN1 or HCN2 genes have been valuable tools to study the function of these subunits in many brain regions including hippocampus, thalamus and cerebellum (1-3).
We aimed to suppress I(h) currents in mouse forebrain in general and expressed a dominant-negative HCN4 subunit (HCN4-DN) under the control of the CaMKII promoter. To gain temporal control on transgene expression we used the Tet-Off system (4). HCN4-DN transgene expression in double transgenic animals was analyzed using in situ hybridisation and imunohistochemistry, and was found to be forebrain-specific and sensitive to the regulation by doxycycline. Patch-clamp experiments on mutant hippocampal CA1 neurons in acute slices from adult mice revealed a strong attenuation of I(h) activity.
To test whether the age-dependent loss of I(h) activity had consequences for brain development and/or behavior, we compared control mice with mice that had HCN4-DN expression from birth (W-mutants) and mice in which HCN4-DN expression was induced after weaning (DW-mutants). Both groups of mutant mice did not have morphological changes in Nissl or Timm-stained histological brain sections. W-mutants developed marked hyperactivity, conspicuous stereotypic behavior and mild motor dysfunction and showed markedly attenuated performance in spatial memory tasks. In contrast, DW-mutants were not hyperactive and even had a tendency to show better spatial memory performance than controls. However, motor dysfunction was also present in DW-mutants. Our results suggest (A) a role for I(h) in central motor coordination beyond its proposed function in motor learning and (B) a need for functional I(h) currents in the postnatal period for proper behavioral performance.

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# Subunit composition of *Drosophila* microvillar TRPC channels: New answers to an old question

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The TRP family of cation channels serve as sensors and transducers of environmental stimuli and also as regulators of ion homeostasis in neuronal and epithelial cells. The eponym of this family is the *Drosophila* TRP (transient receptor potential) channel and its closest relatives TRPL and TRPgamma, all initially described in the rhabdomeres of *Drosophila* retinula cells. In the *Drosophila* compound eye at least TRP and TRPL are activated in response to light, generating the receptor potential. TRP channels are thought to be composed of four individual TRP and/or TRPL subunits. One main question that arises is whether TRP and TRPL subunits are capable of forming heteromeres *in vivo*. The exact composition of the channels has been subject of several electrophysiological and biochemical studies leading to controversial models (Reuss et al., 1997 Neuron 19:1249-59; Xu et al., 1997 Cell 89:1155-64; Leung et al., 2000 J. Neurosci. 20:6797-803; Paulsen et al., 2000 Eur. J. Physiol 439:181-183).

In order to examine the ion channel composition of TRP and TRPL channels in the *Drosophila* rhabdomeric membrane we generated transgenic flies that express eGFP-tagged TRP or TRPL subunits in photoreceptor cells. Performing co-immunoprecipitation (CoIp) experiments from crude *Drosophila* head extracts with anti-eGFP antibodies allowed us to examine which of the endogenous TRPC subunits interact with the tagged subunits. These experiments revealed that TRP as well as TRPL subunits exclusively formed homomeres. Further evidence for the formation of TRPL homomeres was obtained from studies of a TRPL mutant lacking the C-terminus. As revealed by fluorescence studies in intact eyes the mutated channel failed to become targeted to the rhabdomeric membrane. The targeting defect could partially be rescued by overexpression of TRPL-HcRed, but not by the endogenously present TRP. This result suggests that the truncated TRPL interacted with the non-mutated TRPL subunit. Targeting of TRP to the rhabdomere has been shown to depend on the presence of the small G-protein Rab11 (Satoh et al., 2005 Development.132:1487-97). If TRP and TRPL form heteromultimeres, which are assembled in the ER, TRPL targeting should also be affected by Rab11 ablation. However, TRPL transport to the rhabdomere proved to be insensitive to expression of a dominant-negative Rab11 transgene. This result suggests the existence of separate TRP and TRPL oligomeres that are targeted to the rhabdomere independently. Towards identifying regions that are involved in ion channel multimerization, we designed chimeric channels composed of portions from TRP and TRPL. In these constructs the N-teminal region or the C-terminal region or both parts of TRP were replaced by the corresponding regions of TRPL. Our CoIp experiments showed that all three chimeric channels formed multimeres with TRPL.These findings indicate that the cytosolic portions of the channel rather than the transmembrane regions specify the ion channel composition.

In conclusion, our experiments show that TRPL forms a homomeric ion channel in *Drosophila* photoreceptor cells and they argue against the existence of TRP/TRPL heteromultimeres.

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# Effects of BACE1 on Sodium Channel Gating

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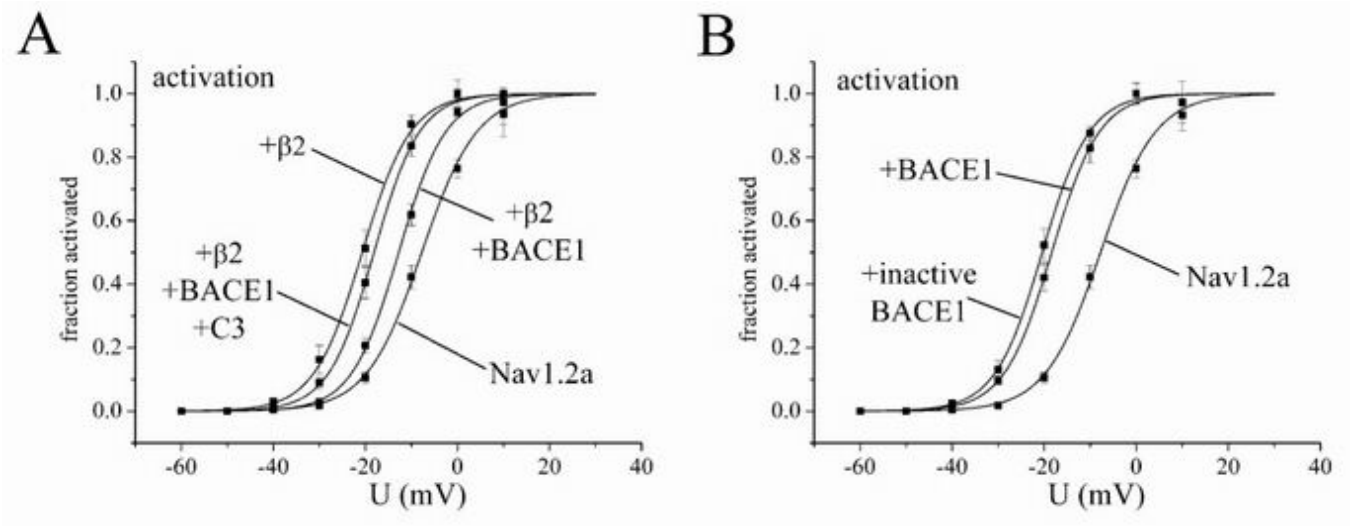
The β-secretase pathway is amyloidogenic in that consecutive cleavage of amyloid β precursor protein (APP) by β-secretase (BACE1) and γ-secretase generates amyloid β proteins, which can aggregate to form amyloid plaques, a neuropathological hallmark of Alzheimer's disease. Recent studies revealed two novel and unexpected substrates of BACE1, namely the β2 and β4 subunits of voltage-gated sodium channels (JBC 280:23009-17, 2005). Here we investigated whether the cleavage of β subunits by BACE1 affects the electrophysiological properties of sodium channels.

We explored this question using whole-cell recordings from HEK293 cells expressing the Nav1.2a subunit alone or in combination with the β2 subunit and BACE1. Consistent with previous reports, co-expression with β2 shifted the activation curve of Nav1.2a by about 12 mV in the negative direction. The negative shift was significantly, but not completely reversed when BACE1 was also co-expressed. The effect of BACE1 on current activation appears to be attributable to its protease activity, since the β-secretase inhibitor compound 3 (C3) abrogated it (Fig. 1A).

In a control experiment, in which we tested the effect of BACE1 on Nav1.2a alone, we made the striking observation that BACE1 might also exert a direct, protease-independent effect on Nav1.2a channel gating. In HEK cells co-transfected only with Nav1.2a and BACE1, but not with β2 subunit, BACE1 shifted the activation curve by about 10 mV to the left. This shift did not require secretase activity, as it was also observed when we replaced BACE1 with its inactive variant (Fig. 1B).

These results suggest that BACE1 is capable of modifying Na channel gating in both a secretase-dependent and -independent fashion.



# TRP channels in microglia: Pharmacological and biophysical properties of TRPM2 and TRPC6

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Microglial cells, the immune cells of the brain parenchyma, continually survey their microenvironment and respond to a variety of chemical stimuli. Diverse substances, such as cytokines, neurotransmitters, lipid compounds, and hydrogen peroxide, evoke calcium signals in microglia. A group of nonselective cation channels, the transient receptor potential (TRP) proteins, have been proposed as chemical and physical sensors within the plasma membrane, mediating direct calcium influx and/or modulating the membrane potential. In microglia, different TRP channels have been detected, including TRPC6, TRPM2, TRPM7, and TRPV1. We are interested in the function of TRPC6 and TRPM2 in microglia. In cultured rat and mouse microglia, TRPC6 transcripts were identified by RT-PCR, while TRPM2 channels have been characterized by means of patch-clamp recording using the specific intracellular agonist ADP-ribose. TRPM2-like single channels showed slope conductances between 65 pS and 85 pS and typical long open times. Furthermore, we investigated several putative blockers and activators of TRPM2 and TRPC6 heterologously expressed in HEK-293 cells. TRPM2 and TRPC6 were inhibited by the previously unrecognized ion channel blocker N-(p-amylcinnamoyl)anthranilic acid (ACA). Extracellular application of 20 µM ACA completely abolished TRPM2-mediated currents in cultured mouse microglia. The single-channel conductance was not changed during ACA treatment, suggesting a reduction of TRPM2 open probability by modulating channel gating. We identified KB-R7943, an inhibitor of sodium-calcium exchangers, as a potent blocker of different TRPC channels, including TRPC6. The cellular compound lysophosphatidylcholine effectively induced TRPC6 currents in HEK-293 cells. We suggest that these ion channel mediators are useful tools for studying the unknown function of the calcium-permeable channels TRPC6 and TRPM2 in microglia.

# Mechanisms underlying diverging T-type Ca2+ currents of thalamic relay- and interneurons in epileptic and non-epileptic rats

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The T-type Ca2+ channels critically contribute to the generation of rhythmic burst activity in the thalamocortical system during states of slow wave sleep and absence epilepsy. Previous studies have demonstrated the importance of GABAergic neurons located in the thalamic reticular nucleus (NRT) in the generation and/or maintenance of spike and wave discharges during absence epilepsy (1). Local circuit interneurons of the dorsal lateral geniculate nucleus (LGN) are the only known population of GABAergic neurons outside the NRT in the rodent thalamus and have been shown to display T-currents and T-current driven low threshold Ca2+ spikes (LTS) which are distinct from those observed in thalamocortical projection (TC) neurons. We thus investigated T-current properties of LGN TC and interneurons in a rat model of absence epilepsy, the WAG/Rij rats and the corresponding control strain, the ACI rats. Whole-cell patch-clamp recordings revealed a depolarised shift in T-current voltage dependency, accompanied by an increase of the inactivation time constant and the time to maximal current amplitude in interneurons compared to TC neurons. This was found in both WAG/Rij and ACI rats, without significant differences between the strains. To investigate the molecular basis underlying this depolarising shift in T-current voltage dependency we employed different molecular biological as well as computational approaches. We probed the expression of the three known pore forming α1 subunits (α1G, α1H and α1I) in samples of identified TC and interneurons of both strains (2). Furthermore, we scrutinized the presence of different splice variants of the α1G subunit, the dominant isoform in thalamocortical relay nuclei, in both cell types of both rat strains. An in silico approach was used to investigate whether a differential T-channel distribution between soma and dendrites could explain the differences in T-type Ca2+ currents between the cell types. Our data suggest that the shift in T-current voltage dependency could be related to the presence of differentially spliced α1G subunits in TC- and interneurons and/or by the exclusive expression of the α1I isoform in interneurons. An increased T-channel density in the dendrites, as thought to be present in LGN interneurons, caused a hyperpolarising shift in somatically recorded T-current voltage dependency, thus arguing against a contribution to the depolarised shift in interneurons. In conclusion we found no differences between T-currents of LGN interneurons in WAG/Rij and ACI rats. The differences between T-currents in TC and interneurons of both strains seem to be due to differentially spliced α1G isoforms or the presence of the α1I isoform in interneurons.

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# Altered expression of HCN channels in developing rat visual thalamus

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The family of hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels comprises of four known gene members. The current carried by HCN channels (Ih) is responsible for neuronal excitability and pacemaker activity in the CNS. Each HCN isoform has distinct characteristics regarding kinetics, voltage-dependency of activation by hyperpolarization, and cAMP sensitivity of the resulting homomeric membrane current. Because of their important role in neuronal rhythmogenesis, HCN channel dysfunction may contribute to neurological disorders like epilepsy. Recent studies of rat models of absence epilepsy have confirmed this idea (Budde et al., J Neusci., 2004, Strauss et al., Eur J Neurosci., Kuisle at al., J Physiol., 2006).

Here, we studied the postnatal developmental expression of the HCN isoforms in the dorsal lateral geniculate nucleus (dLGN), the main thalamic nucleus of the visual pathway using molecular and electrophysiological methods in rats. In situ hybridization and real-time quantitative RT-PCR revealed HCN2, 4 and also HCN1 expression in dLGN. Expression levels of HCN2 and HCN4 channels remained high during postnatal development (P1 - P60). Despite some quantitative changes observed during development, no differences in HCN2 and HCN4 expression patterns were found between epileptic WAG/Rij rats and non-epileptic ACI rats. By contrast, the HCN1 isoform revealed not only remarkable age-dependent changes in expression, but also significantly different expression levels between WAG/Rij and ACI. While HCN1 expression was higher in ACI compared to WAG/Rij around P7, a reversed situation was found at around P21. In addition electrophysiological recordings of Ih at different postnatal ages revealed a reversed developmental change in the voltage of half-maximal activation (Vh). While Vh values were more hyperpolarized in ACI at ages < P15, this parameter was more hyperpolarized in WAG/Rij at ages > P15. These findings indicate that a diminished decrease in HCN1 expression during development facilitates the generation of epileptic discharges in WAG/Rij.

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# Menthol effects on hypothalamic neurons

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Introduction

Menthol is a cooling agent that increases the firing rate of peripheral cold receptors (J Gen Physiol 88:757) by, presumably, activating calcium influx through TRPM8 (CMR1) ion channels (Nature 416:52). Further actions of menthol, e.g. blockade of voltage-gated calcium channels (VGCC) (Neurosci Lett 68:23, Pflugers Arch 409:52) and subsequent decrease of calcium-dependent potassium-currents ($I_{K(Ca)}$), or activation of TRPV3 and blockade of TRPA1 (ANKTM1) ion channels (Mol Cell Neurosci 32:335), have been described. However, all these results refer to peripheral cold receptors or neurons' somata in dorsal root ganglia (DRG). So far, there are no data about menthol effects on warm-sensitive neurons in the peripheral and central nervous system.

Methods

We investigated the effect of menthol on hypothalamic neurons in rat brain slices and in a hypothalamic murine cell line (GT1) using extracellular and patch-clamp recordings. Furthermore, we examined distribution of TRPM8 by means of conventional and real-time reverse transcriptase polymerase chain reaction (RT-PCR) and in situ hybridization (ISH).

Results

TRPM8 mRNA was located in a small number of periventricular neurons in hypothalamic rat brain slices. Extracellular recordings from warm-sensitive hypothalamic neurons in PO/AH, PVN, SON, ARH and SCN showed a decreased firing rate during menthol application (100 µM to 1 mM).

In GT1 neurons TRPM8 mRNA could not be detected. As the GT1 cells mostly did not display spontaneous action potential generation we could not investigate effects of menthol on the firing rate. However, whole-cell voltage-clamp recordings exhibited dose-dependent block of potassium outward currents by menthol (1 µM to 3 mM). At low concentrations menthol blocked sustained delayed-rectifier currents ($I_{K(DR)}$), whereas at high doses the majority of potassium currents, including transient A-type currents ($I_{K(A)}$), was blocked.

Discussion

Inhibition of firing of warm-sensitive neurons in hypothalamic brain slices is in agreement with menthol as a cooling agent since cooling, too, inhibits firing of warm-sensitive neurons. As TRPM8 mRNA was only sparsely distributed in the hypothalamus, diminishment of action potential generation is probably not due to menthol action on TRPM8.

The menthol effect in GT1 neurons is essentially independent from TRPM8 as TRPM8 mRNA could not be detected in the GT1-7 cell line. Furthermore, the observed block of potassium currents by menthol could not be explained by TRPM8 anyway. In contrast to the findings from extracellular recordings in hypothalamic brain slices a block of potassium currents in GT1 neurons probably results in membrane depolarization and subsequent increased firing.

To reconcile our findings, patch-clamp recordings in hypothalamic rat brain slices and calcium imaging are currently in progress.

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# The Impact of Network Activity on Layer 5 Neocortical Pyramidal Neurons from the Rat

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In recent years there has been a growing number of researches investigating the dynamics of single neurons. At the same time there has also been a growing interest in the computational aspects of neural networks. There still is, however, a great gap between these two realms. Our research aims to combine the biological and mathematical methodologies by analyzing measurements taken from pyramidal neurons of layer 5 of the somatosensory cortex. These neurons are known to interact with their surrounding network activity. This surrounding network activity, though being measured in vitro, simulates key parameters of actual in vivo activity (i.e., spontaneous flow of synaptic input). This activity is generated using innovative techniques along side the use of well-known methods (e.g., whole-cell patch-clamp).

We explore intrinsic mechanisms of information processing in the form of synaptic input, and the influence of background activity on it. Therefore, the focus is on measuring backpropagation within each neuron in various locations along the apical dendrite under different levels of network activity. According to preliminary results the backpropagating action potential has not been changed due to increased background synaptic activity, yet, the $Ca^{2+}$ spike, generated when back propagating action potential and distal synaptic input couple, is modulated by that activity.

# A Numerical Solution to the Ion Channel Inverse Problem using Full-Trace Analysis and a Genetic Algorithm

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Trans-membrane protein mechanisms such as ion-channels and their activity are at the essence of neuronal transmission. The most accurate method, so far, for determining ion-channel kinetic mechanisms is single-channel recording and analysis. Nevertheless, single-channel recordings carry several hold-ups and complexities, especially when dealing with voltage-gated channels. Here we show that genetic search algorithms (GAs) can be used to fit whole-cell voltage-clamp data to kinetic models with a high degree of accuracy. The approach takes into consideration the full range of stimulation protocols used when analyzing voltage-gated ion channels. Unlike most previous analyses done protocols results were not analyzed individually, but rather as an entire set of traces from all protocols for a simultaneous analysis. The algorithm was initially tested over simulated current traces produced using several simple Hodgkin-Huxley-like models of voltage-gated potassium and sodium channels. Currents were also produced simulating levels of noise expected from actual patch recordings. Finally, the algorithm was used for finding the kinetic parameters of several voltage-gated sodium and potassium channels models via matching its results to data recorded, in nucleated configuration, from layer 5 pyramidal neurons of the rat cortex. The minimization scheme provides a tool for electrophysiologists in mimicking and simulating voltage-gated ion-channel kinetics on the cellular level.

# Endogenous polyamines regulate cortical neuronal excitability via activity-dependant blockade of voltage-gated Na+ channels

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Our evidence in Layer 5 pyramidal cells in situ indicates that Na+ channels in the soma and proximal dendrites of these neurons produce surprisingly few late openings as compared to dissociated cell preparations. We therefore postulated that there exists a soluble factor extrinsic to the Na+ channel protein that constrains these late openings. We focus our attention on polyamine (PA) substances (spermine, spermidine and putrescine) which are present in all eukaryotic cells, can be released from the cells and are known to affect gating of numerous types of ion channels. Using cell-attached and whole cell recordings from Layer 5 pyramidal neurons in neocortical slices we found that partial depletion of PAs by disrupting their synthesis causes a dramatic increase in the probability of late openings of somatic Na+ channels and in the amplitude of whole cell persistent Na+ current (INaP). Restoration of PA levels by adding the exogenous PAs blocked late channel activities and INaP. Our data suggest that these effects are due to activity-dependent blockade of Na+ channels by PAs. Thus, when Layer 5 pyramidal cells were dialyzed with spermine-containing intracellular solution, there was a dramatic frequency-dependent change in action potential attributes, including a decrease in the maximum rate of rise and a depression in spike amplitudes. These changes, which were observed during 20 Hz spike trains, were very similar to the activity-dependent effects of local anesthetics and some anticonvulsants. Our findings identify a novel mechanism whereby changes in PA metabolism, either associated with normal brain states and stimuli or with pathophysiological conditions, can profoundly influence Na+ channel availability, and thereby modify neuronal excitability.

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# Characterization of *Drosophila* mutants with defects in the subcellular translocation of the ion channel TRPL

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Phototransduction in *Drosophila melanogaster* is a well-established model for G-protein coupled signalling cascades. This signalling pathway is mediated by phospholipase Cβ and terminates in the gating of the light-sensitive channels TRP (transient receptor potential) and TRPL (TRP-like) located in the rhabdomeric membrane. One of these channels, TRPL, translocates back and forth between the signalling membrane and an intracellular compartment. Since in dark-adapted flies TRPL is located in the rhabdomeric membrane and in light-adapted flies it is present in the intracellular compartment, this translocation is light-dependent.

In order to elucidate the mechanisms underlying the translocation of the TRPL ion channel we performed a genetic screen based on the FLP/FRT system using flies expressing a TRPL-eGFP fusion protein. This system allows to generate homozygous mutant clones in the compound eye of the F1 generation by mitotic recombination in a heterozygous background. The TRPL-eGFP reporter gene enables us to detect translocation of the ion channel in living flies. We screened more than 5300 mutagenized flies for defects in translocation of the TRPL-eGFP ion channel, using an optical phenomenon, the so called deep pseudopupil. Six mutants were isolated, one carrying the mutation on chromosome 2L and five bearing the mutation on chromosome 3R. In these mutants TRPL-eGFP is always located in the rhabdomeres irrespective of the light condition in which the flies are raised. The mutation causing the translocation defect of TRPL-eGFP is recessive in all mutants.

To gain insight in the subcellular location of TRPL-eGFP we investigated the mutants by fluorescence microscopy. Using this method the translocation defect of TRPL-eGFP could be confirmed. As revealed by immunohistochemistry native TRPL shows the same translocation defect as TRPL-eGFP in the mutants tested so far.

In order to investigate, if the mutation affects genes that are already known to disrupt translocation of the ion channel TRPL-eGFP, complementation studies are being performed using the following mutants: ninaA, ninaC and ninaD for chromosome 2L and ninaB, ninaE and trp for 3R. Mutants which do not fall into these complementation groups will be mapped by recombination mapping using molecularly defined P element insertions.

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# Effect of hypothyroidism and lack of TH receptors alpha and beta on the Expression of BK channels

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Thyroid hormone (TH) is essential for the development of normal hearing. Lack of TH in a critical developmental period from E17 to P12 leads to morphological and functional deficits in the organ of Corti and the auditory pathway. We investigated the effects of TH deficiency on the expression of the fast activating K+ current (IK,f) carried by the Ca2+- and voltage activated K+ (BK) channels in IHCs using patch-clamp recordings from IHCs of hypothyroid rats.

IHCs of control rats acquired a rapidly activating outward K+ current and showed a steep increase in whole-cell K+ current amplitude from P12 onwards which is consistent with the expression of BK channels around the onset of hearing. In contrast, K+ currents of IHCs of hypothyroid rats did not increase substantially after P12 and did not show rapid current activation until P27. After P27, part of the IHCs from hypothyroid animals expressed IK,f while their neighbours did not. This mosaic pattern of expression could be confirmed by staining the BKalpha subunit with whole-mount immunocytochemistry.

Analysis of the kinetics of IK,f current activation revealed larger (approximately doubled) activation time constants (about twice as large) in hypothyroid IHCs compared to euthyroid controls. In control IHCs, 100 nM iberiotoxin blocked only part of IK,f. The activation time constants of these iberiotoxin-resistant fast K+ currents were similarly elevated as those of the fast K+ currents of BK-expressing hypothyroid IHCs. This confirms heterogeneity of BK current properties in control IHCs (Marcotti et al., 2004, J Physiol 557, 613-633) and suggests incomplete acquisition of mature BK currents in hypothyroid IHCs. The different properties of BK currents could be caused by different splice variants of the BKalpha subunit or differential participation of BKbeta subunits.

Data on expression and properties of BK channels in TH receptor knockout mice (TRalpha-/- and TRbeta-/-) will be presented.

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# *Endocytosis of ion channels in the stria vascularis: Of any importance for hearing?*

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**It has long been accepted that marginal cells of stria vascularis are involved in the generation of the endocochlear potential and the secretion of K+ ions (Wangemann, 1995). K+ is the major cation in endolymph and the charge carrier for mechano-electrical transduction currents and the generation of the endocochlear potential. Accordingly several forms of hereditary deafness are due to mutations of potassium channels, including KCNQ1/KCNE1 in marginal cells of the stria vascularis (Wangemann, 2002; Jentsch et al., 2004). Membrane recycling of apically expressed proteins is a mechanism to regulate the surface expression level of plasma membrane receptors or transport proteins. Once incorporated in endosomes, proteins can recycle back to the trans Golgi network or to the plasma membrane. If these mechanisms may also play a role for the functionally important surface expression of strial potassium channels is elusive.**

**Using mice with gene deletion in a most abundant lysosomal integral membrane protein type 2 (LIMP2) and mice with deletion of Megalin, the low-density lipoprotein receptor protein (LRP) we unraveled the correlation and importance of both proteins for proper strial potassium channels expression and hearing.**

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# Effect of thyroid hormone deficiency on $Ca^{2+}$ currents and exocytosis in cochlear inner hair cells

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Thyroid hormone (TH) deficiency during pregnancy causes mental retardation, dwarfed growth and deafness. It is known that TH is necessary in the phase of final differentiation of the inner ear that extends between birth and the onset of hearing at postnatal day (P) 12 in rats and mice. In this work, the effects of TH-deficiency on $Ca^{2+}$ current dynamics and on exocytosis of the sound-transducing receptor cells - the cochlear inner hair cells (IHCs) - were analyzed. We used two models of TH deficiency, hypothyroid rats and athyroid $Pax8^{-/-}$ mice, and performed current and capacitance measurements.

Neonatal IHCs (P9) of control and hypothyroid rats (hypo) showed comparable voltage-activated $Ca^{2+}$ currents and exocytosis. At P20, $Ca^{2+}$ currents of control IHCs were reduced to 40% of the neonatal peak value, a down-regulation that was lacking in age-matched hypothyroid IHCs. Similar to the $Ca^{2+}$ currents, exocytosis of control IHCs was also reduced at P20, but showed a larger efficiency (i. e. capacitance increase normalized to the $Ca^{2+}$ influx) than in neonatal IHCs. Age-matched hypothyroid IHCs showed exocytosis with a low, neonatal-like efficiency. Analysis of $Ca^{2+}$ currents and exocytosis efficiency in $Pax8^{-/-}$ and wildtype mice confirmed the lack of downregulation of the $Ca^{2+}$ current amplitudes and the missing increase in exocytosis efficiency in the third postnatal week under hypothyroid conditions.

An unexpected finding was that otoferlin, a protein thought to be the $Ca^{2+}$ sensor for vesicle fusion in IHCs, was not present in hypothyroid rats. Robust exocytosis in IHCs of these animals however questions the presumed role of otoferlin for exocytosis.

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# Ionic currents through $Ca^{2+}$ channels in mature mouse Inner Hair Cells under mobil phone field exposure

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The increasing use of mobile phones over the last years lead to an increased public concern with respect to possible health risks involved. In response to those public concerns the WHO orchestrates a world-wide research program to which this study contributes via the German "Mobilfunkforschungsprogramm" organised by the "Bundesamt für Strahlenschutz".

During use, mobile phones are in close proximity to the ear and most of the energy of their communcation signals is directly absorbed by the area around the ear. We therefore investigated whether mobile phone field exposure had any influence on the functioning of the voltage-activated L-type $Ca^{2+}$ channel $Ca_V1.3$ in mouse inner hair cells. The specific $Ca^{2+}$ channel is driven by the receptor potential of the hair cells triggers exocytosis via $Ca^{2+}$ influx and thus plays a crucial role in the signal transduction of inner hair cells.

Using the whole-cell patch-clamp configuration $Ba^{2+}$ currents through the $Ca^{2+}$-channels were measured for 5 mins prior to an exposure, during a 20 min exposure phase and for 15 min after exposure (40 min measurement time). All measurements were carried out under randomized blinded exposure conditions for SAR values of 0.02, 0.2, 2, 20 W/kg and sham, i.e. the specific measurement condition is unkown until the data is completely sampled. Two different exposure signal types simulating GSM 1800 and UMTS communication signals were used.

To isolate the $Ba^{2+}$ currents $K^+$ currents were blocked by the application TEA and 4AP extracellularly and by the use of $Cs^+$ in the pipette.

The following parameters were extracted from I-V-relationships and current traces: Maximum current, voltage of half-activation, steepness of activation, series-resistance and leak-resistance.

After unblinding the conditions a statistical analysis will be carried out to provide evidence whether the exposure with mobile phone communication fields has any influence on the $Ca^{2+}$-channels.

# Analysing the $Ca^{2+}$ currents in inner and outer hair cells of mice lacking the beta3- or beta4 auxiliary $Ca^{2+}$ channel beta subunit

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Voltage-dependent $Ca^{2+}$ channels ($Ca_V$) consist of the pore-forming alpha1 subunit (SU) and auxiliary SUs beta, alpha2-delta and gamma. The intracellular beta SU is responsible for membrane trafficking of the alpha1 SU and modulate current properties like amplitude and kinetics. $Ca^{2+}$ currents in cochlear inner (IHC) and outer (OHC) hair cells are L-type currents and almost exclusively flow through the alpha1 SU $Ca_V1.3$. They are therefore a suitable system to test if there is a preference of this particular alpha1 SU for a certain beta-SU and if the lack of one of the SUs can be compensated by another one. Previous data have shown the presence of mRNA of all four beta SU in hair cell-specific cDNA with a preference of beta1 and beta3 in IHC and OHC cDNA.

$Ba^{2+}$ currents ($I_{Ba}$) were measured in neonatal IHCs and OHCs (P1-P7) and in mature IHCs (P18-P20) in $beta3^{-/-}$ and $beta4^{-/-}$ mice and controls. $I_{Ba}$ amplitudes, voltage-dependence of activation, activation time constants and inactivation after 300 ms depolarization were determined. In $beta3^{-/-}$ mice, there was no difference in these parameters neither in IHCs nor OHCs except a stronger inactivation in $beta3^{-/-}$ OHCs (7.7±4.6%, n=6) compared to control (2.2±1.5%, n=5). In $beta4^{-/-}$ mice, no difference in these parameters could be detected neither in IHCs nor OHCs except a reduction of $I_{Ba}$ in mature $beta4^{-/-}$ IHCs (213±78pA, n=13) compared to $I_{Ba}$ in $beta4^{+/+}$ IHCs (284±78pA, n=12).
These subtle differences did not affect hearing thresholds of 3 week old $beta3^{-/-}$ and $beta4^{-/-}$ mice.
In conclusion, $Ca_V1.3$ channels in IHCs and OHCs either do not contain a considerable degree of beta3/beta4 or the lacking of beta3/beta4 can be compensated by other beta SUs.

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# Functional analysis of mutations in CNGA3: altered channel function or impaired trafficking?

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Mutations in the *CNGA3* gene have been associated with complete and incomplete forms of autosomal recessive achromatopsia, a disorder that is characterized by lack of color discrimination, severely reduced visual acuity, photophobia and nystagmus. *CNGA3* encodes the A-subunit of the cone cyclic nucleotide-gated (CNG) channel. The CNG channel is an essential component of the phototransduction cascade that generates the membrane hyperpolarization signal upon light stimulation. The aim of our study was the functional characterization of CNGA3 channels with the mutations E228K, R283Q, T291R, R439W and E590K in a heterologous expression system.

CNGA3 expression constructs based on the pcDNA3.1 vector were generated for these 5 mutations by means of in vitro mutagenesis using a wildtype full-length cDNA construct as template. Purified plasmid DNA was transiently expressed in HEK293 cells and the calcium permeation of the mutant channels analyzed with calcium-imaging using the calcium-sensitive fluorescent dye Fura-2 and the membrane-permeable cGMP analogue 8-Br-cGMP. Selected mutations were characterized in more detail with the patch-clamp technique. Membrane patches with inside-out configuration were obtained from transfected HEK cells. Dose-response relationships for the cGMP- and cAMP-activation of mutant channels were established and the primary biophysical parameters such as the ligand concentration of half-maximal activation ($K_{1/2}$) and the hill coefficient (h) were determined and compared to the reference values of wildtype channels.

Previous electrophysiological measurements revealed that certain mutations such as R563C do not cause any change in dose-response behaviour, but are associated with severely reduced macroscopic currents. This may be explained by either altered channel function or disturbed folding/trafficking resulting in reduced channel density in the cell membrane. In order to distinguish between these two situations, we used calcium-imaging on transfected HEK cells that had been cultured at different temperatures (28 °C or 37 °C). We found that a lower incubation temperature generally increases the number of calcium-imaging positive cells and that it even rescues some mutants (e.g. R283Q, T291T and R439W) that did not show any reproducible calcium influx in imaging experiments with cells that had been incubated at 37 °C. These findings suggest disturbed folding or trafficking as primary cause of the defect observed with those channel mutants.

# An increase in persistent Na current induced by a mutation in the $Na_V1.2$ channel is associated with neonatal-infantile seizures

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Benign familial neonatal-infantile seizures (BFNIS) is an autosomal dominant epilepsy syndrome characterized by afebrile partial or secondarily generalized seizures occurring in the first weeks and months of life usually disappearing after the first year of life. We identified a novel mutation in the brain sodium channel $Na_V1.2$ (gene: SCN2A) in a 3-generation family with benign familial neonatal infantile seizures (BFNIS). To study the functional consequences of this mutation, it was introduced into a human isoform of the $Na_V1.2$ channel. Wild type (WT) and mutant channels were transiently co-transfected with beta1 and beta2 subunits in a 1:1:1 ratio in the mammalian tsA201 cell line. The co-expression of all subunits was controlled using bicistronic constructs co-expressing GFP and CD8, respectively. The electrophysiological properties of the resulting Na currents were examined using the whole cell patch clamp technique. In comparison to WT channels mutants displayed a 2-fold enhanced persistent current when measured 70 ms after onset of the depolarization, which was completely blocked by application of tetrodotoxin (TTX), a specific blocker of voltage-gated Na channels. In addition, we found a 1.5-fold acceleration in recovery from slow inactivation. In contrast, we did not detect significant change between WT and mutant channels for the voltage-dependencies of steady-state activation, fast and slow inactivation, and for the kinetics of activation, deactivation, fast and slow inactivation and the recovery from fast inactivation. The observed gain-of-function, in particular the increase in persistent Na current, may well trigger epileptic seizures by increasing Na influx resulting in membrane hyperexcitability. The age dependence of this syndrome could be explained by the transient expression of $Na_V1.2$ channels in axons of pyramidal neurons which has been described in previous studies.

# A HYPERPOLARISATION-ACTIVATED CHLORIDE CURRENT IN THE DROSOPHILA MUSCLE

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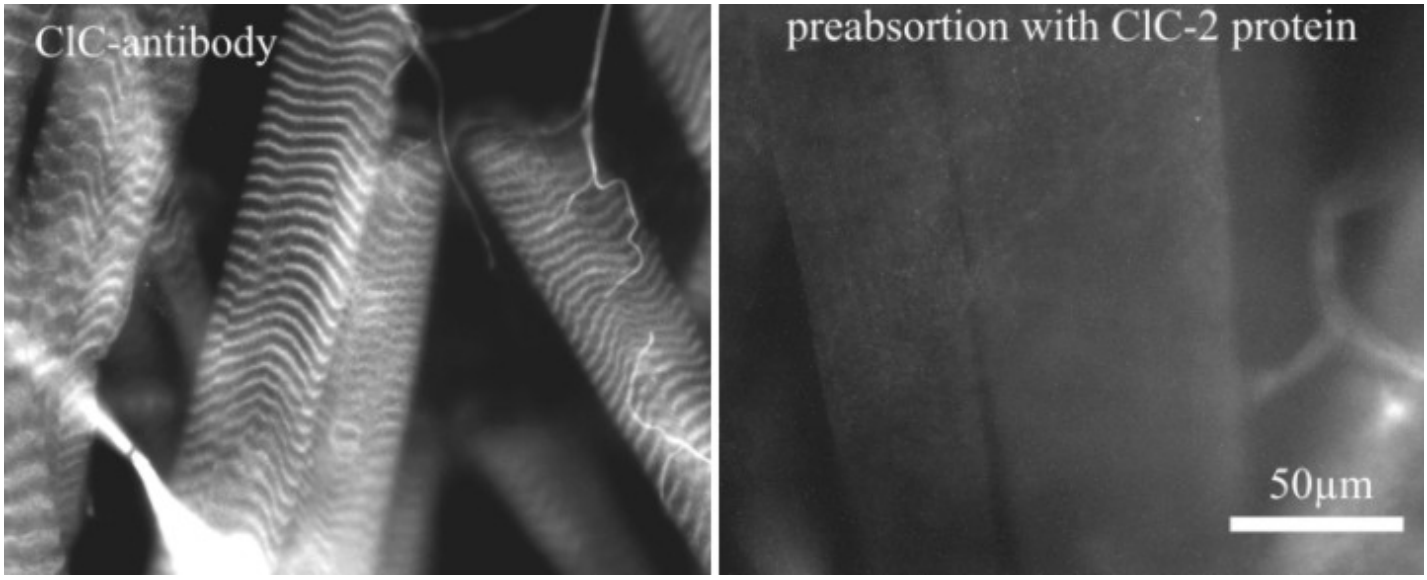
The body wall muscle of Drosophila larvae has evolved as one of the standard preparations for studies of synaptic and non-synaptic aspects of excitability and a number of muscular cation channels have been determined and characterized. So far, no non-synaptic Cl- current has been described for this preparation. The presence of a Cl- conductance, however, would be expected which to regulate the muscle fiber's internal milieu. We give here a first account on a hyperpolarization - activated Cl current in Drosophila larval muscle which seems to be carried by DmClC-a.

A chloride current which slowly activates on hyperpolarization was investigated in Drosophila larval muscle 6 using the two-electrode voltage clamp methode. Sizeable currents were only observed when the intracellular chloride concentration ([Cl-]i) was elevated by diffusion and / or iontophoretic injection of Cl- from the electrodes. The time course of the current was rather variable and usually required two exponentials to be accurately described with tau_slow = 1.77 + 0.51 s and tau_fast = 3.53 + 1.28 s. The instantaneous IV relationship of the current was practically linear and the reversal-potential shifted on lowering [Cl-]o in the positive direction. The current was resistant to 10 mM Cs+, but also to 1 mM Zn++ or 1 mM Cd++ whereas considerably reduced by 9-AC (9 anthracene-carbloxylic acid). Steady state activation of the Cl- current was characterized by V0.5 of ≈ 105 mV and a slope factor of 11 mV. The current was increased on lowering extracellular osmolality and was sensitive to extracelullar pH. RT-PCRs demonstrate the expression of all three ClC - genes in body wall muscles. Only one of these, DmClC-a which is closely related to mammalian ClC-2, is considered a plamalemmal channel and has been recently cloned and heterologously expressed. Additional immunhistochemichal detection with anti-ClC antibodies revealed the expression of the ClC resticted to the M-line of the muscle fibre.
We conclude that this ClC-2 - like channel accounts for the Cl- currents observed in Drosophila muscle. Further experiments will focus on modulation and functional relevance of DmClC-a.



# A novel mutation in the voltage sensor of the Kv7.2 channel causes myokymia

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Myokymia or neuromyotonia is caused by a hyperexcitability of peripheral motor neurons. An impairment of outward potassium currents has been discussed as an underlying mechanism. Recently, a mutation localized within the voltage sensor region in transmembrane segment S4 of the Kv7.2 (*KCNQ2*) channel has been found in a family with benign familial neonatal convulsions (BFNC) and myokymia. Here we describe a new mutation at the same position of the Kv7.2 channel (R207Q) in a patient suffering solely from myokymia since about the age of six years. Neonatal seizures were denied by his mother.

To elucidate the pathomechanism of the mutation, Kv7.2 wild type (WT) and mutated channels were heterologously expressed in Xenopus oocytes and functionally characterized by the two-electrode voltage-clamp method. We found a prominent rightward shift of the conduction-voltage curves and a marked slowing of the time constants of activation. Furthermore co-expression studies of the mutant with the WT channel revealed a dominant negative effect accounting for a large reduction of the relative current amplitudes in the subthreshold range between -50 and -30 mV.

To date about 30 BFNC causing mutations are known in *KCNQ2* and *KCNQ3* genes, clustering in the pore and in the C-terminus of the channel protein. Many of these mutations cause a complete loss-of-function predicting a 50% reduction of current amplitudes by a haploinsufficiency mechanism, since a dominant-negative effect is usually not observed in in vitro studies. In contrast, the R207Q mutation described here, and the previously described R207W lead to a dominant negative effect and probably reduce channel function much more effeciently. Thus, the central nervous system seems to be more vulnerable then peripheral motor neurons since these two are the only mutations reported to be associated with myokymia. The absence of neonatal convulsions in the patient carrying the R207Q mutation could be attributed to a reduced penetrance, as observed in some BFNC patients. This could be explained by a different genetic background which compensates for the function of central but not of peripheral neurons.

# TRESK tandem-pore potassium channels are the major component of background potassium currents in murine DRG neurons

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TRESK (KCNK18) is the most recently identfied member of the tandem pore potassium channel (K2P) family, which represent the molecular basis of background potassium currents. In contrast to the human isoform which is specifically expressed in spinal cord, we detected mouse TRESK mRNA in several epithelial and neuronal tissues including lung, liver, kidney, brain and spinal cord. As revealed by RT-PCR and in situ hybridization most prominent expression was found in dorsal root ganglion (DRG) neurons. Here we identify TRESK-like potassium currents in cultured primary neurons from murine dorsal root ganglia (DRG). In order to unequivocally determine TRESK currents in cultured cells we first expressed and characterized mouse TRESK (mTRESK) channels in Xenopus oocytes. Depolarizing ramp recordings from -150 to +60 mV induced an instantaneously activating outward rectifying potassium current in 2 mM extracellular K+. Mouse TRESK channels, as well as members of the TASK and TALK subfamilies, are regulated by changes in external pH. Upon acidification to pH 6.0, TRESK currents maximally decreased by 60% ± 13 % (n=9) with a pK of 6.9. Application of the antiarrhythmic drug quinidine (100 µM) inhibited the current by 66.7 ± 5.3% with an IC50 value of 73.5 µM. It has been shown that TRESK currents are also regulated by Gq-coupled seven-helix receptors, e.g. muscarinic M1 receptors. We demonstrated that TRESK is very efficiently activated by 5-HT2C receptors in Xenopus oocytes and augmented the initial current by 277 ± 209 % (n=9). As TRESK channels are mainly expressed in the spinal cord and in peripheral neurons, we adressed the question whether these currents are also regulated e.g. by histamine receptors. Application of 100 nM histamine augmented TRESK currents by 58.6 ± 59 % (n=12) when expressed in Xenopus oocytes together with histamine H1 receptors.

Whole-cell recordings from DRG neurons elicited a standing outward current with an amplitude of 1.20 ± 0.12 nA (n=25) when depolarized from a holding potential of -70 mV to -25 mV. This outward current reversed at -85 ± 11 mV as would be expected from the Nernst equation for a potassium selective current. External acidification from pH 7.4 to 6.0 reduced this current by 54% to 0.56 ± 0.07 nA (n=25). To investigate this pH-sensitive current in more detail we prepared DRG neurons from functional TRESK knockout mice with a defective TRESK gene generated by ENU-based mutagenesis (Ingenium Pharmaceuticals). In these neurons, the standing outward current recorded in the whole-cell configuration only amounted to 0.887 ± 0.137 nA (n=11) and acidification to pH 6.0 reduced the current amplitude by 31 % to 0.563 nA ± 0.047 (n=11). Probably the remaining pH-sensitive current in these cells results from other K2P channels, which were identified by RT-PCR (e.g. TASK-1, TASK-2 and TASK-3, TALK-1 and TALK-2). Nevertheless, transcripts of TRESK subunits were found to be most prominent and may thus form the major background potassium current in murine DRG neurons.

# TASK forces stroke: the functional impact of the TWIK-related acid-sensing potassium channels 1 and TASK3 for cerebral ischemia

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Ischemic neuronal damage is a common cause of neurological disability, thus making a better understanding of the underlying cellular mechanisms and the search for neuroprotective agents important. One critical question concerns the high susceptibility of central nervous system (CNS) cells to ischemic injury.
Striking events in the early cascade of stroke formation are the depletion of oxygen ($O_2$) and a decrease in pH in ischemic brain areas. Both aspects - pH decrease and $O_2$ fall - can cause depolarization of the resting membrane potential of CNS neurons. This is associated with increased neuronal excitability and an enhanced $Ca^{2+}$-influx resulting in the activation of a number of cytotoxic cascades. Thus blocking the subsequent neurotoxic biochemical cascade is a critical determinant of cell survival during brain injury. Based on their pharmacological profile and their actions on basic cellular parameters (e.g. membrane potential, action potential generation) the K2P channels TWIK-related acid-sensitive K+-channel 1 (TASK1) and TASK3 are interesting molecular targets of the described cascade leading to cerebral ischemia.
Here, using a combination of patch-clamp recordings and an in vivo animal model for stroke generation, we demonstrate a clear functional impact of TASK1 and TASK3 channels on cerebral ischemia in vitro and in vivo. Inhibition of TASK1 and/or TASK3 mediating an standing outward current in thalamic neurons, e.g. by pH-reduction (42 ± 2%) or $O_2$ depletion (36 ± 5%), could be identified as being largely responsible for membrane depolarization, increased input resistance and switch in action potential generation under ischemic conditions. TASK1 channel modulation by anandamide (10mg/kg) in a transient occlusion model of the middle cerebral artery resulted in an earlier onset of stroke generation and an increase in infarct size of appr. 70% compared to control animals.

# Poster Topic

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**T12-10C** Glia cells and extracellular matrix molecules in the snail (*Lymnaea* and *Helix*) central nervous system: histochemical demonstration
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# A Novel Marker for Oligodendrocytes and Analysis of Extracellular Matrix Signalling for Oligodendrocyte Development

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Oligodendrocyte precursor cells (OPCs) are specified to become the myelin-forming cells of the central nervous system. Although several markers for the study of OPC lineage progression are available, it is of importance to extend this molecular repertoire in order to precisely characterize the particular differentiation state of a developing oligodendrocyte (OL). Here, we present the monoclonal antibody (mAb) 486 as a novel marker for mature OLs. It recognizes a cell surface epitope which is strongly expressed on complex branched and membrane forming OLs in culture. The staining pattern, however, does not completely overlap with any known OL lineage marker that has been analysed so far, such as A2B5, NG2, O4, GalC, MAG and MBP. The mAb 486 also turned out to be a useful marker for developing white matter tracts of the central nervous system as 486-immunoreactivity begins during the respective peaks of myelination in the white matter of the brainstem, the cerebellum and the forebrain. The 486-immunoreactivity persists into adulthood, but to a much lesser extent in white matter tracts than in myelinated grey matter.

Furthermore, we investigated the impact of extracellular matrix components as potential spatiotemporal cues for OPC lineage progression. We focussed on the molecular functions of the ECM-glycoproteins Tenascin C (Tnc) and Tenascin R (Tnr) and of the chondroitin sulfate proteoglycan (CSPG) Phosphacan/RPTPβζ on the differentiation behaviour of OL lineage cells. All of these molecules are dynamically expressed during CNS development. The ECM components Tnc and Phosphacan/RPTPβζ are produced by radial glia and/or immature astrocytes during early brain development, whereas Tnr is detectable in OLs and in subsets of neurones at later stages. Our data show that Tnc and CSPGs are strongly inhibitory for OL differentiation and myelin membrane formation whereas Tnr seems to favour OL maturation. Currently, we investigate the signalling mechanisms that drive the observed changes of the OL differentiation.

In conclusion, the ECM components analysed in our study can be viewed as potent regulators for OL development that probably act via distinct pathways at different stages of OPL lineage progression, from the OPC stage towards a mature, myelin gene expressing cell.

# Modulation of Retinal Neurite Outgrowth by Glial Derived Extracellular Matrix Proteins: Tenascin-C and Chondroitin Sulphate Proteoglycans

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The glial-derived extracellular matrix (ECM) gylcoprotein Tenascin-C (TN-C) is a multifunctional molecule that could either promote or inhibit neurite outgrowth depending upon the neuronal cell types. However, the knowledge about effects of TN-C and its domains in the retinal neuronal network is still limited. To elucidate potential roles of TN-C and different FNIII domains for neurite outgrowth in the visual system we performed in vitro experiments using rat retinal stripes from embryonic stage E18 and retinal ganglion cells (RGCs) at postnatal stage P7. The retinal stripes /RGCs were plated on PDL/TN-C or FNIII domains A1A2, A1D, CD, 78 and D6 (25 and 50 µg/ml). The Morphometric analysis of neurite lengths was performed with Image Tools Software by measuring 10 longest neurites of the retina. TN-C significantly increased neurite outgrowth at 25 µg/ml, whereas it did not show any significant changes in the neurite length from P7 retinal neurites. At E18 D6 and CD have an inhibitory effect at the lower concentration, while at the higher concentration both domains had stimulatory effect. At P7 there was no difference in neurite outgrowth when compared with control. A1D had an increasing effect at both concentrations at E18, whereas at P7 the lower concentration had an inhibitory effect. BD remained neutral at both concentrations at P7, but at 50 µg/ml it showed significant increase. At P7 A1A2 showed significant increase in the neurite outgrowth. These results suggest that in the rat visual system the distinct domains of TN-C have different effects on retinal axon outgrowth depending upon the developmental stages.

Since the purified ECM-molecules do not always represent the biological environment for neurites, we performed cocultures of retinal explants either with glial cells A7, Oli-neu, Mueller glia (MG) and cortical astrocytes (CA) or their matrices. The cell matrix from A7, CA and MG was stimulatory to neurites, whereas the cell matrix from Oli-neu was inhibitory. A7, CA and MG monolayers were also stimulatory towards neurite outgrowth, whereas Oli-neu was more inhibitory than its matrix. Since all the cells express large TN-C, we then tested the hypothesis whether neurite outgrowth from A7, MG and CA was attributed to TN-C. One functional site of TN-C has been localized to the FNIII-D domain, which is involved in the neurite outgrowth. The treatment of the glial cells with an antibody against FNIII-D domain showed a significant reduction of the neurites from only MG cells. To determine if the differential ability of the cells to support neurite outgrowth correlates with CSPG-GAG chains, glial cells were treated with ChABC enzyme. The ChABC increased the neurite outgrowth in CA and A7, whereas MG showed reduction in the neurite length. Olineu did not show any effect before and after the enzyme treatment. Taken together, these results suggest that the ECM molecules produced by glial cells play an important role in the regulation of retinal neurite outgrowth (Supported by DFG, SFB 509 and IGSN).

# Cathepsins S and X, secreted from microglia, trigger neuronal cell death in a conditioned medium transfer model

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Several proteases have been shown to influence neuronal signalling and survival after release from activated microglia. They contribute to microglial migration and the breakdown of invading organisms. However, they may also aid the initiation and maintenance of inflammation and neuronal cell death.
The aim of this study was to investigate whether the lysosomal cysteine proteases cathepsins S and X are secreted from activated microglial cells and trigger microglia-induced neuronal cell death. For these purposes, we established a coculture model, composed of a neuronal cell line intoxicated with the supernatant of the lipopolysaccharide-stimulated microglial cell line BV-2. Furthermore, we tried to influence glial activation and neurotoxicity with different cathepsin inhibitors.
Western blotting, immunohistochemistry and protease activity tests revealed a release of the active forms of the two proteases in stimulated BV-2 cells. In addition, reverse transcriptase (RT-) PCR displayed an up-regulation of cathepsin S mRNA in activated BV-2 cells. Conditioned medium from these cells induced neuronal cell death, while incubation of BV-2 cells with membrane permeable cathepsin inhibitors prevented the secretion of the active forms of cathepsins S and X and diminished microglia-induced neurotoxicity.
The high expression of cathepsins S and X in activated cultured glial cells and our findings that inflammatory mediators stimulate their secretion from microglia suggest an essential role for these two proteases in inflammatory conditions. Inhibition of cathepsins decreases microglia-mediated neuronal toxicity, indicating that cathepsins trigger neuronal cell death in our model. Inflammatory conditions associated with glial activation are believed to have an important impact on neurodegeneration, suggesting that cathepsins S and X might be useful drug targets for the treatment of acute and chronic neurodegenerative diseases like Parkinson´s disease and Alzheimer´s disease.

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# Analysis of $GABA_A$ receptor currents in hippocampal glial cells

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Previous work identified two different types of glial cells with hGFAP promoter activity coexisting in the hippocampus. These cells possess either glutamate receptors or glutamate transporters (GluR and GluT cells). The GluR-type is equipped with functional AMPA and GABAA receptors and receives direct synaptic input from glutamatergic and GABAergic neurons. The functional impact of synaptic innervation of GluR cells is still unclear. Here we further characterised GABAA receptors expressed by GluR cells. To compare pharmacological properties and gene expression profile, the patch clamp technique and single cell RT-PCR was applied to GluR cells in acute slices or to freshly isolated cells.

GABA activated slowly desensitizing receptor responses in GluR cells (desensitization about 2 s). The GABAA receptor agonist, muscimol, mimicked GABA-induced responses. Currents were sensitive to the GABAA receptor antagonist, bicuculline. To elucidate the GABAA receptor subunit composition, we tested the $Zn^{2}+$ sensitivity of receptor responses as well as their modulation by benzodiazepines. Micromolar concentrations of $Zn^{2+}$ blocked GABA responses effectively. Preincubation of modulators of GABAA receptors, benzodiazepines and barbiturates, increased the responses. Single cell transcript analysis subsequent to functional characterization revealed predominant expression of alpha 2 and 4, beta 2/3, and gamma 2 subunits.

To determine the effect of GABAA receptor activation on membrane potential, perforated patches were obtained from GluR cells in situ. In the current-clamp mode, maximal activation of the GABA-mediated chloride conductance depolarized the cells to -20 ± 6 mV (n = 6). Comparison of reversal potentials obtained with different intracellular chloride concentration (whole-cell mode) revealed a physiological $[Cl^-]_i$ of GluR cells of about 60 mM.

During GABAA receptor activation, large tail currents were observed when stepping the membrane voltage from +20 mV to -70 mV. These tails currents were due to activation of the chloride permeable receptors and increased after prolonged depolarization. The data suggest that glial depolarization is accompanied by an accumulation of intracellular chloride.

# Age-dependent regulation of Kir4.1 channel expression in hippocampal astrocytes

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Astrocytes in the CNS possess inwardly rectifying $K^+$ (Kir) channels which are thought to be involved in extracellular $K^+$ buffering. Properties of Kir currents were investigated in mouse hippocampus using the patch clamp technique combined with immunocytochemistry, immunoblotting and single cell RT-PCR. Hippocampal astrocytes were electrophysiologically characterized by large resting $K^+$ currents which dominated the current patterns of these cells beyond postnatal day 3. Single cell RT-PCR revealed expression of the Kir4.1 subunit in all cells, whereas Kir5.1, another glial Kir subunit that co-assembles with Kir4.1, was scarcely expressed. Kir5.1 confers a pronounced pH sensitivity to heteromeric Kir4.1/Kir5.1 channels. Moreover, in Kir4.1-deficient mice, astrocytes were almost devoid of Kir currents and lacked negative resting potentials. Developmental up-regulation of Kir4.1 expression was discovered both on the mRNA and protein levels. This increase in astroglial Kir4.1 channels parallels a significant drop of the extracellular volume fraction of the developing hippocampus, suggesting an important role of Kir4.1 in extracellular $K^+$ buffering.
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# Functional and molecular heterogeneity of 'complex' glia cells in the hippocampus

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Glial cells of the brain for a long time have merely been considered passive, supporting elements. However, data obtained over the past few years have provided substantial evidence for the integration of glial cells into synaptic circuitry. These studies have also unravelled a hitherto unexpected heterogeneity of subpopulation of neuroglial cells with distinct morphological and functional profiles. A subset of these cells in the hippocampus, previously termed 'complex cells' or 'GluR cells', receives direct synaptic input from glutamatergic and GABAergic neurons. They express the proteoglycane NG2 (Bergles et al., 2000; Lin and Bergles, 2003), but also S100β and GFAP transcripts (Jabs et al., 2005), giving rise to a debate on the identity and lineage relationship of these cells. To further explore this issue, we accomplished comparative investigations on wild type and transgenic mice expressing green fluorescent protein (EGFP) under control of the human GFAP promoter (hGFAP/EGFP mice). Patch-clamp recordings were combined with single cell transcript analysis to correlate functional properties with the gene and antigen profile of individual cells. Our data confirm that NG2-positive cells in the hippocampus constitute a heterogeneous cell population, and also suggest the existence of complex glial cells lacking this marker. Future work has to disclose the physiological impact of these distinct glial cell populations.

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# Functional diversity of radial glia-like precursor cells in the adult dentate gyrus

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In the adult dentate gyrus (DG), one of the neurogenic regions in the mature brain, cells with a radial glia (RG)-like morphology represent the putative stem cells that can give rise to new neurons and glial cells. In the course of neurogenesis, RG-like cells divide to generate precursors expressing immature neuronal features. In contrast to precursors that are committed to a neuronal fate, RG-like cells apparently do not receive synaptic input. Therefore, alternative environmental cues must be involved in the control of proliferation and differentiation of RG-like cells in the DG. In the present study, we combined patch clamp analysis, biocytin filling and single cell rt-PCR to test whether RG-like cells are coupled through gap junctions and investigate the glutamate sensitivity of these cells. To identify RG-like cells in situ, two different transgenic mice models were used, expressing EGFP under the control of the nestin and GFAP promoters, respectively. We provide evidence that a majority of RG-like cells in the adult dentate gyrus display gap junctional coupling and predominantly express the astrocytic gap junction protein, CX43. In contrast, no coupling was observed between nestin/EGFP positive precursor cells already expressing neuronal properties. Rapid application of glutamate to patches excised from the soma of RG-like cells identified transporter currents. Future work has to unravel whether gap junctional coupling of RG-like precursors may serve a role in modulating their proliferative activity.

# Activity-induced sodium signals in Bergmann glial cells and Purkinje neurons

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Glial glutamate transporters take up the majority of synaptically released glutamate (Bergles DE & Jahr CE, 1998, J Neurosci 18:7709-7716). They are thus central for the termination of synaptic transmission and protect neurons from excitotoxicity induced by elevated levels of extracellular glutamate. They are electrogenic and use the electrochemical gradient of sodium to move glutamate into the cell (Marcaggi P & Attwell D, 2004, Glia 47: 217-225). Electrophysiological recordings indicated that glutamate uptake results in a fast decline of glutamate in the synaptic cleft and shapes the time course of synaptic conductance (Brasnjo G & Otis TS, 2004, PNAS 101: 6273-8; Bordey A & Sontheimer H, 2003, J Neurophysiol 89: 979-988). Because both glutamate receptor activation and glutamate transport involve movement of sodium, glutamatergic transmission is likely to result in intracellular sodium increases, which will reduce the driving force for glutamate uptake. In the present study, we performed intracellular sodium measurements to analyse activity-induced intracellular sodium transients in processes of Bergmann glial cells and Purkinje neurons.

Experiments were carried out on acute tissue slices of mouse cerebellum (postnatal day 11-15). Fluorescence imaging was performed using a variable scan digital imaging system (TILL Photonics) attached to an upright microscope. Cells were loaded through the patch-pipette with the fluorescent sodium-sensitive dye SBFI. Fluorescence signals were collected in regions of interest and the fluorescence ratio (345/385 nm) was calculated. Calibration of sodium signals was performed as described earlier (Meier SD, Kovalchuk Y & Rose CR, 2006, J Neurosci Methods 155: 251-259. Membrane currents were recorded in the whole-cell patch-clamp mode. Bergmann glial cells were generally held at a membrane potential of -80 mV, holding potential of Purkinje neurons was -70 mV.

Our experiments demonstrate that short bursts of parallel or climbing fibres activation induce inward currents and sodium transients in the mM range in processes of Purkinje neurons and Bergmann glial cells. Activity-induced currents and sodium transients in Purkinje neurons were mainly caused by activation of AMPA receptors in Purkinje neurons, whereas in Bergmann glial cells, sodium transients were predominantly caused by glutamate uptake. In the current clamp mode, synaptic stimulation resulted in a depolarisation by 12 mV in Bergmann glial cells. The intracellular sodium increase as well as the membrane depolarisation result in a calculated decrease in the driving force for glutamate uptake by about 30% in Bergmann glial cells. Because other ion gradients change as well, it is to be expected , however, that the change in driving force for glutamate uptake in the intact tissue is actually smaller than 30%. Intra- and extracellular measurements of concentrations of other ions (pH, potassium) during activity will help to solve this question and to provide a realistic model of glutamate uptake function.

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# GABA-induced calcium signaling in hippocampal astrocytes

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GABA (γ-aminobutyric acid) is the most important inhibitory neurotransmitter in the mature mammalian brain. Besides its function as a classical neurotransmitter, it serves essential functions in the developing nervous system, such as modulation of precursor proliferation, migration and maturation. The cellular actions of GABA are mediated by ionotropic ($GABA_A$ and $GABA_C$) as well as metabotropic receptors ($GABA_B$). Upon $GABA_A$ receptor activation, adult neurons are hyperpolarized due to chloride influx, whereas immature neurons are depolarized due to chloride efflux. The inhibitory effects following $GABA_B$ receptor activation include a reduction of neuronal transmitter release following inhibition of presynaptic calcium channels as well as activation of GIRK channels (G-protein coupled inward rectifier potassium channels) resulting in slow IPSCs.

In both mature and immature astrocytes, GABA induces intracellular calcium transients. Calcium is an important intracellular second messenger and is essential for glia-neuron interaction, as elevations of intracellular calcium may trigger the release of neurotransmitters such as glutamate and ATP from astrocytes. In addition to causing an intercellular calcium wave within the astrocytic network the released "gliotransmitters" signal back to neurons and hence modulate synaptic transmission (Volterra and Meldolesi 2005). The cellular mechanisms of $GABA_A$ receptor-induced calcium signals in astrocytes have been elucidated in previous studies. Activation of $GABA_A$ receptors by the selective agonist muscimol evokes an efflux of chloride and a depolarization-induced opening of voltage-gated calcium channels. $GABA_B$ receptor activation by baclofen also induces intracellular calcium transients, their mechanism, however, is largely unclear. In the present study, we investigated the cellular mechanisms and developmental profile of $GABA_A$ and $GABA_B$-induced calcium signals in hippocampal astrocytes in situ.

We characterized the responses of sulforhodamine 101-positive astrocytes to muscimol and baclofen in acute hippocampal slices of rats (postnatal days 3 to 33) by using whole-cell patch-clamp and ratiometric imaging with the fluorescent calcium indicator dye fura-2. We found significant differences in the calcium responses of the astrocytes to $GABA_A$ and $GABA_B$ receptor activation throughout postnatal development, suggesting an important role of $GABA_B$-receptors during synapse formation and establishment of hippocampal networks. As reported earlier, $GABA_A$-receptor activation by muscimol induced an inward current with linear I/V relationship and influx of calcium through voltage-gated calcium channels. In contrast, calcium signals following application of the $GABA_B$ receptor agonist baclofen were blocked by depletion of intracellular calcium stores as well as sustained activation of G-proteins. These results suggest that $GABA_B$ receptors activate an as yet undescribed signaling pathway in astrocytes by coupling a $G_{i/o}$ -protein to the PLC/$IP_3$-pathway.

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*Volterra, A. and J. Meldolesi (2005). "Astrocytes, from brain glue to communication elements: the revolution continues." Nat Rev Neurosci **6**(8): 626-40.*

# Prolonged glial expression of Sox4 in the central nervous system leads to architectural cerebellar defects and ataxia

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Sox4 and Sox11 which together with Sox12 make up group C of Sox proteins are strongly expressed in the developing central nervous system. Both proteins are predominantly found in cells that have already undergone specification, but have not yet acquired their definitive differentiated phenotype. They are found in neuronal as well as glial precursors. From their expression pattern, Sox4 and Sox11 have been hypothesized to be involved in neuronal and glial maturation. So far, however, such a function has not been confirmed in vivo as the CNS develops normally in Sox4-deficient embryos as well as in Sox11-deficient embryos. Here we have analyzed the role of group C proteins in overexpression studies and have generated transgenic mice in which a Sox4 transgene is expressed under the control of the human GFAP promoter, which drives expression in radial glia as well as astrocytes. The transgenic mice suffer from severe cerebellar ataxia that resulted from a failure of Bergmann glia to undergo correct cytoarchitectural differentiation in the developing cerebellum. Continued Sox4 expression thus interferes with maturation of radial glia. Therefore we propose that occurrence of group C Sox proteins is incompatible with terminal differentiation and may be needed to keep cells in an immature state.

# Overexpression of the High-Mobility-Group Transcription Factor Sox4 Disrupts Oligodendrocyte Differentiation and Leads to Severe CNS Hypomyelination

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Oligodendrocyte development is regulated by a coordinated time-specific expression pattern of several transcription factors. Whereas Olig1, Olig2 and Sox10 are broadly expressed throughout oligodendrocyte development, the Sox4 and Sox11 proteins are transiently expressed during early stages and down-regulated at the onset of terminal differentiation. The function of Sox4 and Sox11 in oligodendrocyte development is not clear and cannot be easily inferred from loss-of-function studies because of the broad co-expression and likely functional redundancy of the two related Sox proteins. To investigate their role by gain-of-function studies, we overexpressed Sox4 in differentiating oligodendrocytes by using 3.2kb of the mouse MBP upstream region. MBP-Sox4 transgenic mice shiver and display severe CNS hypomyelination that may result from an increased apoptosis of differentiating oligodendrocytes. Thus, prolonged Sox4 expression interferes with oligodendrocyte differentiation. At about six weeks shivering subsides and myelination increases, concomitant with the promotor-dependent extinction of the Sox4 transgene. These findings support an important role for Sox4 in oligodendrocyte development before terminal differentiation, for instance by keeping cells in an undifferentiated state.

# Microglial cells release activin A upon stimulation with bacterial TLR-agonists

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**Objective:** Activin A, a member of the TGF-beta family of growth and differentiation factors, is a multifunctional cytokine with roles in the immune system and the inflammatory response. It has neuroprotective properties and can be detected within the central nervous system. Concentrations of activin A are elevated in the cerebrospinal fluid (CSF) of patients with meningitis. Microglial cells, the major constituents of innate immunity within the brain, express Toll-like receptors (TLRs) recognising exogenous and endogenous ligands. Upon stimulation with agonists of TLR2, TLR4, and TLR9, primary mouse microglial cells release nitric oxide (NO) and TNF-alpha. As the sources of the elevated activin A concentrations in CSF during meningitis have not yet been discovered, we examined whether microglial cells release activin A upon stimulation with bacterial TLR-agonists in vitro.

**Methods:** Primary mouse microglial cells (75000 cells/well, 96-well plates, in DMEM supplemented with 10% FCS and penicillin/streptomycin) were stimulated with the TLR2-agonist Pam3Cys (Tripalmitoyl-S-glyceryl-cysteine), the TLR4-agonist LPS (endotoxin), and the TLR9-agonist CpG (oligonucleotides containing unmethylated cytosin-guanosin motifs) in the presence of interferon-gamma (100 U/ml) for 24 hours. Concentrations of activin A in the cell culture supernatants were measured using a specific enzyme linked immunosorbent assay (ELISA; R&D-Systems). Nitric oxide (NO) release was quantified using the Griess reaction. Data were analysed by one-way ANOVA followed by Bonferroni´s multiple comparison test. P-values $<0.05$ were considered statistically significant.

**Results:** Activin A was not detectable in cell culture medium supplemented with interferon-gamma, Pam3Cys, LPS, or CpG, and in the supernatant of microglial cultures treated with interferon-gamma only (control cultures). Strong activation of microglial cells after treatment with the different TLR-agonists was documented by morphological analyses and measurement of NO release. Compared to control cultures, activin A concentrations were significantly elevated in the supernatants of microglial cells stimulated with the different TLR-agonists in the presence of interferon-gamma: 94.95 pg/ml after treatment with 1 µg/ml Pam3Cys ($P<0.01$), 255.20 pg/ml after treatment with 1 µg/ml LPS ($P<0.001$), and 88.12 pg/ml after treatment with 10 µg/ml CpG ($P<0.01$).

**Conclusions:** Microglial cells release activin A upon stimulation with bacterial TLR-agonists. This finding provides further evidence for a role of activin A in the innate immune response. It suggests that microglial cells are a source of elevated activin A concentrations observed in the CSF during bacterial meningitis.

# Calcium influx into astrocytes mediated by the inwardly rectifying $K^+$ channel Kir 4.1 (KCNQ10) at low external $K^+$ concentration

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Lowering the external $K^+$ concentration leads to calcium transients in astrocytes, but not in neurons. This phenomenon can be used to discriminate between these two cell types. It was hypothesized, that potassium inwardly rectifying channels (Kir) mediate low $K^+$ induced calcium influx [1]. Possible candidates are Kir4.1 channels, which represent the major Kir conductance in astrocytes in the ventral respiratory group [2]. To investigate whether Kir4.1 channels are involved in the low $K^+$ induced $Ca^{2+}$ signal we analysed low $K^+$ induced calcium influx in Kir4.1 knock-out mice (Kir4.1-/-) and in COS cells transfected with the Kir4.1 channel subunit. Acute brain slices from wild-type and knock-out mice, which were loaded with the fluorescent $Ca^{2+}$ indicator Oregon Green BAPTA1-AM, were exposed to low external potassium concentration (0.2 mM $K^+$). In Kir4.1-/-, the number of responding cells was dramatically reduced and the duration and half-width of the $Ca^{2+}$ transients in responding cells was significantly smaller in comparison to wild-type mice. We observed this change in brainstem, cortex and hippocampal slices. To support our data, we additional used COS-1 cells with heterologeous expression of the EGFP tagged Kir4.1 channel subunit. Upon lowering the external $K^+$-concentration, Kir4.1 expressing cells generated cytosolic $Ca^{2+}$ transients, which could be blocked by external $Ba^{2+}$ (100µM). Our results indicate that under conditions of low external $K^+$-concentration the Kir4.1 channels become $Ca^{2+}$ permeable.
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# Propagation Speed of Cortical Spreading Depression Correlates with Cortical Myelin Sheath Thickness

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Cortical spreading depression (CSD) was first described by Leao in 1944 and is characterised by a spreading wave of depolarisation, depression of spontaneous cortical activity and severe disturbance of ion homeostasis. It advances with a speed of 3-5 mm/min in every direction from the origin. CSD appears to be associated with different neurological diseases of the central nervous system, such as migraine and ischemic stroke.

Here, we investigated whether myelin sheath thickness may influence the propagation speed of CSD using transgenic mice overexpressing the axonal growth factor neuregulin 1 (NRG-1) type I which exhibit hypermyelination in the cortical grey matter.

NRG-1 type I overexpressing animals showed a significantly increased latency of the onset of CSD between the two epidural recording electrodes (tg: 88.4 ± 5.8s, wt: 51.8 ± 7.5s; $p < 0.005$), resulting in a decrease of the propagation speed when compared to littermate controls.

We conclude that myelination may be an important resistance factor influencing the initiation and propagation of CSD. CSD, unlike action potentials, is not conducted by neurons along myelin sheaths, but propagates uniformly in every direction from its origin. Therefore, we hypothesise that myelin has an insulating effect, which prevents the spread of the disturbance of ion homeostasis as a diffusion barrier. Alternatively, the buffering capacity for CSD-propagating agents like K+ or glutamate may be altered by myelin sheath thickness in the cerebral cortex.

In conclusion, our results suggest that changes in myelination play a role in the physiology of CSD and may therefore be involved in the pathogenesis of CSD-related diseases like migraine.

# Simvastatin affects oligodendroglial process formation and myelin production
# Can Simvastatin be recommended for therapy in MS?

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Statins inhibit cholesterol synthesis via inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (EC 1.1.34). Because of their lipid lowering properties they are therapeutically used for reducing the incidence of diseases such as arteriosclerosis, stroke and heart failure. Besides cholesterol lowering statins exhibit pleiotropic effects which result in anti-inflammatory and immunomodulatory properties. Based on these findings, statins were discussed as therapeutics for multiple sclerosis (MS). However, myelin production, another challenge of MS treatment, could be negatively affected since inhibition of HMG-CoA-reductase interferes with oligodendroglial process formation. To address this question, in vitro and in vivo experiments were performed. Cultured pig oligodendrocytes (OL) were exposed to Simvastatin (Sst, 2-10µM). An obvious morphological effect after addition of Sst was that oligodendroglial process formation was retarded; already formed processes were retracted. Sst could reduce oligodendroglial cholesterol by about 20-30% after 6 days. Addition of mevalonate but not PEG-cholesterol reversed the morphological changes indicating that lacking intermediates such as farnesylpyrophosphate (FPP) or geranylgeranylpyrophosphate (GGPP) cause the negative outcome due to Sst treatment. FPP and GGPP are necessary for posttranslational modification of small G-proteins (p21Ras, Rho), an important requirement for their membrane docking and proper signaling. Treatment with Sst caused a decrease of membrane bound p21Ras, RhoA and RhoG. Activation of MAPK, a final step in p21Ras signaling, was significantly reduced when OL were exposed to Sst. Finally Sst treatment caused a diminished synthesis of myelin proteins as evidenced by 14C-incorporation studies. For in vivo investigations, a mouse cuprizone-model was used in which demyelination occurs predominantly in the corpus callosum. Replacement of a cuprizone diet by normal food induces remyelination spontaneously. Histochemistry revealed that Sst caused a retardation in remyelination when fed during this time. This finding was supported by TEM and SDS-PAGE of myelin proteins.

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# Hypomyelination due to inactivation of cholesterol biosynthesis in Schwann cells

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Cholesterol is a major structural component of the mammalian cell membrane. It is also involved in a variety of intracellular processes, including signal transduction and the formation of lipid rafts. In the brain up to 70% of cholesterol is associated with myelin.
To investigate the function of cholesterol in myelin, conditional mouse mutants lacking functional cholesterol biosynthesis specifically in myelin-forming glia cells were generated using the cre/loxP system. These mice show a severely hypomyelinated phenotype, ataxia, tremors and approximately 30% mortality by one month of age. Myelination in the CNS slowly normalizes, leading to an improvement of the shaking phenotype with age.
Hindlimb paralysis indicates progressive pathology of the PNS. Indeed, myelination of sciatic nerve keeps lagging behind and has still not reached wildtype levels by one year of age. In contrast to the CNS, composition of peripheral myelin has changed. The glial defect also leads to a reduction of axon caliber. These differences in pathology between CNS and PNS might be caused by a lack of non-targeted cells that are able to supply cholesterol to mutant Schwann cells. This mouse model will provide insight into how PNS and CNS manage to compensate for the loss of glia-derived cholesterol.

# Induced deletion of AMPA-type glutamate receptors in Bergmann glia

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Cerebellar Bergmann glial cells possess arborized processes which irregularly extend lamellae toward synapses on Purkinje-cell spines formed either by parallel or climbing fibers. The glial appendages do not only seal the synaptic cleft, they also constitute microdomains for signalling pathways. On these membranes, Bergmann glia expresses highly localized either glutamate transporters (GLAST close to the synaptic cleft) or AMPA-type glutamate receptors (GluR A and GluR D close to the shaft of the presynaptic terminal). While the role of GLAST in controlling extracellular glutamate levels is rather obvious, we have only a limited knowledge of glial AMPA receptor function.

To investigate the particular impact of glial transmitter receptors in vivo, we generated a transgenic mouse line in which the tamoxifen-sensitive Cre recombinase fusion protein CreERT2 is expressed under the astroglia-specific human GFAP promoter. Such transgenic mice were further crossbred with genetically modified mice in which exons 11 of the GluR A and GluR D genes were flanked by loxP sites.

Here, we demonstrate that in vivo GluR A and D can be ablated from Bergmann glial processes using the cre/loxP system in an inducible and timely controlled manner. Preliminary electrophysiological data suggest that homomeric GluR A or GluR D change their reversal potential relative to wildtype GluR A and D heteromeric channel complexes. Further data on Bergmann glia-selective ablation of both, GluR A and GluR D, glutamate receptors will be presented.

# Analysis of NG2 expression at the synaptic glia-neuron interface

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Glial cells of the central nervous system such as astrocytes or oligodendrocytes are active partners in neurotransmission by contacting neuronal compartments, releasing transmitters like ATP or glutamate, and expressing numerous receptors and proteins for cell recognition. These molecules are actively involved in neuron-glia interaction at synapses.

Very recently, brain cells expressing the proteoglycan NG2 have been identified as a separate glia population. Although they express distinct functional and structural differences to astrocytes and oligodendrocytes, they also share an important feature, i.e. the bidirectional interaction with neighboring neurons. Astrocytes and NG2-glia have in common that, both of them, contact synapses of the brain parenchyma. In addition, they are sensitive to neuronal activity by expressing various transmitter recepors. While the morphology of astrocytes in different brain regions varies considerably, structural heterogeneity of NG2-glia is less pronounced. NG2-glia expresses predominantly the AMPA-type of glutamate receptors (GluRA, B and D), while glutamate transporters and NMDA receptors are lacking. Analysis of the sensitivity of astrocytes and NG2-glia to glutamate will be a major key to understand the roles of these two glia population in brain function.

Here, we used quantum dot (QD)-conjugated antibodies to visualize the dynamic properties of the glial cell-type defining proteoglycan NG2 within the glial cell membrane. We found that the NG2 molecules can exist in three different motility classes: NG2 can be completely immobile for minutes, can randomly diffuse at rather localized membrane sites, or can be transported at a speed of up to 1 µm/s from somatic regions into cellular processes.

# Expression of HNK-1 by subpopulations of olfactory neurons and Schwann cells in the adult nasal mucosa *in situ* and its regulation *in vitro*

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Olfactory ensheathing cells (OECs) are promising candidates for the development of novel transplantation-based therapeutic strategies of human nervous system injury and disease. Recently, the adult dog was introduced as an intermediate animal model to translate the evidence from the rodent system into clinical practice. In the present study, we comparitively analyzed the molecular expression profile of adult canine nasal mucosa OECs and Schwann cells *in situ* and *in vitro* using immunostaining with a panel of monoclonal antibodies. Cell type-specific markers would be crucial for the antibody-based establishment of pure cell preparations suitable for autologous transplantation. We found that the HNK-1 epitope, previously suggested to be a pan-specific Schwann cell marker is in fact expressed by myelin-forming Schwann cells only, whereas OECs and non-myelin-forming Schwann cells did not contain HNK-1. Contrary to previous findings, we could not detect HNK-1 in cultured Schwann cells implying its down-regulation after loss of axonal contact. In addition to this, HNK-1 was found to be expressed by subpopulation of mature olfactory neurons. Taken together, our data show that HNK-1 in the adult canine mucosa is expressed by subpopulations of olfactory neurons and Schwann cells *in situ*, and down-regulated in Schwann cells *in vitro*. These data strictly argue against the use of anti-HNK-1-antibodies for the purification or depletion of Schwann cells from nasal mucosa cell suspensions or cell cultures.

# Purification and *in vitro* characterization of olfactory ensheathing cell-like CNS glia (aldynoglia) from adult canine brain

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Olfactory ensheathing cells (OECs) are regeneration-promoting glial cells of the olfactory system. A variety of *in vivo* studies has shown that OECs can promote axonal regeneration and remyelination *in vivo* following transplantation into the lesioned central nervous system. Studies in the rat demonstrated that an OEC-like glial cell population (aldynoglia) can also be found in other brain areas (Gudino-Cabrera & Nieto Sampedro, 2000). The aim of the present study was to purifiy OEC-like aldynoglia from the adult canine brain (Beagle, 3-6months-old) and to compare their *in vitro* characteristics with olfactory bulb-derived OECs (Krudewig et al., 2006). The adult dog was recently introduced as an intermediate model system towards clinical use of regeneration-promoting glia. Aldynoglia was immunopurified using anti-$p75^{NTR}$-antibodies and analyzed with regard to antigenic expression, proliferation and differentiation. Aldynoglial cells displayed a spindle-shaped, bipolar morphology reminiscent of OECs and proliferated in response to established OEC mitogens, e.g. fibroblast growth factor-2 (FGF-2), and heregulin-1ß (HRG-1ß). Aldynoglia stained positive for typical OEC markers e.g. $p75^{NTR}$, sulfatide (O4), S100 and the ganglioside A2B5. What was found to be different from OECs was the expression of GFAP, which was detected in 5-10% of aldynoglial cells compared to 40-50% in OECs. Taken together, our data show that adult canine aldyoglia is a glial cell type closely related to OECs. Future studies have to reveal their *in situ* localization. Aldynoglia may serve as a target for promoting endogenous neural repair.

# Thyroid Hormone Action during Development of Cerebellar Purkinje Cells and Bergmann Glia

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Thyroid hormone (TH) plays an important role during cerebellar development. Perinatal TH deficiency leads to severe developmental perturbations affecting virtually all cerebellar cell types. A prominent hallmark of congenital hypothyroidism is a striking reduction of dendritic growth and branching of Purkinje cells, the principle neuron of the cerebellar cortex. Yet, the molecular mechanisms by which thyroid hormone stimulates Purkinje cell dendritogenesis are still poorly understood.

In order to elucidate the role of thyroid hormone in cerebellar differentiation we analyzed Purkinje cell development in Pax8-/- mice. These animals are born without a functional thyroid gland and, thus, represent an ideal animal model of congenital hypothyroidism. As expected, Purkinje cells of Pax8-/- mice exhibit stunted dendrites, an effect that can be reversed by TH treatment of the animals. By using laser capture microdissection microscopy we collected 24 000 cells of the Purkinje cell layer derived from Pax8-/- mice and wild-type littermates at postnatal day 12. Following RNA isolation, amplification and Affymetrix gene array analysis, candidates were selected based on their putative involvement in regulating cytoskeletal changes. As one of the differentially regulated gene products that are currently under investigation, binder of Rho GTPases 4 (borg4; cdc42 effector protein 4) represents an interesting candidate since it is a putative downstream target of Rho family GTPases and may therefore be implemented in signalling cascades regulating dendrite formation. In our array analysis, borg4 expression was found to be strongly downregulated under athyroid conditions. Moreover, substitution of Pax8-/- mice with thyroid hormone completely restored borg4 expression to wild-type levels as assessed by Northern Blot analysis and real-time PCR. Most interestingly, in situ hybridization analysis revealed that borg4 is selectively expressed in Bergmann glia cells and not in Purkinje cells. Since interactions between Purkinje cells and Bergmann glia were recently shown to be important for Purkinje cell dendritogenesis, we will further focus on studying the impact of thyroid hormone-regulated borg4 expression for Bergmann glia fiber formation, a process that might represent a prerequisite for the morphological and functional maturation of the Purkinje cell.

# Satellite glial cells in sensory ganglia of mice- a possible role in pain sensation

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There is evidence that injury to nerve increase endoneurial fluid pressure and decreased blood flow in sensory ganglia, leading to sensory dysfunction. We propose that satellite glial cells (SGCs) in sensory ganglia can be activated by mechanical (pressure) changes in their surrounding. We have previously shown that these cells are influenced by nerve damage, and also respond to inflammatory substances such as ATP by an elevation of intracellular calcium. We hypothesized that SGCs responding to such stimulation can transmit signals to other cells. Application of mechanical stimulus to an isolated single SGC elevated intracellular calcium concentration in that cell. This response was not limited to the stimulated SGC, but also generated intracellular calcium elevations in neighboring SGCs. Signal propagation from the stimulated SGC to neighboring cells was reduced by more than 90% after adding of the purinergic receptor antagonist suramin (100 microM). In the presence of a blocker (ARL-67156, 100 microM) of endogenous extracellular ATPases, responses in neighboring SGCs had higher amplitude and longer duration. The gap junction blocker carbenoxolone (50 microM) did not alter signal propagation among SGCs. These results indicate that mechanical stimulation of a SGC evokes calcium increase in neighboring SGCs. The signal mediating this propagation appears to be a purinergic agonist (presumably ATP). Intercellular communication via gap junctions does not seem to be involved. Our results demonstrate for the first time the presence of calcium waves in SGCs in sensory ganglia. We suggest that calcium waves can be a step in the generation of pain sensation.

# GABA-induced calcium signals in astrocytes and interneurons of the mouse olfactory bulb

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Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the adult CNS. The hyperpolarizing response is due to an increase in the chloride conductance of the neuronal membrane allowing chloride ions to flow down their electrochemical gradient into the cell. For many brain regions it is well-known that GABA also exerts excitatory effects on neurons during development, caused by the high neuronal intracellular chloride concentration in immature neurons. However, the effect of this neuronal excitation on astrocytes had not been studied yet. We used confocal laser scanning microscopy to investigate GABA-induced $Ca^{2+}$ signalling in Fluo-4-loaded astrocytes and juxtaglomerular interneurons of acute olfactory bulb slices of young mice. Both interneurons and astrocytes showed a $Ca^{2+}$ increase induced by bath application of 100 µM GABA, which was sensitive to the GABA(A) receptor antagonist bicuculline and the inhibitor of voltage-gated $Ca^{2+}$ channels, diltiazem. The GABA(B) receptor antagonist CGP52432 did not affect the $Ca^{2+}$ response in interneurons and astrocytes. The GABA-induced $Ca^{2+}$ response in interneurons remained unaffected by depleting the intracellular $Ca^{2+}$ stores via application of cyclopiazonic acid (CPA), whereas the $Ca^{2+}$ response in astrocytes could be blocked completely by CPA. We conclude that the $Ca^{2+}$ response induced by GABA in interneurons is attributable to $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels, and is due to $Ca^{2+}$ release from intracellular stores mediated by metabotropic transmitter receptors in astrocytes. *Supported by DFG (SFB 530, TP B1; LO 779/2-6)*

# Modulation of synaptic activity in the cerebellar cortex by Bergmann glial cells

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Bergmann glial cells (BG) are radial elements in the cerebellar cortex that provide enveloping of synapses of the molecular layer circuits. A former study demonstrated that electrical BG stimulation decreases the frequency of spontaneous postsynaptic currents (sPSCs) in the neighboring Purkinje neuron (PN) by about 40 % (Brockhaus & Deitmer, 2002, J Physiol 545, 581). This effect is long-lasting (1 h) and is primarily due to suppression of synaptic output from inhibitory interneurons. In this study, we focused on the signaling pathway underlying this modulatory action and provide evidence for a crosstalk with the purinergic pathway which also influences the PN synaptic activity. Experiments were performed on sagittal slices of the rat cerebellum. Bergmann glial cells were depolarized during voltage-clamp, and sPSCs were recorded from PN in patch-clamp whole-cell mode at a holding potential of -70 mV. At first, the cellular signal arising from the stimulated BG was studied. The effect of BG stimulation on PN synaptic activity was prevented by the AMPA receptor blocker GYKI 52466, but not by NS-102, an inhibitor of kainate receptors. Blocking the glial glutamate transport by pipette loading of β-threo-hydroxy-aspartate or D-aspartate omitted the modulatory effect on neuronal activity. These findings suggest that the BG responds to depolarization with glutamate release by reversed uptake. We then addressed the question how AMPA receptor activation might lead to a change in synaptic activity of the interneurons. Because of the long-lasting effect and a recent report on the interaction of AMPA receptors with intracellular messengers (Takago et al, 2005, PNAS 102, 7368), a metabotropic mechanism was favored. Treatment of the slice with U73122, a blocker of phospholipase C, prevented the glial modulatory influence on PN activity. In contrast, this influence persisted when the adenylyl cyclase was inhibited by SQ 22.536, as well as during inhibition of protein kinase A by KT 5720. Instead, the BG effect on PN synaptic activity was sensitive to blocking of protein kinase C by GF 109203X. We also found that the glial modulatory effect exclusively occurred from postnatal day 14 onwards. Since this parallels the developmental regulation of the effect of ATP on synaptic activity in the molecular layer of the cerebellar cortex (Casel et al, 2005, J Physiol 568, 111), the putative interrelation of the glial effect with the purinergic pathway was investigated. Interestingly, the positive effect of externally given ATP on the sPSC frequency of PN was substantially reduced following BG stimulation or application of submicromolar doses of AMPA. Similar results were obtained when the degradation of endogenous ATP was prevented by ARL 67156. Conversely, the purinergic receptor antagonist PPADS failed to reduce the PN synaptic activity after a preceding BG stimulation or AMPA application. These findings demonstrate the strong impact of Bergmann glial cells on the neuronal activity of the cerebellar cortex. They probably respond to depolarization by glutamate release leading to a metabotropic action of AMPA receptors on interneurons mediated by the PLC-PKC-pathway.

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# Communication between axons and olfactory ensheathing cells in the rodent olfactory bulb

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The olfactory system undergoes continuous renewal of olfactory receptor neurons (ORNs) and axonal regrowth into the adult central nervous system (CNS). This unique ability in the vertebrate CNS is believed to be due to a special glial matrix accompanying the axons along their way to the CNS. The predominant glial cell type of the olfactory nerve, the olfactory ensheathing cell (OEC), has been used to treat lesions in the CNS by cell transplantation. Little is known, however, about the physiological properties of the OECs. We have examined OEC calcium signaling in the olfactory bulb of neonatal rodents using confocal and two-photon microscopy. Electrical stimulation of ORN axon bundles evoked $Ca^{2+}$ transients in the OECs which are mediated by the release of $Ca^{2+}$ from intracellular stores. This axon-glia communication seems to be mediated by metabotropic glutamate receptors and purinoceptors. Stimulation-evoked $Ca^{2+}$ transients in OECs could not only be blocked by CPA, but also by antagonists of the metabotropic glutamate receptor mGluR1 (CPCCOet) and the purinergic receptor P2Y1 (MRS2179). Immunohistological and pharmacological analysis confirmed the existence of these two receptors in the OEC. We conclude that axonal activity triggers $Ca^{2+}$ signals in OECs via activation of mGluR1 and P2Y1, possibly by release of glutamate and ATP by axons.

# Ca2+ Responses of Müller cells induced by light stimulation of photoreceptor cells

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Müller glial cells respond with an intracellular calcium rise to light stimulation of the retina. Under dark adapted conditions this is a very slow calcium rise, which applies to all Müller cells and starts right after the beginning of the light stimulation. After further strong light stimulation Müller glial cells react with a second calcium response which is faster and starts at the level of the ganglion cell layer, similar to that published by Newman (2005). Pharmacological experiments so far showed for the slow calcium rise, that about two third of the signal transfer from neurons to Müller cells occurs at the level of the photoreceptor cells. Glutamate seems to be involved in the signal transduction, but neither via ionotropic nor via metabotropic glutamate receptors, but rather via glutamate transporters. Blocking of the glutamate transporters by TBOA reduces the signal by some 30%. The glutamate dependence of the slow calcium rise might also be due to a feedback inhibition mediated by the mGluR8 receptor, which is presynaptically located on photoreceptors (Koulen et. al., 1999). Chloride channels seem also to be involved in the generation of the slow calcium rise, since it can be blocked by the chloride channel blockers NPPB, Flufenamic acid and Niflumic acid.

The fast calcium rise starts after a delay of about 2.5 minutes at the level of the ganglion cell layer. It originates in the smooth endoplasmatic reticulum of the Müller glial cells, which could be shown by the application of cyclopiazonic acid. Cyclopiazonic acid reduces the number of Müller cells displaying fast calcium rises to zero, but leaves the first slow calcium rise of the cells unaltered.

# Non-myelinating glia cells with close contact to aquaporin-1-positive unmyelinated nerve fibers in peripheral mouse nerves and ganglia

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Excitation of nociceptive neurons by inflammatory mediators is a possible explanation for the development of pain during inflammation of a peripheral nerve trunk. Another typical phenomenon in the context of inflammation is edema, a swelling of the nerve due to an accumulation of a protein-rich fluid in the interstitium. Recently, the presence and probable function in nociception of aquaporin-1, a membrane protein for the transport of water, has been demonstrated in unmyelinated peripheral nerve fibers [1]. Therefore, it is likely that edema itself may contribute to the excitation of nociceptive axons. Another cell type involved in water and electrolyte homeostasis in nervous tissue are glia cells. In the present study, we have explored the anatomical coupling between aquaporin-1-immunopositive axons and glia cells in peripheral nerve trunks and dorsal root ganglia of mice. Tissue sections from transgenic mice which express enhanced green fluorescent protein (eGFP) under the control of the human glial fibrillary acidic protein (GFAP) promoter [2] were studied using confocal microscopy. In these preparations, single non-myelinating Schwann cells can be identified in dorsal roots, sural nerve, vagus nerve, and spinal ganglia. The structure of the glia cells differs clearly depending on the location. A spindle-shaped cell type can be found in the nerve trunks whereas a complex structure of the glia cell with many processes is observed in the center of the ganglia. Unmyelinated axons were immunopositive after staining with an antibody against aquaporin-1 (AQP1). Such axons with parallel orientation have close contact to spindle-shaped hGFAP-eGFP Schwann cells in the dorsal roots, sural and vagus nerve. The shape of the complex glia correlates in most cases with a network of AQP1-positive thin axons in the center of dorsal root ganglia. These data demonstrate a tight anatomical coupling between AQP1-channels of unmyelinated axons and the membrane of non-myelinating glia (Schwann) cells in peripheral nerve trunks and dorsal root ganglia. It is possible, therefore, that water movements between nociceptive axons and non-myelinating glia may contribute to the development of pain in nerve trunk inflammation.

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[1] Oshio et al.; Biochem Biophys Res Com 341: 1022-1028, 2006

[2] Nolte et al. ; GLIA 33: 72-86, 2001

# Effect of Proteasomal Inhibition by MG-132 on Inclusion Body Formation in Astrocytes

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The small heat shock proteins (sHSPs), HSP25/27 and αB-crystallin, are more prominent in glia than in neurons, act as molecular chaperones and specifically interact with cytoskeletal elements. They represent prominent constituents in inclusion bodies originating in astrocytes and oligodendrocytes, and have been described to be involved in Alexander's disease, a human neurodegenerative disorder with astrocytic inclusions called Rosenthal fibres (RF). These protein aggregates also contain GFAP (glial fibrillary acidic protein) and ubiquitin, indicating that inclusion body formation might be causally related to an impairment of the ubiquitin proteasome pathway (UPS).

In the present study, we have examined if defects in the UPS system contribute to the protein aggregation process in astrocytes. Cultured astrocytes, prepared from the brains of newborn rats, were treated with the proteasomal inhibitor MG-132 (5μM). Under control conditions, astrocytes constitutively express high levels of HSP25, but only very low amounts of αB-crystallin. Treatment with MG-132 for 24h caused the upregulation of HSP25 and αB-crystallin, which both were increasingly found in the detergent insoluble fraction. Indirect immunofluorescence analysis displayed small aggregates of HSP25 throughout the cytoplasm and in the perinuclear region. These aggregates also contained αB-crystallin, ubiquitin and HSP70. The effects of MG-132 were reversible and did not cause apoptotic cell death, as observed in oligodendrocytes. However, after 24h and 48h recovery, respectively, in the absence of MG-132, cells were observable with aggresome-like structures near the microtubule organizing center, consisting of HSP25, HSP70 and αB-crystallin. GFAP filaments were surrounding these inclusions. After 72h recovery the aggresomes were removed and HSP25 again was diffusely present throughout the whole cell, and HSP70 and αB-crystallin were down regulated in most of the cells.

Hence, proteasomal inhibition leads to HSP induction and the formation of inclusion bodies, as observed in neurodegenerative diseases. In astrocytes these aggregates are not toxic, and their formation might be a rescue mechanism to protect cells from unwanted interactions with amyloid proteins.

# α-Synuclein Aggregate Formation in Oligodendroglia OLN-t40 Cells Stably Transfected with α-Synuclein

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α-Synuclein (α-syn) is the major building block of cytoplasmic inclusions in neurodegenerative disorders named synucleinopathies, such as Parkinson`s Disease and multiple system atrophy (MSA). In MSA glial cytoplasmic inclusions (GCIs) originating in oligodendrocytes are prominent. During disease progression a shift in α-syn solubility is observable, and oxidative modification of α-syn has been linked to neurodegeneration and fibril formation. Furthermore, an overlap of pathological features of patients with tauopathies, e.g. frontotemporal dementias including Pick`s disease and progressive supranuclear palsy, has been reported, and it has been argued that α-syn aggregation can induce the fibrillization of tau.

To investigate the possible involvement of oxidative stress in α-syn aggregate formation, we have engineered OLN-t40 cells, a cell line with oligodendroglial characteristics stably expressing the longest human isoform of the microtubule associated protein Tau, to express wild type α-syn or the A53T α-syn mutation. α-Syn expression in both cell lines caused the appearance of small punctuated α-syn aggregates, which did not stain with thioflavine S, and were negative for tau and heat shock proteins (HSPs). These aggregates were more abundant in cells expressing the A53T α-syn mutation and therefore OLN-t40-A53T cells were used in the following studies.

Oxidative stress, exerted by hydrogen peroxide, led to an enlargement of the cytoplasmic protein inclusions, and to the recruitment of Tau and HSP90, HSP70 and αB-crystallin to the inclusions. However, oxidative stress did not cause an increase in α-syn in the detergent insoluble fraction, possibly indicating that α-syn did not further accumulate and was of prefibrillary nature, and still degradable by the proteasomal system. To test this, cells were treated with hydrogen peroxide first and then with the proteasomal inhibitor MG-132. The results show that oxidative stress followed by proteasomal inhibition leads to a decrease in α-syn solubility and to the occurrence of larger thioflavine S-positive inclusions, which was not seen either after oxidative stress or proteasomal inhibition alone.

In summary, oxidative modification in combination with proteasomal impairment, contributes to alterations in the solubility of α-syn, and leads to the formation of inclusions resembling GCIs. HSPs were upregulated and together with tau recruited to the GCIs, possibly further contributing to the formation of degenerative inclusions.

# Glutamate receptor distribution on NG2/EGFP expressing glial cells identified by freeze-fracture replica EM immunogold labelling in the mouse brain.

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Restricted location of membrane proteins enables the formation of highly compartmentalised microdomains that are specialised for distinct functions. Electron microscopic freeze-fracture replica immunogold labelling of these domains is a highly sensitive technique at ~ 20 nm resolution to study their surface distribution and their relationship to other structures (Fujimoto J Cell Sci 1995 108 3443, Hagiwara et al. J Comp Neurol 2005 489 195). However, the cellular origin of membranes in these replicas can only be established in exceptional cases, when the special laminar position of cellular profiles is visible or when intrinsic molecular markers are preserved. To overcome this limitation, we developed a generally applicable strategy for membrane identification using transgenic expression of a membrane anchored EGFP.

A unique population of glial cells found in large numbers throughout the central nervous system (CNS) expresses the NG2 proteoglycan (NG2+ cells) and also the alpha receptor for platelet-derived growth factor (PDGFαR). In the postnatal brain, differentiation of NG2+ cells into oligodendrocytes, type-2 astrocytes and neurons has been suggested and activation and proliferation of adult NG2+ cells has been reported following CNS injury. Some NG2+ cells receive functional GABAergic and glutamatergic synaptic junctions from surrounding neurons (Lin et al. Neuron 2005 46 773). The distribution of neurotransmitter receptors and other proteins in NG2+ cell membranes and their relationship to neuronal processes might help to explain their intercellular relationships. Using bacterial artificial chromosome-mediated transgenesis we created mice that express a lymphocyte protein tyrosine kinase (Lck)-EGFP fusion protein under control of the NG2 promoter. In these mice, membrane bound EGFP fluorescence was present in NG2/PDGFαR immunoreactive cells located throughout the brain.

Consistent with the insertion of the myristoyl moiety into the cytoplasmic leaflet, the protoplasmic P-face of NG2+ cell membranes was densely immunogold labelled down to the thinnest processes in freeze-fracture replicas. These results indicate that restricted expression of membrane anchored EGFP within defined classes of cells allows the unambiguous identification of membranes for high-resolution two-dimensional quantitative mapping of membrane proteins in the brain. The facing extracellular E-face replica of this EGFP labelled surface showed the presence of small clusters of AMPA type glutamate receptors coincident with intramembrane particles (IMPs) and some IMPs immunopositive for AMPA type glutamate receptors outside clusters. The clustered distribution of AMPA type glutamate receptors on NG2 expressing glial cells indicate that NG2+ cells may interact synaptically with neurons in vivo.

# Glia cells and extracellular matrix molecules in the snail (*Lymnaea* and *Helix*) central nervous system: histochemical demonstration

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Non-neuronal elements in the nervous system, such as glia cells and extracellular matrix molecules, play a fundamental role in maintaining homeostatic environment which contribute in optimizing the activity of nerve cells, the proper formation of neuronal networks, and the regulation of regeneration processes. In invertebrates, however, our knowledge on both glia cells and chemical components of the extracellular matrix is rather limited. Therefore the purpose of the present study was to analyze the distribution of these elements in the central nervous system (CNS) of two model snail species, *Lymnaea stagnalis* and *Helix pomatia*, living in different habitats, by the application of different histochemical methods on cryostat sections.

At pH 4.0, the basic thiazin dye, toluidine blue resulted in a selective, fluoromethachromatic staining of glial cells at 540/580 λ excitation/emission wavelengths. This phenomenon was also observed in cultured glia cells isolated from *Lymnaea ganglia*. Acridine orange, applied to demonstrate nuclei of glial cells at pH 7, by using 540/580 λ excitation/emission filters, revealed the glia cells located partly around neuronal perikarya but mainly concentrated between the layer of neural perikarya and the neuropil in *Helix*, whereas they displayed a more homogenous distribution in the *Lymnaea* ganglia. One of three different GFAP antibodies applied labeled intracellular filamentous structures in cultured *Lymnaea* glia cells, but not in ganglion sections. A remarkable difference was detected between the *Lymnaea* and *Helix* CNS, regarding the presence of proteoglycans in the neuropil. Staining with alcian blue at low pH and with acridine orange at neutral pH indicated that acidic proteoglycans, among them presumably mainly hyaluronic acid, were present more abundantly in *Helix*. Non-acidic proteoglycans and glycolipids also seem to occur more frequently in the ganglion neuropil of *Helix* as it was demonstrated by acriflavine-Schiff staining. In both species, concanavalin-A resulted in labeling along the membrane of neural perikarya and it was also bound to the elements of the neuropil. Another lectin, wheat germ agglutinin, strongly labeled the extracellular matrix located between the perikaryonal layer and the neuropil. Applying lectin probes, the involvement of the core carbohydrates of proteoglycans, the terminal N-acetyl-glucosamine molecules, which are often connected to sialic acid and glycolipids, was also demonstrated in the composition of extracellular matrix of the snail ganglia.

Our results, demonstrating for the first time extracellular matrix molecules in the gastropod CNS, indicate significant differences existing in the extraneuronal environment of the aquatic *Lymnaea* and terrestrial *Helix* CNS. The present study provides, in addition, a basis for a detailed analysis of the role of glia cells and different extracellular matrix molecules in the developing snail central nervous system.

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# Poster Topic

## T13: Plasticity

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*A. Gundlfinger, C. Leibold, R. Kempter and D. Schmitz, Berlin*

**T13-2A** Homeostatic gain changes and ocular dominance diversity can account for the differential expansion of the left- and right-eye receptive fields of cortical neurons after monocular retinal lesions
*JM. Young, MB. Calford and K. Obermayer, Berlin and Newcastle (AUS)*

**T13-3A** Synaptogenesis and neurogenesis are related phenomena in the dentate gyrus of the mature brain in gerbils (Meriones unguiculatus)
*M. Butz, Bielefeld*

**T13-4A** High- and Low-Frequency Repetitive Transcranial Magnetic Stimulation (rTMS) Down-regulates the Expression of the Calcium-binding Proteins Parvalbumin and Calbindin
*K. Funke, J. Trippe, S. Aydin-Abidin, E. Weiler, W. Girzalsky, UT. Eysel, R. Erdmann and A. Benali, Bochum*

**T13-5A** *Stem cell induced cortical plasticity reduces brain damage after perinatal asphyxia in rats*

*M. Geißler, K. Kreikemeier, S. Neuhoff, C. Meier and HR. Dinse, Bochum*

**T13-6A** The role of presynaptic NMDA-receptors in lesion-induced plasticity of the visual cortex in rats
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**T13-7A** Defined role of p75, TrkB.T1 and TrkB neurotrophin receptors in structural plasticity of hippocampal neurons
*K. Michaelsen, M. Zagrebelsky and M. Korte, Braunschweig*

**T13-8A** Role of the NogoA/NgR/p75$^{NTR}$ signaling system in modulating structural plasticity in the mature mouse hippocampus
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**T13-9A** Online interaction with in vitro neuronal networks
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**T13-2B** EPAC: A novel modulator of presynaptic short-term plasticity in cultured hippocampal neurons.
*I. Gekel and E. Neher, Göttingen*

**T13-3B** Stress induced regional and hemispheric morphological modifications of pyramidal cells in the rat prefrontal cortex
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**T13-4B** Morphological analyses of the hippocampus of tenascin-C deficient mice
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**T13-8B** Constitutive expression of cyclins and cdks and their function beyond cell cycle
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**T13-9B** Biochemical and immunochemical characterization of perineuronal nets
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**T13-1C** Sculpturing synapto-dendritic circuitry via Jacob and its interaction partners
*J. Sahin, I. Zdobnova, F. Pöll, H. Sann, ED. Gundelfinger, W. Zuschratter and MR. Kreutz, Magdeburg*

**T13-2C** L-type $Ca^{2+}$ channels, CaMKII, and cAMP cooperate in mediating postsynaptic secretion of BDNF and NT-3
*R. Kolarow, T. Brigadski, C. Kuhlmann, H. Luhmann and V. Lessmann, Mainz*

**T13-3C** Small interfering RNA mediated efficient knockdown of BDNF in cultured hippocampal neurons
*GC. Niemann, T. Brigadski and V. Lessmann, Mainz*

**T13-4C** Changes in the spectral main sequence of human saccades during saccade adaptation
*S. Klingenhoefer and F. Bremmer, Marburg*

**T13-5C** A balance of protein synthesis and proteasome-dependent degradation determines the maintenance of LTP
*RM. Fonseca, RM. Vabulas, FU. Hartl, T. Bonhoeffer and UV. Nägerl, Martinsried and München*

**T13-6C** Sensorimotor gating of the goldfish startle response: behavioral and neural characteristics.
*T. Preuss and H. Neumeister, New York (USA)*

**T13-7C** Molecular Correlates of Tinnitus in the Auditory System: BDNF, Arg3.1/Arc, and the Role of GABA
*R. Panford-Walsh, W. Singer, L. Rüttiger, HS. Geisler, U. Zimmermann, I. Köpschall, K. Rohbock, E. Oestreicher, H. Haas and M. Knipper, Tübingen*

**T13-8C** *Identification of markers for neuronal plasticity in the auditory system*
*W. Singer, R. Panford-Walsh, J. Tan, L. Rüttiger, H. Haas, I. Köpschall, K. Rohbock, U. Zimmermann and M. Knipper, Tübingen and Melbourne (AUS)*

# Multidimensional Long-Term Potentiation at the Hippocampal Mossy Fiber Synapse

Anja Gundlfinger[1,3], Christian Leibold[1,2,3], Richard Kempter[1,2,3] and Dietmar Schmitz[1,3]

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NMDA-receptor dependent forms of synaptic plasticity are presumed to underlie the formation of memory. Several glutamatergic synapses throughout the central nervous system, however, express long-term potentiation (LTP) that is entirely independent of NMDA-receptor activation, yet the functional role of such forms of plasticity still needs to be determined. Here, using electrophysiological and computational techniques, we have developed a model of transmission dynamics to quantify NMDA-receptor independent plasticity at the hippocampal mossy fiber synapse. Short-term plasticity (STP) at this synapse is best described by two facilitatory processes that act on timescales of a few hundred milliseconds and about 10 seconds. We found that these distinct types of facilitation are differentially influenced by LTP such that the impact of the fast process is weakened as compared to that of the slow process. This attenuation is reflected by a selective decrease of not only the amplitude but also the time constant of the fast facilitation. We henceforth argue that multidimensional LTP serves as a mechanism to adapt the mossy fiber to its synaptic input rather than to form associative memory.

# Homeostatic gain changes and ocular dominance diversity can account for the differential expansion of the left- and right-eye receptive fields of cortical neurons after monocular retinal lesions

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Cortical neurons initially respond to the loss of subcortically mediated feedforward input by expanding their receptive fields, apparently due to an increase in the efficacy of the input arriving via intrinsic horizontal connections. This appears to be a strategy aimed at recovering from a deficiency in synaptic drive whereby subthreshold input connections are rendered suprathreshold and can thus engage in activity-dependent Hebbian competition. It is plausible that this sub- to supra-threshold transformation is achieved by some kind of response gain modulation, where the efficacy of all synapses are scaled such that excitatory neurons become more responsive and/or local inhibitory neurons become less responsive to input (effectively producing disinhibition). However, studies have repeatedly observed that binocular neurons in primary visual cortex, after being partially deafferented by a monocular retinal lesion, appear to show receptive field expansion via their lesioned eye receptive fields only. Such a bias appears to be inconsistent with the hypothesis that a global increase in response gain underlies this receptive field expansion. Here we examined experimental results from monocular lesion experiments and compared them to the behaviour of a network model. The feedforward and horizontal connectivity of each modeled neuron population had a specific ocular bias, and the distribution of ocular dominance within the population was based on in vivo data. We found that if the modeled neurons underwent gain changes proportional to their input loss the distribution of the ratios of lesioned eye to non-lesioned eye receptive field expansion matched the distributions observed in vivo. Our results support the hypothesis that single neurons or neuronal circuits respond to a substantial reduction of excitatory input by undergoing an increase in gain which indiscriminately amplifies responses to all excitatory inputs.

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# Synaptogenesis and neurogenesis are related phenomena in the dentate gyrus of the mature brain in gerbils (Meriones unguiculatus)

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Abstract. We propose a biological network model to describe neurogenesis and synaptogenesis in the hippocampal denatate gyrus as interdependent processes. Simulating the synaptic reorganisation process among proliferating and pre-existing neurons by our network model predicts an optimal synaptogenesis for a moderate cell proliferation whereas an increased cell proliferation leads to a reduced synaptogenesis. This effect is also observed experimentally in the dentate gyrus of gerbils: Impoverished in contrast to semi-naturally reared animals are known to have a permanently increased dentate cell proliferation in adulthood. Our recent quantitative immun-histochemical data now proofs a significantly reduced synaptogenesis - quantified by the densitiy of lysosomal processes in the DG. Furthermore, impoverished reared gerbils treated with a single juvenile Methamphetamine intoxication have an adult cell proliferation rate again lowered to normal levels. In accordance with the theoretical predictions, as we could also show, these animals have a less decreased synaptogenesis than the non-treated impoverished reared group. Thus, the experimental data strongly support the theoretical explanation model for the synaptic integration and survival of new neurons, in particular, in the dentate gyrus of the rodent brain.

# High- and Low-Frequency Repetitive Transcranial Magnetic Stimulation (rTMS) Down-regulates the Expression of the Calcium-binding Proteins Parvalbumin and Calbindin

Klaus Funke[1], Jörn Trippe[1], Selcen Aydin-Abidin[1], Elke Weiler[1], Wolfgang Girzalsky[2], Ulf T Eysel[1], Ralf Erdmann[1] and Alia Benali[1]

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Repetitive transcranial magnetic stimulation (rTMS) has been found to alter cortical excitability, thereby providing therapeutic ways to treat neuropsychiatric disorders associated with cortical hypo- or hyperexcitability. The cellular mechanisms fundamental to changes in cortical excitability are not known. Synaptic plasticity in terms of long-term depression and potentiation have been proposed since low-frequency rTMS (around 1 Hz) has been found to lower cortical excitability while high frequencies (> 5 Hz) increase it. Cortical activity is specifically controlled by diverse populations of inhibitory interneurons, not only in terms of activity level but also with regard to the temporal structure, e.g. gamma and theta oscillations. In the current study, we therefore investigated the acute effects of 2 different rTMS protocols on the expression of the calcium-binding proteins (CaBP) Parvalbumin (PV), Calbindin (CB) and Calretinin (CR). Changes in CaBP expression may signal activity-dependent adaptive processes in distinct types of interneurons. Rats received either a 1 Hz rTMS (3 blocks of 20 min, 3600 stimuli in 70 min), or 5 blocks of an intermittent-type theta-burst rTMS protocol (TBS, 10 trains of 3 pulses/60ms [50 Hz, intertrain interval 200 ms, 5 Hz resp.] repeated 20 times every 10 seconds, 3000 stimuli in 63 min) at an intensity previously found to be peri-threshold for eliciting action potential activity (about 30% of max. output). Stimuli were applied via a figure-of-8 coil (MagStim) centered 10 mm above occipital cortex of rat with mediolateral current direction to induce primarily callosal activity. Following rTMS, rats were perfused and visual, somatosensory, motor and frontal cortices of each hemisphere were processed for PV, CB, CR, zif-268 and NeuN immuno-labelling. In all cortical areas analysed, the number of NeuN cells was unchanged indicating no loss of neurons while the number neurons expressing PV strongly decreased after TBS but not after 1 Hz rTMS and CB expression decreased only with 1 Hz stimulation. CR was not affected at all, but both rTMS protocols increased zif-268 expression - indicative of increased pyramidal cell activity. Selective decrease in cortical PV after TBS was confirmed by Western Blot. Our results indicate that rTMS might activate different populations of cortical interneurons depending on the stimulation frequency and pattern used. TBS including 5 and 50 Hz frequencies specifically affected PV which is known to be expressed in fast-spiking interneurons of basket and chandelier type and which are thought to control gamma- and theta-oscillations, just those frequencies included in the TBS. A decrease in PV is also found in schizophrenics and can be induced in a rat model by transiently blocking interneuron activity with the NMDA receptor antagonists phencyclidine (PCP). The decreased CB concentration after 1 Hz rTMS (which lowers cortical excitability) is also in line with CB knockout mice showing a depressed synaptic facilitation. Supported by the DFG (SFB 509 TP C12).

# *Stem cell induced cortical plasticity reduces brain damage after perinatal asphyxia in rats*

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Every year many neonates suffer brain damage arising during birth because of perinatal asphyxia leading among others to spastic paresis. Previous studies indicated that treatment with stem cells obtained from human umbilical cord blood (hUCB) reduces motor deficits after hypoxia, as illustrated by walking pattern [1]. Here we investigate the influence of hUCB cells after perinatal brain damage on neural recovery by studying cortical reorganisation in primary somatosensory cortex (SI) in rats with and without hUCB treatment. To investigate the neuronal deficits after perinatal brain damage we used seven week old rats. One group (lesioned rats) underwent a hypoxic ischemic insult, induced by the Levine-procedure [2] at postnatal day seven. This model entails ligation of the left common carotid artery followed by hypoxia. Another group of rats (stem cell rats) underwent the same treatment as the lesioned rats, but additionally received an injection of hUCB cells. Control rats were neither lesioned nor hUCB treated. To investigate the potential of hUCB cells on neural behaviour, we recorded multiunit-activity in layer IV of the hind paw representation of SI. Parameters analysed were the size of the cortical hindpaw map, receptive field size, and cortical excitability measured by means of a paired pulse stimulation (pps) paradigm. For pps we used different interstimulus intervals (ISIs) between 30 and 200 ms. Evaluation of peristimulus histo¬grams was performed for each ISI individual¬ly by cal¬cu¬la¬ting the ratio of amplitudes (2nd/1st). The electrophysiological recordings were supplemented by histological analysis to document migrated stem cells. Immunohistological methods were used to show apoptosis. Furthermore we performed behavioural experiments (footprint analysis, cylinder test [3]), to assess motor deficits. In contrast to control animals, lesioned animals were characterized by reduced cortical maps, and enlarged receptive fields. In the stem cell group, the size of receptive field was fairly normal, and the cortical representations were enlarged compared to lesioned animals. The paired pulse behaviour (ppb) observed in control animals at short ISI was characterised by a strong suppression of the second response, which was gradually alleviated with increasingly longer ISIs. Rats with hypoxic ischemia showed irregular ppb, indicating a distorted balance of excitation and inhibition. Rats from the stem cell group showed an intermediate ppb resembling in many features that observed in control animals, indicating a positive effect of stem cells to the damaged tissue including recovery and reorganisation of neuronal pathways.

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[3] Schallert T. (2000), CNS plasticity and assessment of forelimb sensorimotot outcome in unilateral rat model of stroke, cortical ablation, parkinsonism and spinal cord injury, Neuropharmakology 39: 777-787

# The role of presynaptic NMDA-receptors in lesion-induced plasticity of the visual cortex in rats

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NMDA receptors are fundamental for neuronal plasticity in the CNS. There is emerging evidence for the existence of presynaptically located NMDA receptors in different areas of the brain. Studies on the functional properties revealed a relatively high expression of the NR2B-subunit at these receptors, and most studies link presynaptic NMDA receptors to the expression of long-term depression. We have recently shown that the center of a focal, cortical lesion is surrounded by an area of facilitated LTP, which is sensitive to antagonists of NMDA-receptors containing the NR2B-subunit. To further investigate the possible role of presynaptic NMDA receptors for plasticity in our lesion model, we studied the effect of an NMDA receptor antagonist on AMPA receptor mediated miniature excitatory postsynaptic currents (mEPSCs). Whole cell patch-clamp recordings were performed from neurons in cortical layers II/III at distances of 2.5-4mm lateral to the lesion. AMPAR mediated mEPSCs were acquired, and the mean frequency of these signals was increased in lesion-treated slices (sham-OP: 9.87±0.56 Hz; lesion: 12.26±0.5Hz, $p<0.05$; n=24) with no changes in the amplitude of the mEPSCs. Next, postsynaptically located NMDA receptors were blocked by dialysis with dizocilpine maleate (MK-801) in the recording pipette. Under these conditions the NMDA receptor-mediated component of evoked synaptic responses was completely blocked. When D-AP5 was applied to the extracellular solution in the presence of intracellular MK-801, it caused a significant decrease in the frequency of AMPA receptor mediated mEPSCs in lesion-treated animals (before D-AP5: 11.69±0.58Hz; in D-AP5: 9.32±0.68Hz, $p<0.05$, n=11) compared to sham-operated controls (before D-AP5: 8.7±1.01Hz; in D-AP5: 8.13±0.67Hz, n=12). However, the amplitudes of these signals were not different. We conclude that the reduced frequency of mEPSCs in the presence of D-AP5 post lesion is caused by blockade of presynaptically located NMDA receptors. We suggest that these receptors mediate the recently observed enhancement of presynaptic glutamate release, thereby facilitating the enhanced LTP at the border of the lesion (supported by the DFG, SFB 509, C4).

# Defined role of p75, TrkB.T1 and TrkB neurotrophin receptors in structural plasticity of hippocampal neurons

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Neurotrophins and their receptors are important modulators of both functional and structural plasticity in the developing and adult nervous system. It is of particular interest that they are able to modulate both positive and negative changes depending on the type of receptor they bind to - either the Trk receptors or the pan neurotrophin receptor $p75^{NTR}$. According to our working hypothesis it is the balanced activation of these two distinct receptor types that is responsible for the often opposing effects of neurotrophins.

Whereas numerous studies report the essential role of BDNF and its receptor TrkB in the regulation of hippocampal long-term potentiation (LTP), the $p75^{NTR}$ is known to be involved in synaptic weakening (LTD) (Rösch et al., 2005). Furthermore there is evidence that BDNF signalling via TrkB is promoting neurite outgrowth and elongation, in contrast to this over-expression of the $p75^{NTR}$ has been shown to have a negative effect on dendritic morphology and spine density (Zagrebelsky et al., 2005). However the molecular and cellular mechanisms mediating these structural changes remain so far elusive and also the role of the truncated form of the TrkB receptor (TrkB.T1) in these processes is not clear.

To investigate the possibly opposite role of the two distinct receptors involved in structural plasticity we analysed the dendritic morphology and spine density of organotypic hippocampal cultures of mice over expressing the full-length (TrkB) or TrkB.T1. We found an altered dendritic complexity and increased spine numbers in cultures prepared from p5-p6 mice of both strains after 18 DIV compared to cultures derived from WT mice. Remarkably, a detailed spine analysis showed an increase of mushroom spines in particular for the TrkB.T1 over-expressing pyramidal neurons whereas the other spine types thin and stubby were not altered. Neurons over expressing the TrkB full-length receptor showed a similar but milder phenotype.

As a second step we investigated morphological changes of neurons over-expressing TrkB receptors under conditions known to evoke $p75^{NTR}$-dependent functional plasticity. Therefore we chemically induced LTD by the addition of a low NMDA conc. to cultures 16 DIV and analysed their dendritic structure and spine density 48 h later. Interestingly this treatment seemed to reduce the changes induced by the over-expression of both TrkB receptor types. Along with these findings an additional $p75^{NTR}$ over-expression approach will help us to better define how the balanced activation of $p75^{NTR}$ and TrkB receptors modulates structural plasticity.

# Role of the NogoA/NgR/p75$^{NTR}$ signaling system in modulating structural plasticity in the mature mouse hippocampus

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Functional and structural dynamics in neurons play a crucial role in the development and adult synaptic plasticity of neuronal circuits. While it is known that dendritic spine number and dendritic complexity can change during activity-dependent plastic processes (Yuste and Bonhoeffer, 2001) mechanisms and molecules involved in this structural plasticity are largely unknown. Neurotrophins have been shown to modulate neuronal morphology and to induce functional changes at synapses. Specifically, BDNF, through its receptor TrkB, is a crucial component of activity-dependent strengthening of hippocampal synapses (for a review see Kovalshuk et al. 2004) as well as of positive structural changes at dendrites and spines (Tyler and Pozzo-Miller 2001). In contrast, the p75 Neurotrophin Receptor (p75$^{NTR}$) has been reported to be involved in maintaining hippocampal long-term depression suggesting a possible role in synaptic weakening (Rösch et al., 2005). In addition, we described a role for the p75$^{NTR}$ in negatively regulating dendrite complexity/spine density in the mature hippocampus (Zagrebelsky et al., 2005). Our goal is to understand the cellular mechanism by which the p75$^{NTR}$ modulates negative plasticity. Besides binding several ligands, the p75$^{NTR}$ interacts with Trk receptors, controlling positive structural changes, and with the Nogo receptor (NgR) mediating the negative action of myelin associated neurite growth inhibitors. We want to asses whether p75$^{NTR}$ exerts its effect on dendrite morphology by mediating the negative action of the NogoA/NgR signalling system and how Neurotrophins could modulate this interaction. We first compared dendrite length and complexity as well as dendritic spine density in wild type (WT) *versus* NogoA knockout (KO) mice. Dendritic length and complexity are increased in the apical dendrite distal portion of NogoA KO *versus* WT neurons. Interestingly, in the most distal portion of the apical dendrites spine density is significantly lower in KO versus WT neurons. In addition, we compare the morphology of WT neurons treated with specific NogoA-blocking antibodies to the one of WT neurons treated with control antibodies. Our data show a decrease in spine density in neurons treated with NogoA blocking antibodies versus those treated with control antibodies. These results suggest that NogoA might indeed modulate the morphology of mature hippocampal pyramidal neurons. On going experiments comparing the changes in dendrite morphology induced by the application of NogoA blocking or control antibodies and with an opposite approach over expressing NogoA in p75$^{NTR}$ KO mice will be crucial in clarifying the role of the NogoA/NgR/p75$^{NTR}$ signalling systems in the mechanisms controlling structural plasticity in mature pyramidal cells.

# Online interaction with in vitro neuronal networks

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The whole brain as well as single neurons process information parallel at multiple spatial and temporal scales. Random ex-vivo neuronal networks provide means to study generic neuronal and network function. Cell cultures develop and adopt oscillations and spikes over a broad range of time scales. They are structurally rich and experimentally stable over several weeks. The goal of our work is to understand the principles underlying functional network differentiation, modification and stability. By applying electrical stimulation that interacts with the network connectivity, we want to identify the mechanisms that induce synchronized bursting and selective learning in neuronal cultures.

We use neocortical neurons cultured on microelectrode arrays (MEAs) that develop into random networks and are accessible for simultaneous recording and stimulation through multiple electrodes. After about one week in vitro, these networks change their activity from uncorrelated spiking to more and more complex synchronized bursts. The bursts may have physiological relevance in the culture, e.g. they may induce synaptic plasticity. However, they also resemble epileptiform activity and may result from the lack of physiological input to the culture. To attain control over the state of network activity, we first dissociate or reduce bursting by applying multi-site electrical stimulation to the networks.

Additionally, this leads to a second aspect which is to use a defined stimulation paradigm to embed functionality into the networks from outside. We show that the Stimulus Regulation Principle, a universal learning principle advocated by behaviorists over 60 years ago, is inherent to cultured neuronal networks. In particular, we 'teach' the network predefined activity patterns or 'spatial spiking tasks'. We apply low-frequency stimulation that interferes with the network and promotes changes in synaptic connectivity. By removing the stimulus whenever a predefined response is observed, we preclude the acquisition of any new stimulus-response association. This change in the drive underlying the exploration in the space of possible responses can be seen as the physiological counterpart of behavioral reward and realize selective learning in neuronal networks.

These experiments require fast and flexible interaction with the networks. The stimulation induces changes in network dynamics requiring adaptation of the stimulation patterns over timescales ranging from milliseconds to days. The efficacy of a stimulation pattern and the storage capacity also depends on the state of the network. For these reasons, we have established a LINUX-based activity-controlled feedback system for neuronal networks grown on MEAs. Selected features of the neuronal activity can be extracted online and used to control a stimulus generator connected to the MEA system. This closed-loop setup allows direct interaction with cultured neuronal networks to better understand their functional principles.

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# Glycoprotein M6a: Expression and regulation by chronic stress in the brain

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Morphological changes in neurons are implicated in the pathogenesis of depression and are thought to reflect aberrant adaptive neuroplasticity initiated by an altered programme of gene expression in response to stressful or aversive stimuli. The glycoprotein M6a, a presumptive structural component of neural membranes, was previously reported to be downregulated by chronic psychosocial stress in the hippocampus of male tree shrews (Tupaia belangeri), but restored to normal levels following treatment with the antidepressant clomipramine (Alfonso et al. 2004). This finding and the recently discovered role of M6a in neurite outgrowth and filopodia formation (Alfonso et al. 2005) suggests M6a may be involved in neuroplastic synaptic events comprising the brain's response to chronic stress.
In the present study M6a expression was investigated in the hippocampus and prefrontal cortex of normal and chronically stressed rats using quantitative in situ hybridization and real-time PCR. Strong hybridization signals were detected in specific hippocampal subfields (including dentate granule cells and pyramidal neurons of the CA fields) and in pyramidal cells of the prefrontal cortex (including prelimbic and infralimbic cortices). Hippocampal expression of M6a was significantly downregulated by chronic restraint stress. In contrast, prefrontal M6a expression was found to be mildly upregulated by chronic stress in layer II pyramidal neurons of the prelimbic and infralimbic cortices.
Immunocytochemical techniques, including light and confocal microscopy, were used to characterise the localization of M6a protein within these brain regions. In the stratum lucidum of the hippocampus, M6a immunoreactivity is found to colocalise with calbindin-immunoreactive mossy fibers projecting from dentate granule cells, but not with MAP-2-immunoreactive dendrites of the CA3 pyramidal neurons targeted by mossy fiber terminals. M6a was similarly found to be localised on axon membranes, but not on dendrites in the prefrontal cortex regions examined.
The present data demonstrates that in the brain, glycoprotein M6a is localised to axonal membranes and provide evidence that M6a expression is differentially regulated by chronic restraint stress in the hippocampus and prefrontal cortex, suggesting that M6a may be involved in stress-induced remodelling of presynaptic terminals in the brain.

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# EPAC: A novel modulator of presynaptic short-term plasticity in cultured hippocampal neurons.

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At excitatory mammalian cerebral synapses cAMP is known to play a crucial role for the development of short-term plasticity by enhancing glutamate release from presynaptic terminals. Traditionally this effect of cAMP is attributed to the direct activation of PKA. Here we functionally characterize the novel cAMP target EPAC in cultured autaptic granule cells of the dentate gyrus. Specifically, we examine the EPAC-induced modulation of short-term plasticity and of synaptic vesicle dynamics. Forskolin activates adenylyl cyclases and increases evoked EPSC amplitudes and mEPSC frequency up to 2-fold and 4-fold, respectively. 50% of the increase in evoked EPSC amplitudes and 100% of the increase in mEPSC frequency of the forskolin effect can be accounted for by EPAC activation using the specific EPAC agonist 8-pCPT-2'-Me-O-cAMP. After measuring the increase in mEPSC frequency in the presence of 8-pCPT-2'-Me-O-cAMP and tetrodotoxin we immediately perfuse the granule cells with 500 mM sucrose. We measure a reduced readily releasable pool (RRP) size compared to control conditions. We observe a similar reduction in the RRP size when we record evoked EPSCs at a frequency of 0.2 Hz in the presence of 8-pCPT-2'-Me-O-cAMP and when we subsequently release the RRP by application of sucrose solution. We conclude that EPAC activation enhances vesicular release probability. Potential changes in the number of readily releasable vesicles resulting from EPAC activation will be addressed by high frequency stimulus trains under suppressed EPAC activity conditions. In preeliminary high frequency train experiments we initially deplete the RRP size by sucrose application. During the following refilling phase of the RRP the general adenylyl cyclase inhibitor MDL 12,330A completely abolishes the electrically evoked neurotransmitter release. However, a second sucrose response is still evoked in the presence of MDL 12,330A at the end of the high frequency train. Further research will show if different cAMP microdomains modulate distinct steps in vesicular dynamics involving PKA or EPAC. Finally, we present experimental evidence that MAPK activity is important for EPAC signalling. EPAC-induced potentiation of evoked EPSC amplitudes is sensitive to the MEK inhibitor U0126. At the concentration used, U0126 does not change basal neurotransmission. Moreover, our results demonstrate that phorbol-12,13-dibutyrate (PDBu) enhances evoked EPSC amplitudes in autaptic granule cells of the dentate gyrus up to 2.5-fold. PKC activity accounts on average for 60% of the measured PDBu effect. Pretreatment of autaptic granule cells with 8-pCPT-2'-Me-O-cAMP increases the subsequently elicited PDBu effect by maximally 80%. Hence granule cells of the dentate gyrus possess a mechanism for cAMP-to-PKC signaling which presumably overlaps with the so far proposed Rim1/Rap1/PLCε and Rap1/MAPK signaling pathways. We provide evidence that EPAC activity is clearly involved in the regulation of vesicular dynamics.

# Stress induced regional and hemispheric morphological modifications of pyramidal cells in the rat prefrontal cortex

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The rat prefrontal cortex (PFC) is thought to play an important role in the stress response. We investigated whether there might be morphological differences between pyramidal neurons in the three PFC sub-areas (infralimbic, prelimbic and anterior cingulated cortex) and between the hemispheres, and whether chronic stress changes pyramidal cell morphology. Using a whole-cell patch-clamp method with 400-µm thick slices from the PFC we selectively filled neurons in the three PFC sub-areas with neurobiotin. Cells were three-dimensionally reconstructed and the length of their apical and basilar dendrites, as well as the number of dendritic branches of distinct branch orders was determined. In control rats, all three PFC sub-areas showed inter-hemispheric differences in the length of apical dendrites at certain distances from the soma and stress abolished these differences between the hemispheres. In the prelimbic cortex (PL) of controls, the number of apical dendritic branches of the branch order 3 was larger in the right compared to the left hemisphere. Stress reduced the number of branches of branch order 3 selectively in the right PL. Stress also reduced the total length of apical dendrites in the right PL yielding a significant difference to the respective data from the right hemisphere of controls. These data support and extend previous findings describing functional differences between PFC sub-areas and the importance of the right hemisphere in the stress response.
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# Morphological analyses of the hippocampus of tenascin-C deficient mice

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Tenascin-C (TNC) is an extracellular matrix glycoprotein involved in neural development and plasticity. Constitutive TNC deficiency in mice causes aberrations in the cellular composition of the adult neocortex, such as increased numbers of excitatory neurons and astrocytes, and decreased numbers of parvalbumin-positive (PV+) interneurons, which are associated with enhanced evoked-related cortical responses (Irintchev et al., 2005, Cerebral Cortex 15, 950). Previous electrophysiological studies have also revealed abnormalities in synaptic plasticity and rhythmic activity in the hippocampus of TNC deficient (TNC-/-) mice but it is unclear whether these functional abnormalities are associated with structural aberrations. We quantified PV+ interneurons, principal cells and astrocytes in the dentate gyrus and CA1 and CA3 subfields of the dorsal hippocampus of 5-month-old TNC-/- mice and wild-type (TNC+/+) littermates using stereology and immunofluorescence. The volume of the hippocampus and its subfields, and synaptic coverage of principle cells by GABAergic terminals were also evaluated. The results showed a reduction of the CA1 subfield volume in TNC-/- mice compared with TNC+/+ littermates as well as increased numerical density (number per unit volume) of the PV+ interneurons in this area but not in CA3 or the dentate gyrus. Total numbers of PV+ neurons, principal cells and ratios of PV+ to principal cells, as well as linear densities of PV+ and PV- inhibitory terminals around principal cell somata were similar in the two genotypes. Both densities and total numbers of astrocytes were significantly increased in all subfields of the hippocampus of TNC-/- mice. These findings indicate different impacts of TNC deficiency on major populations of neurons, but not astrocytes, in the hippocampus as compared with the neocortex and might help explain some functional abnormalities previously observed in TNC deficient mice.

# Auditory sensorimotor integration and training of motor functions after stroke

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Background and Purpose: Previous studies have shown that just three weeks of piano training can induce neuronal representations of skilled finger movements activated by auditory stimulation. This study investigated whether or not this kind of cross-modal mechanism for auditory sensorimotor integration can be employed in the rehabilitation of motor functions following a stroke.

Methods: We evaluated a music-supported training program designed to induce an auditory sensorimotor co-representation of movements in 32 stroke patients (17 affected in the left and 15 in the right upper extremity). Patients without any previous musical experience participated in an intensive step-by-step training, that began with the paretic extremity, and was followed by training of both extremities. Training was applied 15 times over 3 weeks in addition to conventional treatment. Fine as well as gross motor skills were addressed by using either a MIDI-piano or electronic drum pads. 30 stroke patients (15 affected left and 15 right) undergoing exclusively conventional therapies were recruited as a control group. Behavioral pre- and post-treatment motor functions were monitored using a computerized movement analysis system (Zebris) and an established array of motor tests (e.g. Action Research Arm Test, Box and Block Test). To investigate the activity of cortical regions in the control of movement, we studied event-related desynchronization/synchronization (ERD/ERS) and event-related coherences from all 62 patients performing self-paced movements of the right index finger (MIDI-piano) and of the whole arm (drum pads).

Results: Patients showed significant improvement after training with respect to speed, precision and smoothness of movements as shown by 3D movement analysis and clinical motor tests. Furthermore, compared to the control patients, motor control in everyday activities improved significantly. Neurophysiological data showed a significantly larger decrease of EEG signal (power) before time of movement onset in the music-supported training group in the post training register, which is associated with increased corticospinal excitability, whereas there are almost no differences in the control group. The music-supported training group presented a pronounced enhancement of the coherences after the training compared to the control group, especially in the drum condition.

Conclusion: This innovative therapeutic strategy is an effective approach for the motor skill neurorehabilitation of stroke patients.

# Immediate early gene expression in the cortico-hippocampal network after neocortical ischemic lesions

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Immediate early gene expression in the cortico-hippocampal network after neocortical ischemic lesions

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Focal ischemic lesions of the cerebral cortex stimulate endogenous progenitor cells in the dentate gyrus resulting in an increase in neurogenesis. Up to now only little is known about the mechanisms which mediate this neurogenic response in the dentate gyrus. However, some evidence indicates that the cortico-hippocampal network via the entorhinal cortex plays a role. Here, we analyzed the activation of the cortico-hippocampal network after small cortical infarcts using immunohistochemistry with antibodies against different immediate early genes (c-fos, pCREB) in transgenic pNestin-eGFP mice allowing the determination of distinct progenitor subtypes. Small ischemic infarcts were induced in the forelimb sensorimotor cortex using the photothrombosis model. Sham-operated animals served as controls. Animals were allowed to recover for 6 and 72 hours post surgery. C-fos and pCREB-positive cells were immunocytochemically detected and phenotyped with additional markers (nestin-GFP, doublecortin, NeuN, and GFAP) using confocal laser scanning microscopy. Stereological evaluation revealed a significant increase in number of c-fos positive cells in the entorhinal cortex of animals with cortical infarcts at 6h and 72 h post ischemia compared with sham-operated controls. In the dentatus gyrus the increase in c-fos-positive cells was delayed showing no differences between lesioned and control animals at 6 h after the infarct but a significant increase at 72 h. Further immunocytochemical characterization of c-fos- and pCREB-positive cells was performed. Our data indicate that small cortical infarcts in the forelimb sensorimotor cortex activate the cortico-hippocampal network via the entorhinal cortex including specific subtypes of progenitor cells in the dentate gyrus.
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# Calcium-induced exocytosis from actomyosin-driven, motile varicosities formed by dynamic clusters of organelles

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Varicosities are ubiquitous neuronal structures that appear as local swellings along neurites of invertebrates and vertebrates. Surprisingly little is known about their cell biology. We use here cultured *Aplysia* neurons, and demonstrate that varicosities are motile compartments that contain large clusters of organelles. The varicosities content propagate along neurites within the plasma membrane "sleeve", split and merge, or wobble in place. Confocal imaging, retrospective immunolabeling, electron microscopy and pharmacological perturbations reveal that the motility of the varicosities' organelles content occurs in concert with an actin scaffold and is generated by actomyosin motors. Despite the motility of these organelle clusters within the cytoplasm along the neurites, elevation of the free intracellular calcium concentration within the varicosities by trains of action potentials induces exocytosis followed by membrane retrieval. Our observations demonstrate that varicosities formed in the absence of postsynaptic cells behave as "ready to go" prefabricated presynaptic terminals. We suggest that the varicosities' motility serves to increase the probability of encountering a postsynaptic cell and to rapidly form a functional synapse.

# Constitutive expression of cyclins and cdks and their function beyond cell cycle

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Maturation of brain is accompanied by the generation of neural precursors, migration of postmitotic neuronal cells to their final location and the subsequent terminal differentiation to a neuron. An obligatory step for a neuron to become differentiated is the withdrawal from the cell cycle. The timing of cell cycle exit and neuronal differentiation is likely to be controlled by the activity of cell cycle-related proteins, like cyclins, cyclin-dependent kinases (cdks) and cdk inhibitors (cdkis). Once neurons are terminally differentiated, they lose their ability to proliferate. Maintenance of mitotic quiescence is essential for the functional stability of the complexely wired neuronal system. In the present study, we have evaluated the expression of cell cycle-related proteins in the adult mouse neocortex by immunohistochemical, immunobiochemical and molecularbiological methods. We have found the expression of cell cycle-related proteins, like cyclins D, A, B and E as well as the cdks 1, 2 and 4 in terminally differentiated pyramidal neurons. The expresssion of cyclins and cdks could be localized not only to the nucleus, but also to the perinuclear cytoplasm and in dendrites of pyramidal neurons. Additionally, we demonstrate that cdks and their corresponding cyclins are co-localized in neurons. The cdk inhibitors of Ink4 family and Cip/Kip family have been detected in the nuclear and cytoplasmic compartment as well as in dendrites of the same cell types. Expression of cdk inhibitors prevents cell division and is essential to maintain differentiated neurons in a quiescent state. Immunohistochemical findings were verified by the detection of RNA messages for a number of cyclins, cdks and cdkis in pyramidal cells by means of single cell PCR. The functional impact of the detection of cell cycle-related proteins in differentiated neurons has not been elucidated yet. Our hypothesis is, that cyclins and cdks may contribute to structural changes that underlie neuronal plasticity. The ability to undergo structural and functional reorganization is an unique feature of neurons, that distinguish them from other cell populations of the organism. We could show by immunoelectron microscopy and immunobiochemical methods an association of cyclins and cdks with the microtubule cytoskeleton. Furthermore, microtubule-associated protein tau is complexed with cyclins D, E, A and B as well as with cdks 1, 2 and 4. We also demonstrate that the cyclin/cdk complexes exhibit kinase activity towards microtubule-associated proteins. Findings suggest, that the expression of cell cycle-related proteins in terminal differentiated pyramidal neurons are associated with additional physiological functions beyond cell cycle regulation that might be involved in processes of neuronal plasticity.

# Biochemical and immunochemical characterization of perineuronal nets

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Perineuronal nets (PNN) are specialized extracellular matrix structures enwrapping CNS neurons. They are important regulators for neuronal and synaptic functions. Here, we have classified the appearance of these structures in hippocampal primary cultures. We used brevican, a chondroitin sulfate proteoglycan that is an integral component of PNN as molecular marker. We found three major classes of brevican-containing PNN-like structures in dissociated neurons. Type 1 is the most abundant PNN appearance observed in cultured neurons. It is formed around somata of excitatory cells and is typically wrapped around the axon initial segment. Type 2 is formed on a different excitatory cell type and shows very little immunoreactivity for brevican. As type 3 we classified the most pronounced net formed around inhibitory GAD65-positive cells. Further we found excitatory and inhibitory synapses embedded in the PNN. To assess the size and content of perisynaptic brevican containing PNN-like structures we prepared synaptosomes from rat brains. We found that the major portion of synaptosome-attached brevican is proteolytically cleaved and highly glycosilated. Extraction with EDTA suggests this attachment to be calcium dependent. The size of Brevican containing complexes from synaptosomes was determined by gel filtration and they were up to 1,8 MDa. Altogether, we show that mature primary cultures can form different types of PNN, and that basic features of these extracellular matrix structures described in the brain may also be studied in vitro. Further, we characterized the size and the binding properties of brevican-containing PNN-like structures to the synaptosomes and plan now to tackle the question of its molecular content and attachment to the membrane.

# Sculpturing synapto-dendritic circuitry via Jacob and its interaction partners

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The neuronal Ca2+-sensor Caldendrin is a postsynaptic density (PSD) component with high similarity to Calmodulin (CaM). Jacob, a newly identified Caldendrin binding partner, is a novel protein present in the PSD and neuronal nuclei that is abundantly expressed in limbic brain and cortex. Upon NMDA receptor activation Jacob is recruited to the nucleus resulting in a rapid stripping of synaptic contacts and a drastically reduced complexity of the dendritic tree. Caldendrin, but not CaM, controls Jacob's extra-nuclear localization by Ca2+-dependently competing the binding of Importin-α to Jacob's nuclear localization signal. The extranuclear overexpression of Jacob leads to an increased number of dendrites and depending upon alternative splicing the growth and formation of PSD-like structure that eventually build dendritic protrusions with active synaptic contacts. These Jacob-induced PSD-like clusters are large (on the average 4µm in width), they can be induced before synaptogenesis takes place und they recruit major PSD-scaffolding proteins like ProSAP/Shanks and MAGuKs. At later stages NMDA- and AMPA receptors are recruited and clustered in these protrusions. To learn more about the underlying mechanisms we identified crucial regions in Jacob that are essential for the overexpression phenotypes. We then checked for potential binding partners that could be involved in the extranuclear morphogenetic effects of Jacob. This strategy disclosed a number of novel Jacob binding partners including the formation of Jacob homodimers. Importantly, Jacob interacts with α-internexin, a type IV intermediate filament abundantly present during neuronal development. Most interestingly, confocal laserscan microgrpahs of hippocampal primary cultures show that the distribution of α-internexin in dendrites is discontinuous and that Jacob-induced protrusions are preferentially located at those discontinuities where α-internexin is not present. Further biochemical studies suggest that the α-internexin interaction could provide an important dendritic docking site for Jacob where both, its retrograde transport to the nucleus as well as its morphogenetic effects on PSD-growth are regulated.

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# L-type $Ca^{2+}$ channels, CaMKII, and cAMP cooperate in mediating postsynaptic secretion of BDNF and NT-3

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In spite of the wealth of knowledge regarding their biological actions, little is known about the mechanisms involved in activity-dependent secretion of neurotrophins (BDNF, NT-3, NT-4/5 and NGF). We now investigated the intracellular signalling cascades that mediate depolarization induced synaptic secretion of neurotrophins (NT).

GFP-tagged recombinant BDNF and NT-3 were overexpressed in hippocampal neurons, and time lapse video microscopy of GFP fluorescence was employed to explore the intracellular messengers involved in the secretion process. Experiments were performed in the presence of inhibitors of glutamatergic and GABAergic synapses to avoid any indirect effects of drugs via modulation of synaptic transmission. DsRed-labeled PSD95 was coexpressed to enable investigation of synaptically localized neurotrophin vesicles. Depolarization-induced (50 mM K+) secretion of NTs was critically dependent on the influx of extracellular $Ca^{2+}$ ions. Inhibition of L-type calcium channels with nifedipine (10 to 50 µM) blocked NT secretion. Depletion of internal $Ca^{2+}$ stores by thapsigargin (10 µM) or inhibition of the CaM kinase II using KN-62 (10 µM) both strongly inhibited NT secretion. Intracellular elevation of cAMP (with 100 µM 8Br-cAMP) had no effect on NT secretion. However, inhibition of PKA using Rp-cAMP-S (100 µM) led to a delayed onset and a reduced efficacy of NT secretion. The inactivation of trk receptors by k252a (200 nM) did not impair the secretion of either BDNF or NT-3. Together, these data indicate that depolarization-induced secretion of NTs is triggered by the influx of extracellular $Ca^{2+}$ via L-type voltage dependent $Ca^{2+}$ channels, and efficient release relies on additional release of $Ca^{2+}$ from internal stores and subsequent activation of CaM kinase II. Furthermore, basal PKA activity appears to be necessary for an efficient release of NTs. In addition, short term autocrine activation of trk receptors doesn't modulate NT secretion.

Elevation of intracellular levels of nitric oxide (NO) with sodium nitroprusside (100 µM) partially inhibited NT secretion, while exogenously applied BDNF increased intracellular levels of NO. Inhibition of NO synthesis with L-NMMA (300 µM) had no effect on NT secretion. These data indicate a cellular crosstalk between postsynaptic BDNF and NO signalling in synaptic plasticity in hippocampal neurons.

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# Small interfering RNA mediated efficient knockdown of BDNF in cultured hippocampal neurons

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RNA interference (RNAi) is a conserved cellular mechanism for posttranscriptional regulation of gene expression. Gene silencing by means of transfection with small interfering RNA (siRNA) can be used as a powerful tool to knockdown a selected protein at desired stages of development.
The neurotrophin brain-derived neurotrophic factor (BDNF) is a secreted neuronal protein important for survival, differentiation and synaptic plasticity. Recent evidence suggests that synaptic functions of BDNF are local and require activity-dependent synaptic secretion. Many different synaptic functions of this neurotrophin were discovered in response to either bulk application of exogenous BDNF or by generalized gene deletion in BDNF k.o. mice. However, fine tuning of synaptic efficacy by BDNF is likely to depend on restricted BDNF supply in the local synaptic environment. Thus, in an attempt to suppress BDNF expression and release in selected single postsynaptic neurons, we now have developed an efficient method to knockdown BDNF expression in cultured hippocampal neurons, using lipofection of BDNF-selective siRNA.
To verify efficient delivery of siRNA into neurons, several transfection procedures were tested. Microcultures of postnatal (P0-P3) hippocampal neurons from GFP transgenic mice were cultured in serum-free Neurobasal/B27. Neurons were transfected at 8 DIV with a validated siRNA against GFP. The efficiency of GFP silencing in the neurons was quantified by measuring the residual GFP fluorescence at 1-10 days posttransfection (9-18 DIV), compared to controls transfected with scrambled siRNA. Optimal transfection efficiency (>80% of neurons transfected) was achieved by a combination of hiperfect mediated lipofection and calcium-phosphate precipitation. No signs of neuronal death were detectable at 1-10 days after transfection. GFP expression was unchanged one day after transfection but gradually decreased thereafter, with silencing to <35% (<20%) of GFP fluorescence at 3 (6) days posttransfection. No recovery of GFP-fluorescence was observed up to 10 days following siRNA transfection.
An optimal siRNA sequence for silencing of mouse and rat BDNF was determined using standard algorithms. Silencing efficiency was tested in BDNF-GFP overexpressing HeLa cells. One day after transfection with BDNF siRNA the BDNF-GFP fluorescence was dramatically decreased to <10% of control values of cells transfected with scrambled siRNA. NT-3-GFP, NT-4-GFP and GFP overexpressing cells did never show any signs of fluorescence decrease, supporting the specificity of our BDNF siRNA.
Coexpression of BDNF-GFP and BDNF siRNA in mouse hippocampal neurons also indicated knockdown of the BDNF-GFP protein in neurons. To test for knockdown of endogenous BDNF in neurons, cultures were transfected with the BDNF siRNA at 8 DIV, and BDNF mRNA levels were analyzed at 14 DIV by PCR. These experiments revealed suppression of endogenous BDNF mRNA in mouse hippocampal neurons. This BDNF knockdown approach will enable us to investigate the role of endogenous postsynaptic BDNF for synaptic plasticity in hippocampal slices.

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# Changes in the spectral main sequence of human saccades during saccade adaptation

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It is well known that saccadic eye movements can be characterized by a given correlation between the amplitude of the saccade, its duration and peak velocity, called the "main sequence". Recently, Harwood et al. (J.Neurosci., 99) showed that the Fourier transforms of saccades exhibit certain characteristic minima in the frequency domain that correlate likewise with the duration of the saccade. They called this dependency the "spectral main sequence". Interestingly, the frequencies at which these minima occur in the spectrum hold information about the neuronal control signals that drive the eyes, i.e. the pulse-step signal of the oculomotor neurons (Harris et al.,J.Neurophysiol., 90).

Despite the stereotypy of saccadic eye movements, the underlying neuronal control system is capable of rapidly adapting to conditions (e.g. changes in the mechanics of the eyeball) that otherwise would lead to movement inaccuracy and poorer vision - an effect usually referred to as saccade adaptation. In the laboratory environment this adaptation process can be easily induced within few trials by an intrasaccadic step of the saccade target, usually going unnoticed by the subjects. This step can either be directed into or against the direction of the saccade, leading to an increase or decrease of the saccadic gain, respectively. Up to now, it is still an open question, whether or not such gain adaptation changes the dynamics of the saccades and if so, how this change is implemented at the neuronal level. The goal of our present study was to further investigate the influence of the saccade adaptation on the spatio-temporal profile of the eye movement. We sought to address this issue by examining both the "classical" and the spectral main sequence of human saccades.

Subjects were tested under three different conditions: in blocks of control trials without gain modification or in blocks in which we induced either a gain increase or decrease of saccade amplitude. Adaptation was induced by displacing the saccade target perisaccadically by an amount of 20% of saccade amplitude. Eye movements were recorded with an infrared eye tracking system (EyeLink II, SR Research). In order to calculate the (spectral) main sequence, we varied the mean saccade amplitudes across blocks of trials, with amplitudes ranging from 10 to 25 degrees of visual angle.

Our eye movement analysis revealed the expected pattern for the classical and spectral main sequence. Yet, comparisons between the control and the adaptation conditions revealed significant differences for the classical measures as well as for the spectral data.The most pronounced effect we observed in the spectral data was a shift of the first minimum towards lower frequencies in the gain decrease condition.Our findings make specific predictions concerning the changing discharge pattern of the pulse-step generator in the process of saccade adaptation.These predictions can be tested neurophysiologically.

# A balance of protein synthesis and proteasome-dependent degradation determines the maintenance of LTP

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Long-lasting changes in synaptic strength are thought to play a pivotal role in activity-dependent plasticity and memory. There is ample evidence indicating that in hippocampal long-term potentiation (LTP) the synthesis of new proteins is crucially important for enduring changes. However, whether protein degradation also plays a role in this process has only recently begun to receive attention. Here, we examine the effects of blocking protein degradation on LTP. We show that pharmacological
inhibition of proteasome-dependent protein degradation, just like inhibition of protein synthesis, disrupts expression of late (L-)LTP. However, when protein degradation and protein synthesis are inhibited at the same time, LTP is restored to control levels, calling into question the commonly held hypothesis that synthesis of new proteins is indispensable for L-LTP. Instead, these findings point to a more facetted model, in which L-LTP is determined by the combined action of synthesis and degradation of plasticity proteins.

# Sensorimotor gating of the goldfish startle response: behavioral and neural characteristics.

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Prepulse inhibition (PPI), a well-documented sensorimotor-gating phenomenon observed in many animals, including man, is characterized by a decrease in magnitude of a startle reflex if a weak, non-startling stimulus (acoustic, visual, or tactile) is presented 20-600 ms before the startling auditory stimulus. However, the underlying cellular mechanisms of PPI have remained largely elusive.

The Mauthner-cell (M-cell) system, a reticulospinal network that mediates the startle escape behavior of many teleosts, is potentially well suited to address such mechanistic questions because it is accessible for quantitative behavioral studies as well as *in vivo*in electrophysiology. However, a detailed behavioral description of PPI in fish and its physiology is missing.

We performed behavioral experiments in goldfish (N=12) in order to determine whether the expression of an auditory-evoked escape response, specifically the escape probability, can be modified by an auditory prepulse (a-A PPI paradigm). In addition, we also substituted a visual looming stimulus for the auditory startling stimulus (a-V PPI paradigm). The rational for the latter was to test if the putative PPI effect evoked by an auditory prepulse can also influence escapes triggered by a different modality. For both experiments the prepulse (S1) was presented 20-600 ms before the startling stimulus (S2).

Both stimulus paradigms produced the same significant ($p<0.001$) decrease in escape probability, i.e., an auditory prepulse reduces the occurrence of both visual and auditory evoked startle escapes by about 80 %. The effect was seen over the whole range of S1-S2 interstimulus intervals (ISIs).

We also performed electrophysiological experiments (N=5) where we recorded intracellularly from the M-cell soma and lateral dendrite using the a-A PPI paradigm and compared the PSPs evoked by a range of S2 stimuli with and without prepulse. The results show, on average, a 16 ±2.5 % (SEM) decrease of peak amplitude for ISIs ranging from 20-150 ms. However, ISIs between 20-50 ms typically produced up to 25% attenuations of the PSP evoked by S2. In addition, we found that an auditory prepulse produces postsynaptic inhibition in the M-cell that can last up to 300ms.

Taken together the results suggest that a PPI-type attenuation, with temporal characteristics comparable to those of PPI in mammals, occurs during visual and auditory evoked escapes in goldfish. The cross-modal nature of the observed attenuation suggests a downstream inhibitory mechanism, presumably at the level of the M-cell system.

# Molecular Correlates of Tinnitus in the Auditory System: BDNF, Arg3.1/Arc, and the Role of GABA

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Phantom auditory sensation (tinnitus) is known to be associated with altered neuronal excitability. Brain derived neurotrophic factor (BDNF), the activity-dependent cytoskeletal protein (Arg3.1/Arc), and the immediate early gene c-Fos are known to be affected by changes in excitability and plasticity. Using RT-PCR, in situ hybridisation and immunohistochemistry, the expression of these genes was monitored following acoustic trauma and local (cochlear) or systemic application of salicylate, which are all known to induce tinnitus in humans and rodents. Induction of tinnitus was verified in an animal behavioural model. Regardless of the traumatic paradigm used, a common pattern became evident: (1) BDNF mRNA expression was increased in the spiral ganglion neurons of the cochlea and (2) Arg3.1 expression was significantly reduced in the the auditory cortex (AC). Cochlear application of a GABAA receptor agonist resulted in the complete reversal not only of trauma-induced changes in cochlear BDNF expression, but also in cortical Arg3.1 expression, indicating that the tinnitus-associated changes in cochlear BDNF expression trigger the observed decline of cortical Arg3.1 expression. These findings introduce Arg3.1 and BDNF expression as a molecular “fingerprint” for trauma induced plasticity changes in the central auditory system. Furthermore, our data demonstrate the potential role of an inhibitory efferent feedback on sensory neurons in the modulation of Arg3.1 plasticity changes in the cortex.

# *Identification of markers for neuronal plasticity in the auditory system*

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**Aberrant neuronal activity is known to lead to changes in neuronal plasticity. However, molecular changes following sensory trauma and the subsequent response of the central nervous system are only poorly understood. We focused on finding a molecular tool for monitoring the features of excitability which occur following acoustic trauma to the auditory system. Of particular interest are genes that alter their expression pattern during activity-induced changes in synaptic efficacy and plasticity. The expression of brain-derived neurotrophic factor (BDNF), the activity-dependent cytoskeletal protein (Arg3.1/arc), and the immediate early gene c-Fos were monitored in the peripheral and central auditory system hours and days following a traumatic acoustic stimulus that induced not only hearing loss but also phantom auditory perception (tinnitus), as shown in rodent animal behavior models. A reciprocal responsiveness of activity-dependent genes became evident between the cochlea and the primary auditory cortex (AI): as c-Fos and BDNF exon IV expression was increased in spiral ganglion neurons, Arg3.1/arc and (later on) BDNF exon IV expression was reduced in AI. Expression of Arg3.1/arc is changed in layer II of the AI with a reduction in the tonotopic area representing lower frequencies (10kHz). We extended our studies to the transmembrane proteins neurexin and neuroligin, to examine wether these genes are involved in pre- and postsynaptic activity changes and therefore also mirror trauma-induced changes in synaptic responsiveness. The data are discussed in the context of using activity-dependent genes to monitor trauma induced plasticity changes in the auditory system.**

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# Poster Topic

## T14: Visual system I: Invertebrates

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**T14-7B** A bee in the corridor: regulating the optic flow on one side
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**T14-4C** Lateral interactions between VS cells of the fly visual system give rise to two distinct receptive fields in single VS cells
*YM. Elyada, J. Haag and A. Borst, Martinsried/Munich*

**T14-5C** Light dependent modulation of Kv -Currents in Photoreceptors of *Drosophila*
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**T14-6C** Functional properties of UV photoreceptors in the compound eye of the cockroach
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*MJ. Henze, M. Wernet and T. Labhart, Zürich (CH) and Stanford (USA)*

**T14-8C** The optics of the polarizing tapetum in the postero-median eyes of the gnaphosid spider *Drassodes cupreus*
*K. Müller, L. Bigler and T. Labhart, Zürich (CH)*

# Synaptic properties remain stable during ongoing transfer of visual motion information in the fly brain

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Synaptic signal transmission forms a key mechanism underlying information processing within neuronal networks. It is well known that synapses may change their transfer characteristic in an activity-dependent way, e.g. due to synaptic potentiation or to depletion of the transmitter pool. In this study we focus on graded synaptic connections between identified visual motion-sensitive neurons in the fly brain in order to ask whether these synapses are subject to processes that alter the properties of signal transfer. In particular, we investigate whether the dynamic characteristics of the synapses are modified during ongoing presynaptic activity which is evoked by prolonged motion stimulation. This adapting stimulus consists of a time-varying motion-sequence defined by a white-noise velocity profile. In order to characterise the synaptic filter properties, we apply a linear reconstruction method to recover the presynaptic membrane potential from the postsynaptic spike trains at different time segments during ongoing synaptic activity. We demonstrate that the presynaptic signal can be reconstructed almost equally well in different consecutive time segments of increasing adaptational state. This is true although both the pre- and the postsynaptic responses had lower coherence with the dynamic motion stimulus in the adapted than in the non-adapted state. The lowered coherence values are paralleled by a decline in neural overall activity (presynaptic depolarization and postsynaptic spiking, respectively) in the course of adaptation.

# Activity-dependent adaptation in fly visual motion-sensitive neurons

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Motion-sensitive neurons in the blowfly brain present an ideal model system for the study of adaptation and its functional significance in a behavioural context. Several different adaptation phenomena have been described, but as yet it is largely unknown which of them are based on physiological processes in the motion-sensitive neurons themselves and which originate in previous processing stages [1-3]. One form of adaptation is specifically generated during motion in the preferred direction and is associated with a prominent after-hyperpolarization (AHP) following cessation of stimulus motion [2;3]. The AHP is accompanied by a decrease in neuronal input resistance. This suggests that direction-selective adaptation is generated in the motion-sensitive neurons themselves by the opening of activity-dependent ion channels. We tested this further by registering membrane potential changes of the neurons after the injection of depolarizing currents and by measuring currents in response to depolarizing voltage-clamp commands. Unlike visual stimulation, these protocols activate only intrinsic adaptation processes, but not those located in previous processing stages. $Ca^{2+}$-dependent ion channels have previously been proposed to underlie direction-selective adaptation, because the AHP and dendritic $Ca^{2+}$ accumulation are correlated in their strength and timecourse [3]. To test the involvement of $Ca^{2+}$ in adaptation directly, we elevated the cytosolic $Ca^{2+}$ concentration in single neurons by UV photolysis of caged $Ca^{2+}$. As monitored by $Ca^{2+}$ imaging, this procedure leads to high elevations of the cytosolic $Ca^{2+}$ concentration, equal to those after several seconds of stimulation with preferred-direction motion. However, artificial $Ca^{2+}$ elevations in the absence of visual motion did neither elicit an AHP nor a conductance change. This result renders regulation of direction-selective adaptation by the concentration of bulk cytosolic $Ca^{2+}$ improbable.

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# Motion adaptation improves the detectability of spatial discontinuities in the environment

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It is generally agreed that many features of the responses of motion sensitive neurons depend on stimulus history and, thus, may be regarded as adaptive. However, the functional significance of most of the observed phenomena has not yet been clarified. This is because adaptive processes have been studied by very different stimulus paradigms and conceptual approaches. The functional significance of motion adaptation is addressed here in the blowfly visual system by a novel approach: We analysed by intracellular recording from major output neurons of the blowfly visual system, the HS-cells, how the neuronal representation of spatial aspects of the environment changes during motion adaptation with naturalistic image sequences, i.e. optic flow that has been actively generated by a free-flying fly in a three-dimensional world. Image sequences were rendered employing flight trajectories recorded in known environments.
From previous experiments it is known that blowflies actively structure their visual input by their saccadic flight and gaze strategy, thereby separating rotational and translational optic flow components to a large extent. This behaviour based on such separation allows HS-cells to provide, during the intersaccadic intervals, information about translational self-motion of the animal and, thus, about the spatial layout of the environment [1]. In our experiments we manipulated the naturalistic optic flow sequences generated by free-flying flies by introducing or removing an object into the environment before the optic flow sequences were rendered. With and without object, the overall neuronal response in the intersaccadic interval decreases during prolonged stimulation. However, the sensitivity of HS-cells for the object considerably increases as a consequence of motion adaptation. These results suggest that motion adaptation improves the detectability of spatial discontinuities in the environment.

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# Multisensory integration in the visual system of the fly: encoding common control information from two sensory systems in the activity of a single neuron.

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Summary

In the blowfly Calliphora vicina, lobula plate tangential cells (LPTCs) estimate self-motion by integrating local motion information from the compound eyes (Hausen, 1993). Each LPTC is sensitive to a particular (preferred) rotation of the fly's head. The fly can also sense rotation using its three ocelli (simple eyes), by comparing the light intensities measured at each ocellus (Taylor, 1981). Although both the compound eyes and the ocelli code rotations, there are considerable differences in anatomy, operation, and encoding characteristics. Nonetheless, both systems are known to elicit similar motor activity in response to identical stimuli (Hengstenberg, 1993).

How are the signals from the compound eyes and ocelli combined and transformed into an appropriate format for the motor system? We present extracellular recordings from the neuron V1 which show that integration occurs in LPTCs. We drive V1 with complementary inputs form the ocelli and compound eyes to assess the integration of inputs to the cell, and investigate the advantages of integration. These findings indicate that an exceptionally well-characterised set of visual interneurons can be used to study multimodal integration.

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# The function of the IRM proteins in cell sorting in *Drosophila melanogaster*

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The development of specialised cells or organs requires complex communication between cells in order to give them a specific identity. This involves long and short range signalling mediated by either diffusible factors or direct cell interaction. In a variety of processes the IRM (Irre Cell Recognition Module) proteins (Kirre, Rst, SNS and Hbs) play key roles in the recognition between different cell types.
Using the MARCM method and specific Gal4 strains for *rst* and *kirre* we identified cells in the optic lobes expressing*rst* and/or *kirre*, respectively. With this method it is also possible to drive expression of proteins interfering with IRM mediated signalling in single cells.
During the last step of cell sorting of interommatidial cells in pupal eye development, the IRM is involved in the formation of the semi-crystalline structure of the compound eye. In loss and gain of function analysis we show the necessity of Rst, Kirre and Hbs and also demonstrate the involvement of the Notch signalling pathway.
In the wing imaginal disc IRM proteins play a role in the regular spacing of the sensory organ precursors at the wing margins. The expression of *rst* and the subsequent intracellular relocalisation of the protein at the apical membrane is a prerequisite for the correct placement of the mature sensory bristles. This suggests comparable molecular mechanisms of cell sorting in the eye and the wing imaginal disc.

# Light microscopical localisation of an insect neurons input and output synapses

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Our aim is to obtain a map of the synaptic connections a neuron makes by intracellularly labelling the neuron and staining its synaptic sites using a combination of light microscopy and immunocytochemistry. Using confocal laser scanning microscopy to create 3d reconstructions of the neuron and its synapses, we aim to avoid lengthy ultrastructural reconstructions from serial thin sections. Staining of proteins associated with synaptic vesicles is not useful for detecting individual synaptic sites using the light microscope because the vesicles can spread over quite a large area and cover several synaptic active zones. In contrast, we show here that the Bruchpilot protein, which is confined to the active zone of synapses, can be used to detect active zones of the compound eye photoreceptors of locusts using light microscopy. Whag et al.(1) describe that Bruchpilot is a homologue to mammalian ELKS/CAST and localised at active zones of Drosophila. Our own electron microscopical investigation in the locust has shown that an antibody directed against Drosophila bruchpilot (nc82) localises at the active zone, near the presynaptic dense bar. Thus, it can be used to detect the active zones of individual synapses. Our light microscopical investigations in which we stained photoreceptors of the locust with anti taurine and Bruchpilot with nc82, show that the photoreceptor cells are surrounded by Bruchpilot positive, elongated particles which are the active zones of putative feedback synapses and synapses between other neurons. Some of these particles co-localise with the photoreceptors and are putative output synapses of photoreceptors.

1. Wagh, D. A. et al. Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in Drosophila. Neuron 49, 833-44 (2006).

Acknowledgements:
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# The aspheric, divided superposition eye of the *Ascalaphus* owlfly

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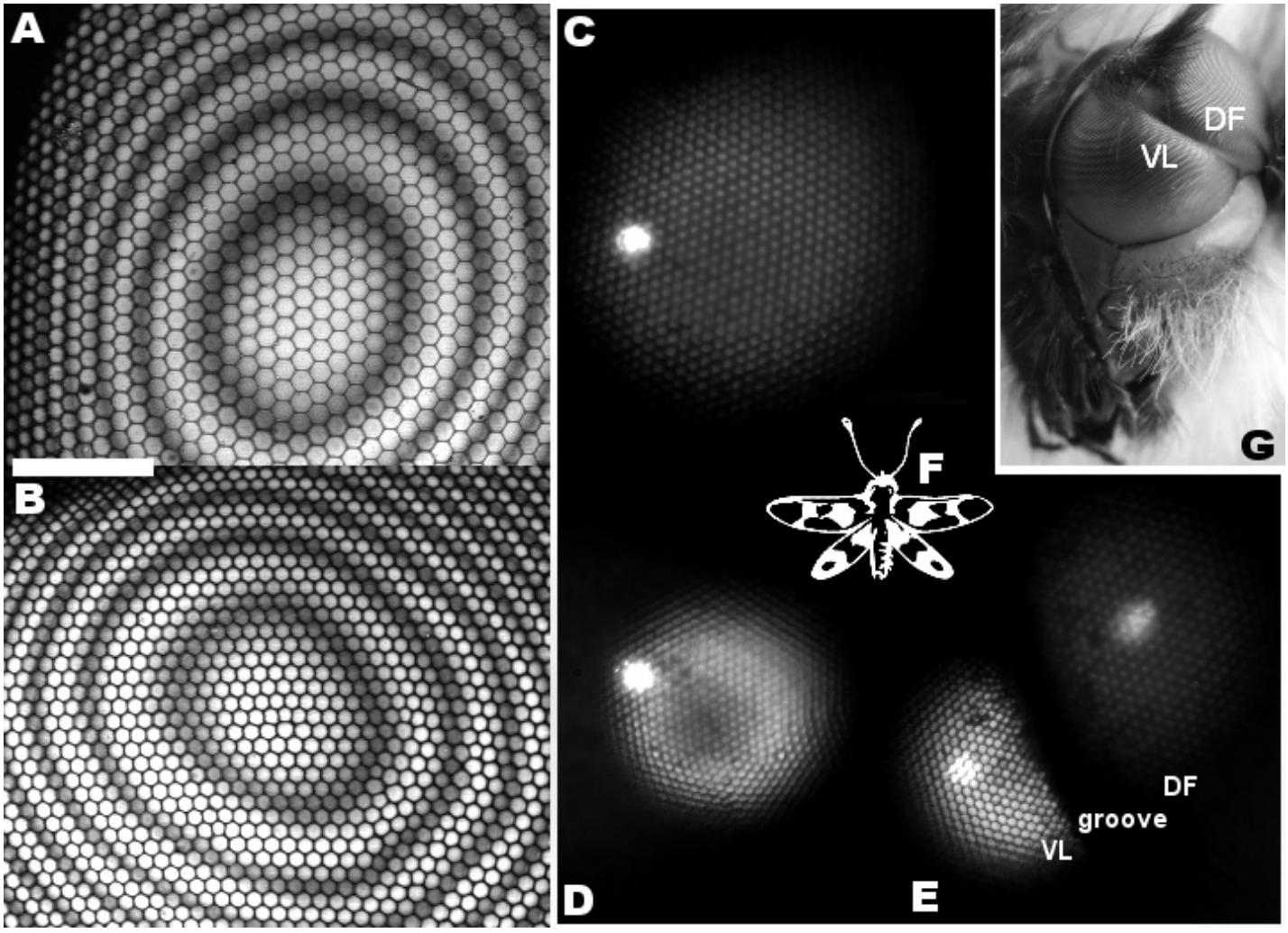
The owlfly *Libelloides macaronius* (Neuroptera) is a predator living in arid meadows of SE Europe. Imagos are hunting small airborne insects only when they are heated up by unobscured sunshine. Their eyes are partitioned into the exclusively UV-sensitive dorso-frontal (DF) and the ventro-lateral (VL) part. Owlflies have a superposition eye with a wide clear zone that lacks longitudinal pigment migration.

We measured the eye shape, visual fields and superposition optics of both eyes. The DF eye has bigger facets than the VL eye, and a larger number of them contribute to the eye shine. The DF eye is ovoidal and has an acute zone where the interommatidial angles are very small. The DF and VL visual fields slightly overlap. Optically dysfunctional facets exist in the groove between the DF and VL eyes.

Several diurnal fast-flying insect groups (e.g. hawkmoths, skippers) have retained the ancestral superposition eye design, which suggests that superposition optics may be superior to apposition in terms of the overall visual performance (sensitivity vs. acuity). In the case of a small black spot against a bright background, lower shot noise may enhance contrast detection. We also address the effect of partial coherence on the receptor acuity.

**Figure:** Surface geometry of the DF **(A)** and VL **(B)** eye (isohypse 20 μm, bar 200 μm). The eyeshine in the DF **(C)** and VL **(D)** eye caused by a small pencil of light (artefact spots). A double exposure of the DF/VL overlap area **(E)**. A sketch of the imago **(F)**. A macrophoto of the right eye **(G)**.



# In-vivo photochemistry of butterfly visual pigments

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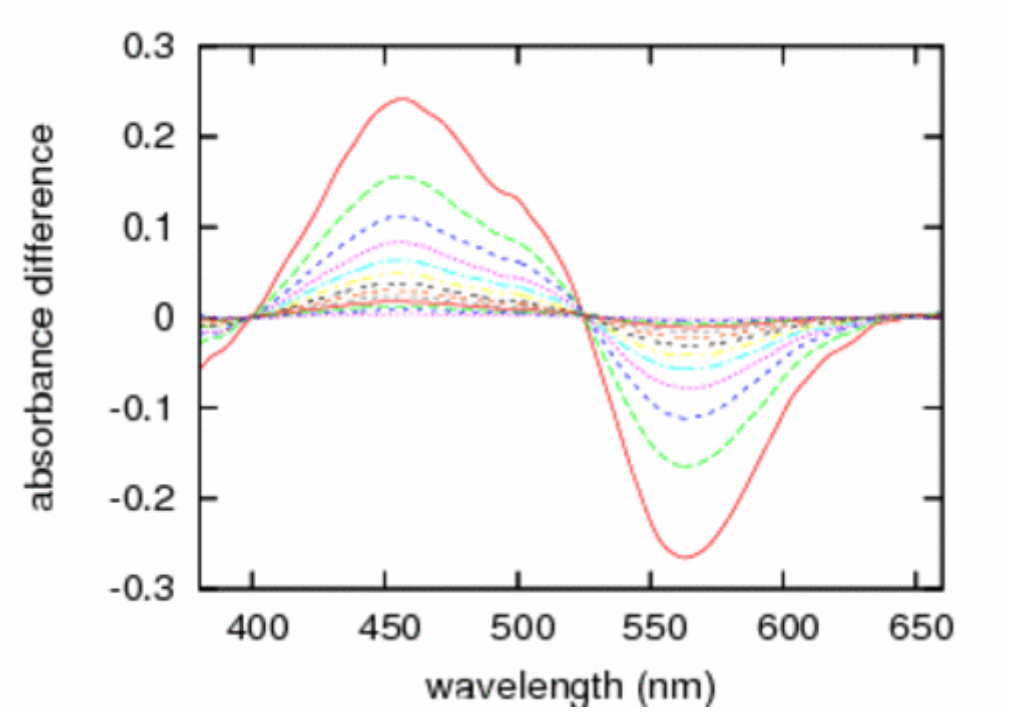
The photochemistry of the visual pigments in butterfly eyes can be studied in vivo, because of the existence of a tapetum. This is a reflecting structure, located proximally of the rhabdom, the light guide, which contains the visual pigment molecules. Incident light that enters a facet lens and is focused by the crystalline cone into the rhabdom propagates there until it is absorbed by the visual pigment molecules. The light fraction that escapes absorption, even after being reflected by the tapetum and having traveled twice through the rhabdom, leaves the eye as eye shine. We have measured the eye shine from the so-called deep pseudopupil, the superimposed image of numerous rhabdoms, using a special epi-illumination microscope. We collected the reflected light with a fiber optics spectrophotometer and applied bright and broad-band, white light to the dark-adapted eye, so that a photosteady state of the visual pigments was reached within the response time of the pupil mechanism. From the light-induced changes in the reflectance spectra we calculated time-dependent absorbance difference spectra, as shown in the figure. The curves are absorbance differences between the measured spectra and the equilibrium spectrum. The time interval between the curves is 80 ms. The spectra represent difference spectra of virtually exclusively the green visual pigment, and they are proportional to the difference of the absorption spectra of the rhodopsin and metarhodopsin.

We investigated two nymphalid species: the painted lady (*Vanessa cardui*) and peacock butterfly (*Inachis io*), as well as two pierid species: the small white (*Pieris rapae*) and green-veined white (*Pieris napi*). The main, green-sensitive visual pigments appear to have isosbestic wavelengths at 523 nm (*Vanessa cardui*), 525 nm (*Inachis io*), 536 nm (*Pieris rapae*), and 539 nm (*Pieris napi*). Using visual pigment templates, the absorption spectra of the rhodopsins and metarhodopsins have been estimated. The rhodopsin spectra of the nymphalids and pierids differ strongly, which is presumably related to their different life styles.



# Signal-to-noise ratio and quantum catch in the tuning of visual sensitivity in *Mysis relicta*

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Spectral photon catch depends on the wavelength distribution of available light, the spectra of ocular light filters, and the absorbance spectra of the visual pigments. Other things equal, vision in dark environments would always benefit from increased photon catch and decreased background noise. One evident selection pressure on visual pigments for dim-light vision is therefore that spectral absorbance should in some sense "match" the spectral distribution of available photons. Indeed, the visual pigments of freshwater species tend to have longer wavelengths of maximum absorbance ($\lambda_{max}$) than those of terrestrial or marine species, qualitatively consistent with the relatively long-wavelength-biased illumination

Visual-pigment absorbance spectra were examined in opossum shrimp from different light environments. Four Finnish populations, two from the Baltic Sea and two from freshwater lakes, represent Mysis relicta, sensu stricto. The sibling species M. salemaai are represented by two Baltic Sea populations. In M. relicta, the visual pigments of the two lake populations were similar ($\lambda_{max}$ = 554.3 ± 0.8 nm and 556.4 ± 0.4 nm), but significantly red-shifted compared with the sea populations (at 529 and 535 nm) and with M. salemaai (at 521 and 525 nm). All these pigments had only A2 chromophore and the lake/sea difference indicates adaptive evolution of the opsin.

We propose that the red-shift of $\lambda_{max}$ in the lake populations is achieved by changes in the opsin amino-acid sequence, which leads to a decrease in the energy gap between the ground state and the first excited state of the chromophore. This will carry a cost in terms of increased thermal noise, and that evolutionary adaptation of the visual pigment to the light environment is directed towards maximizing the signal-to-noise ratio rather than the quantum catch.

# Self-motion estimation and flight control in blowflies and locusts - a comparative study

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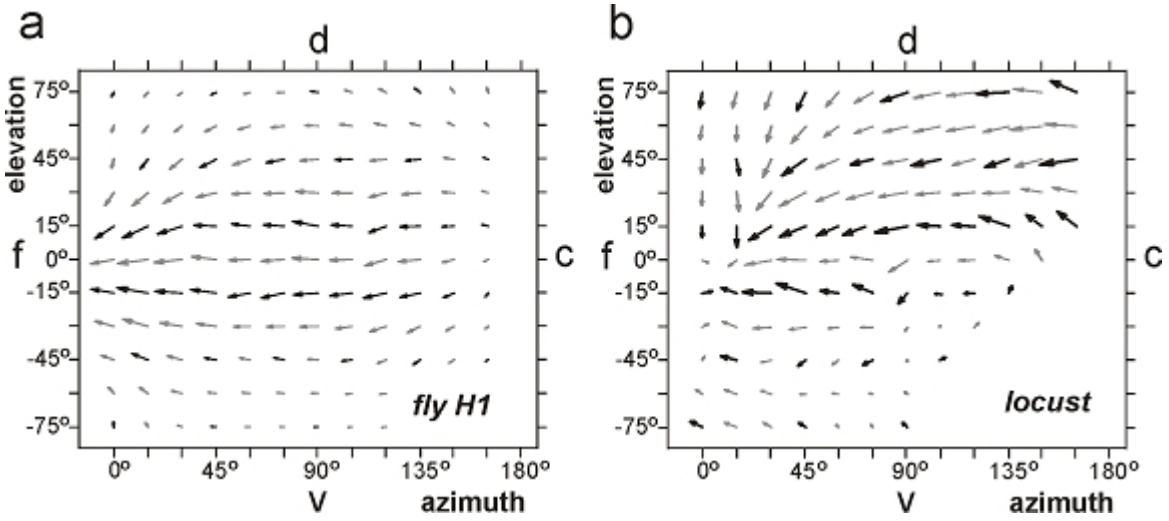
For visual locomotor control insects extract self-motion information from the optic flow over the retina. In the fly, lobula plate tangential cells (LPTCs) selectively integrate local directional motion information detected throughout the animal's visual field. As a result, each LPTC is tuned to a particular self-motion component.
Besides analysing self-motion along and around the 3 cardinal body axes, some LPTCs prefer rotations around intermediate axes. These LPTCs seem to be tuned to sense flight manoeuvres the animal frequently performs, e.g. banked turns. If there was a relationship between an animal's default flight modes and the preferred self-motion components of optic flow processing interneurons, insects that perform different flight manoeuvres should be equipped with different sets of optic-flow processing interneurons.
To test this hypothesis we characterized local response properties of motion sensitive, directional selective wide-field neurons in the lobula of the locust *Schistocerca gregaria*. The receptive field organization of these putative optic-flow processing interneurons is then compared to corresponding results previously obtained from the fly *Calliphora vicina*. As both species are flying insects we would expect to find some optic flow processing interneurons with similar properties but also some interneurons which do reflect the specific differences in flight style.
Here we present the receptive field organization of the LPTC H1 obtained from extracellular recordings in the fly lobula plate (Fig a) and of a visual interneuron recorded in the locust lobula (Fig b). Both cells were characterized using the same stimulation procedure (Krapp & Hengstenberg, Vis.Res., 1997). The arrows' orientation and length indicate the neurons' local preferred direction and motion sensitivity, respectively, plotted as a function of elevation and azimuth within the right visual field. The gradual change of the local preferred directions and motion sensitivities is typical for optic-flow processing LPTCs. Both cells would be strongly excited during clockwise yaw-rotation of the animal.
Our results so far suggest that in the locust a smaller number of wide-field neurons is tuned to rotations about horizontal axes than in the fly. This gives support to the hypothesis that the sensory coordinate system for visual self-motion estimation is adapted to control species-specific flight modes. Future experiments will involve intracellular recordings to analyse and identify the wide-field interneurons in the locust visual system.



# The nervous system in the visual sensory organs of a Cubozoa (Box jellyfish)

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Cubozoans belong to phylum Cnidaria and are located at the base of metazoan evolution. Cnidarians were the first animals to possess a distinct multicellular nervous system, which makes the system basic in structure and function compared to "higher" animals. The most prominent neuronal condensation found in the cnidarian medusa is represented by one or two nerve rings that run along the bell margin.

Among cnidarians, Cubozoans stand out by having the most advanced visual sensory organ in the phylum, called rhopalia, and a total of 24 eyes! Each medusa carries four rhopalia, which are bilaterally symmetric club like structures each holding a statocyst, four pigment cup eyes and two camera-type of eyes. The camera-type of eyes are surprisingly complex structures to be present among such "simple" organisms as the cnidarians and are similar to those found in higher organized animals, such as cephalopods and vertebrates.

With such a basic nervous system, how are the cubozoans able to handle the visual information gathered by the many and surprisingly complex rhopalial eyes? The nerve ring, representing the central nervous system, has long been thought to be the area for visual processing in cnidarians. But what about the rhopalia? Recently we have shown that there is a bilaterally symmetric neuronal system in the rhopalia of the cubozoan species Tripedalia cystophora that could form a basis for visual processing. The system, detected by antibodies towards proliferation cell nuclear antigen (PCNA), is composed of clusters of nerve cell bodies and nerve fibre pathways. The fibre pathways reach between the eyes and between the eyes and the base of the stalk on each side of the system, they also form commissures connecting the two sides of the system.

Here we show three additional systems in the rhopalia by using antibodies towards the molluscan neuropeptide FMRFamide, its C-terminal fragment RFamide and towards the synaptic protein syntaxin. These systems also show a bilaterally symmetric neuronal distribution and have clusters of nerve cell bodies and fibre pathways and therefore support our earlier findings. We also answer the question if these systems are distinct from, or integrated with, each other.

With a total of four rhopalial nervous systems detected by different antibodies, we draw the conclusion that the rhopalial nervous system is the part of the central nervous system in cubozoans where it is likely that most processing of visual information takes place.

# Photoreceptor Reliability Assessed by Response Discriminability

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Neuronal signals are always corrupted by noise from different sources, be it channel or synaptic noise or, in case of the visual system, noise included within the stimulus itself, i.e. photon noise. Studies on the reliability of neuronal signalling try to unravel the sources of the noise and the ways the system deals with it. The coding performance of photoreceptors is commonly analysed by information theoretical approaches, i.e. the Shannon information capacity. Here we formulate a discrimination task as an alternative way to assess the response reliability. We show that responses to white noise stimuli can be safely discriminated even though the respective responses hardly show any information capacity. We further use the discrimination measure to investigate the impact of synaptic filtering on the response discriminability. We show that the effect of synaptic filtering depends on the kind of background the white noise stimuli are superimposed. In case of a constant background discriminability does not depend strongly on the applied filter. White noise stimuli riding on a dynamically varying background can be better discriminated when the responses are passed through a linear filter resembling the real synaptic transfer properties. Thus, the first synapse in the fly visual system does not only optimise the channel capacity in a information theoretical sense, as was shown before, but is also a way to increases the response discriminability.

# Optomotor response depends on behavioural state of fly, Calliphora vicina

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For an animal to survive in a complex and variable environment it is important to respond to a particular stimulus in a context specific way. The context dependence of behaviour is analysed here for the optomotor response of the fly. Optomotor responses are performed by flies and other animals to counteract an unwanted slip of the retinal image. These are eye- and head movements as well as flight steering manoeuvres.
Head movements of the fly are not only controlled visually but are also influenced by haltere input. The halteres are transformed hindwings of dipterans. They oscillate when the fly is flying or walking and detect rotations of the fly. Additionally, sensory feedback elicited by oscillating halteres is proposed to gate visually induced head movements. The oscillating activity of the halteres makes them suitable as indicators for the motor activity state of the fly.

In our analysis head pitch movements and haltere oscillations of tethered blowflies were monitored by high-speed digital cinematography while the animal was stimulated by visual motion stimuli.

When the fly was in the high activity state the head was jittering and the gain of the optomotor response was much higher than in low activity state. Additionally our data indicate sensory feedback from oscillating halteres not to be the (only) source of high gain and jittering responses. Instead a signal reflecting the motor command could control both the haltere oscillations and the modifications of the head movements.

# Topographic organization of *E*-vector orientation columns in the central complex of the locust brain

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The central complex is one of the most intriguing, but least understood area in the insect brain. It consists of a group of neuropils (protocerebral bridge, upper and lower divisions of the central body) in the center of the protocerebrum and is characterized by a highly regular neuroarchitecture. The three major subunits of the central complex are composed of 16 vertical columns, arranged in rows and, in addition, a number of horizontal layers in the central body. The function of columns and layers has been unknown for a long time. Recently, the layers of the upper division of the central body in *Drosophila* (termed fan-shaped body) have been associated with memory storage for specific aspects of visual space (orientation and elevation of stimuli, Liu et al. 2006, Nature 439:551-556).

In desert locusts, several types of central-complex neurons are sensitive to the *E*-vector orientation of dorsally presented polarized light (Vitzthum et al. 2002, J Neurosci 22:1114-1125). When the blue sky is visible, *E*-vector orientation in the zenith directly corresponds to the azimuthal position of the sun. Locusts perceive zenithal polarized light through a specialized dorsal rim area of the compound eye; signals are transmitted further through several brain stages and are finally processed in the central complex (Pfeiffer et al. 2005, J Neurophysiol 94:3903-3915).

Here we show through intracellular recordings combined with Neurobiotin injections that the columnar arborizations of identified neurons in the protocerebral bridge (PB) correspond to their preferred *E*-vector orientation in a topographic fashion. This spatial representation of *E*-vector tunings was observed in tangential neurons of the PB with columnar arborization domains and in three sets of columnar neurons. In all of these cell types the number of the invaded column correlated linearly with the preferred *E*-vector orientation. Within the eight columns of each hemisphere a range of about 180° (i.e. all possibly occurring *E*-vectors) was covered. The regression lines for tangential and columnar cells were out of phase by about 90°. Thus, maximal excitation in tangential cells corresponded to maximal inhibition of columnar cells with the same columnar domains, suggesting an inhibitory connection. This was supported by the fact that in tangential neurons the involved arborizations were presumably presynaptic, whereas the ramifications of the columnar neurons appeared postsynaptic. Finally, the tangential cells provided a first possible connection of the central-complex polarization system to the proposed circadian clock of the locust, the accessory medulla (via the posterior optic tubercle).

The map-like representation of zenithal *E*-vectors in the columns of the central complex may be suited to provide the animal with information about its head direction relative to the sun's azimuth. Together with the recent evidence from *Drosophila*, our data suggest that recognition of objects in space is achieved in insects by corresponding neural activity in a given column (coding for azimuth) and a particular layer (coding for particular visual features) of the central complex. Supported by DFG-grant HO 950/16-1.

# Standardized atlas of the brain of the desert locust

Angela Eva Kurylas[1], Torsten Rohlfing[2], Arnim Jenett[3], Sabine Krofczik[4] and Uwe Homberg[1]

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In recent years three-dimensional (3D) visualization of neuronal structures has greatly advanced our understanding of brain anatomy and neural connectivity. It allows one to create and expand standardized anatomical databases with increasing levels of detail and complexity. In insects, standardized 3D brain atlases are available for the fruitfly, *Drosophila melanogaster* (Rein et al. 2002, Curr Biol 12:227-231) and the honeybee, *Apis mellifera* (Brandt et al. 2005, J Comp Neurol 492:1-19). In addition to the honeybee and the fruitfly, the desert locust, *Schistocerca gregaria* is another well-established model system in neuroscience, especially for research on the olfactory and visual system, endocrine functions, and motor control (Burrows M, 1996, The neurobiology of an insect brain, Oxford University Press, Oxford).
Towards establishing a multi-user database for analysis of the locust brain, we present here a standardized 3D brain atlas of the locust. Ten anti-synapsin stained locust brains, processed as wholemounts, were imaged by laser confocal microscopy.Thirty-four neuropil structures were then labeled using the 3D-software Amira 3.1 (Mercury Computer Systems Inc., San Diego, CA). For standardization, we compared two different methods: an iterative shape averaging procedure derived and improved from Brandt et al. (2005), and the Virtual Insect Brain Protocol (http://www.neurofly.de), which uses a global and local (piecewise) rigid followed by localnonrigid registration. We decided in favor of the first method, since the resulting average image exhibited superior resolution. The locust brain atlas includes all major neuropil structures of the proto-, deuto- und tritocerebrum. In addition, first examples for fitting single neurons into the standard brain are provided. A Neurobiotin-injected, polarization-sensitive neuron from an intracellular recording was reconstructed together with the segmented corresponding neuropils again marked with anti-synapsin. Based on the transformation parameter calculated for the segmented neuropils, we registered the neuron into the standard brain. The availability of the locust 3D standardized brain atlas is an important step to visualize the complexity of neuronal networks in this animal. Therefore, this atlas will serve as a highly valuable base for further analysis of the anatomical and functional organization of the insect brain.

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# A bee in the corridor: regulating the optic flow on one side

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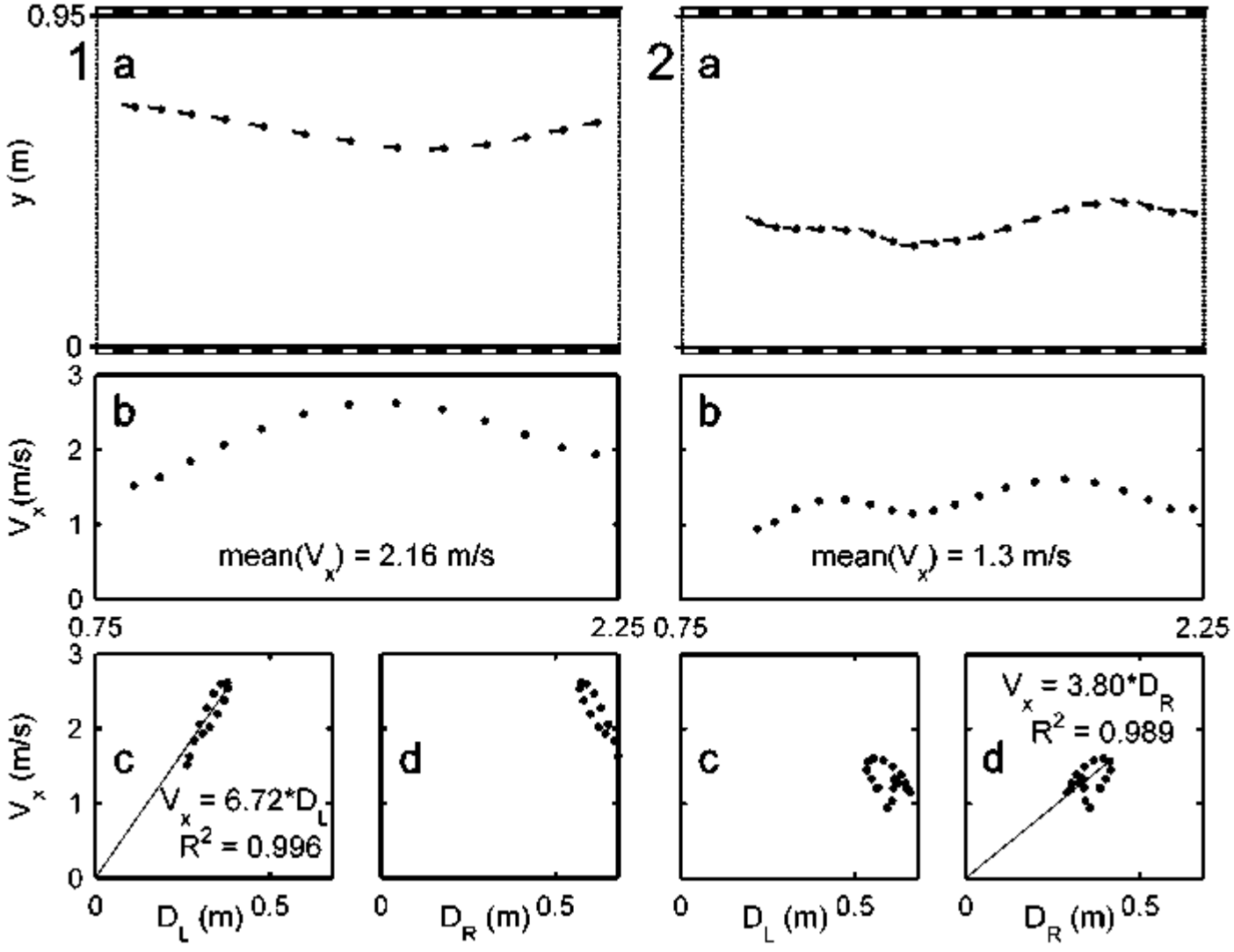
To work out the information flow underlying the honeybee's anti-collision system, we performed a frame-by-frame analysis of the trajectories of individual bees (*Apis Mellifera*) flying in a *wide* outdoor flight tunnel (Fig.1a,2a). Forward speed $V_X$ and distance D to one of the two walls happen to be proportional to each other (Fig.1c,2d), attesting that the angular velocity $V_X/D$ (Optic Flow, OF) of the image of that same wall is held constant. Like the landing bee holding the downward OF constant (Srinivasan et al. 1996), the bee holds either the left (Fig.1c) or right (Fig.2d) OF constant. The bee's behaviour is well accounted for by a lateral *optic flow regulator* scheme. Simulations showed that this scheme can make a (fully actuated) hovercraft automatically adjust its *distance* to a wall by regulating the OF on one side (Serres et al., *IEEE Biorob* 2006).

Fig 1-2: Two bees' trajectories in a wide 0.95x3x0.25m corridor. A digital camera (2.2m above the corridor) records the trajectory over 1.5m at 20 fps. Both walls are lined with vertical grey-and-white stripes (10cm wide, contrast 0.27). Entrance and reward locations are on the left (1) or right (2). Top recordings: bee's position; middle plots: bee's forward speed; bottom plots: forward speed versus distance to the right and left walls. Bees 1 & 2 happen to hold the OF (slope in Fig.1c and 2d) at 6.72rad/s (385°/s) and 3.80rad/s (217°/s), respectively.



# A bee in the corridor: centring or wall-following ?

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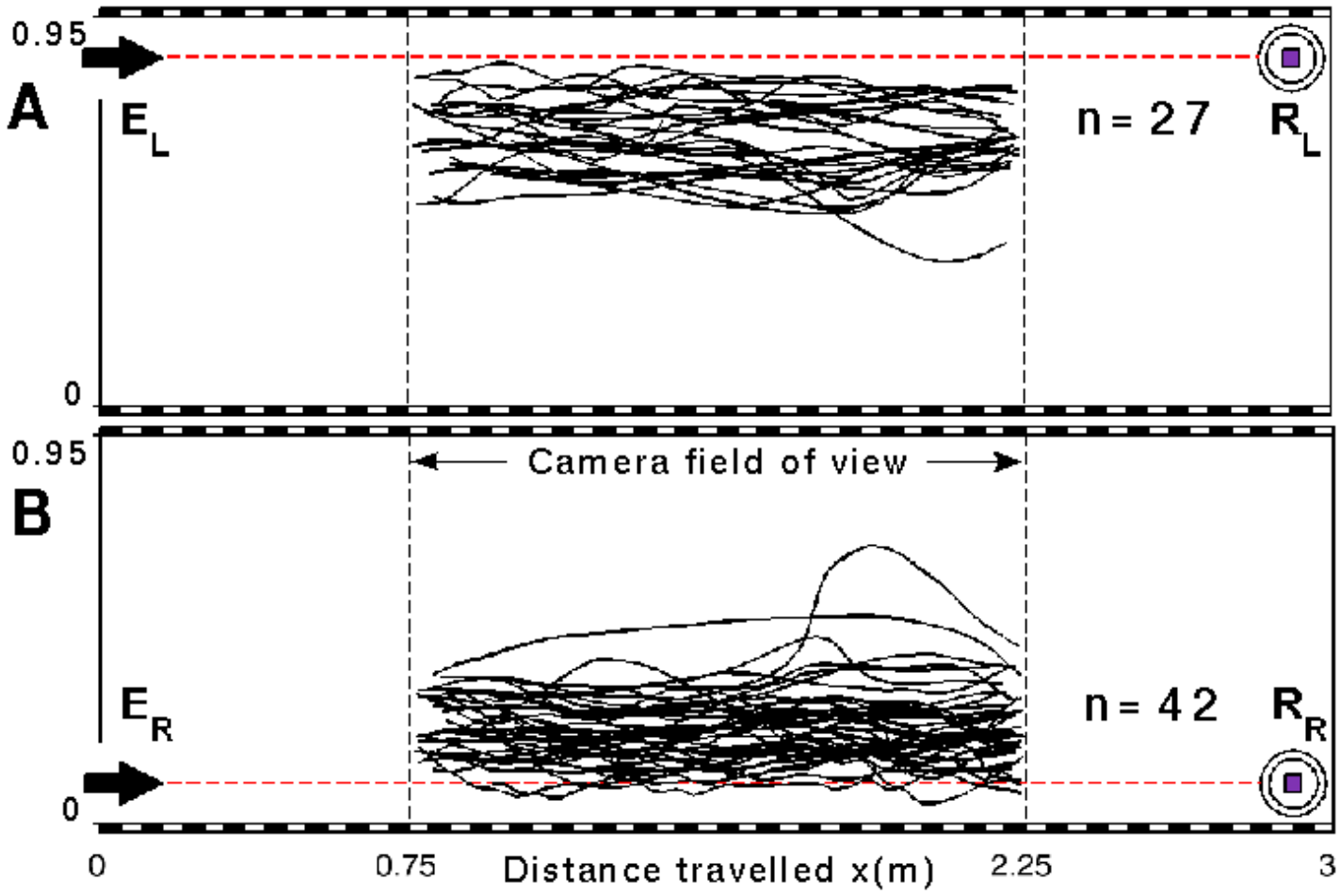
To understand the logics behind the honeybee's anti-collision system, we filmed bees (*Apis Mellifera*) flying through a *wide* outdoor flight tunnel (0.95m-wide, 3m-long). We observed that bees do not centre systematically, in contrast with previous observations made in a narrower, 0.12m wide corridor (Srinivasan et al., 1991). Bees may instead follow either wall (Fig. A,B), depending on the entrance ($E_L$ or $E_R$) and reward ($R_L$ or $R_R$) locations. The 'optic flow balance' hypothesis (Srinivasan et al., 1991) does not account for this *wall following behaviour*. The bee's sideway motion is well accounted for by an *optic flow* based feedback loop that we called an *optic flow regulator* (Ruffier and Franceschini *Rob. Aut. Syst.* 2005; Serres et al., *IEEE Biorob* 2006). This scheme would require the bee to measure neither its forward speed nor its distance to the walls.

Fig. A-B: Bees' trajectories in a straight corridor (3x0.95x0.25m). A high-resolution digital camera placed 2.20m above the corridor recorded the trajectory of single flying bees at 20 fps over a distance of 1.5m centred halfway through the corridor. Both walls are lined with a periodic grating that consists of vertical grey-and-white stripes with a spatial period of 10cm and a contrast of 0.27. The entrance ($E_L$ or $E_R$) and reward ($R_L$ or $R_R$) locations are near the left (A) or right wall (B). The bees' trajectories happen to be significantly shifted (*p-value*$<10^{-10}$) with respect to the midline. Their mean ordinate is 0.65±0.08m in A and 0.24±0.08m in B. (*n* = number of bee trajectories)



# Integration of lobula plate tangential cells' signals by DNOVS1, an identified premotor neuron

Juergen Haag, Adrian Wertz and Alexander Borst

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Many motion sensitive tangential cells of the lobula-plate in blowflies are well described with respect to their visual response properties [1] and the connectivity amongst them [2]. They have large and complex receptive fields with different preferred directions in different part of their receptive fields matching the optic flow that occurs during various flight maneuvers [3]. However, much less is known about how tangential cells connect to postsynaptic neurons descending to the motor circuits in the thoracic ganglion, and how optic flow is represented in these downstream neurons. Here we describe the physiology and the connectivity of a prominent descending neuron called DNOVS1 [4]. We found that DNOVS1 is electrically coupled to a subset of the VS-cells and responds, as VS-cells, with a graded shift in membrane potential. The specific wiring leads to a preference for rotational flow-fields around a specific body axis.

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[4] Strausfeld NJ, Bassemir UK (1985) Lobula plate and ocellar interneurons converge onto a cluster of descending neurons leading to neck and leg motor neuropil in Calliphora erythrocephala. Cell Tissue Res 240:617-640.

# Electrophysiological characterization of directionally selective visual interneurons in *Drosophila melanogaster*.

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The neuronal computations underlying directionally selective visual motion detection have been widely studied in many different organisms. The vast majority of these studies validate the fundamental work of Hassenstein and Reichardt (1956) who deduced the "correlation type motion detector" model from the turning response of the freely moving beetle *Chlorophanus*. Given a precisely defined visual input, the model computes a directionally selective output which serves as a command signal to drive the observed behavioral responses of the animal. The model predicts specific response features of directionally selective local elements as well as of spatially integrating large field neurons. These predictions originate from the two most prominent features of the model: asymmetric temporal filtering and a non-linear, multiplicative-like interaction between two input channels separated along the axis of image motion.

Motion selective large field cells have been extensively characterized in the lobula plate of the blowfly *Calliphora vicina*, however, almost nothing is known about the cellular implementation of the detector itself, which is located presynaptic to them. To close this gap we established an electrophysiological approach in *Drosophila melanogaster*, which involves the neurogenetic dissection of the network presynaptic to the lobula plate neurons. We show the first patch clamp recordings of Vertical Sensitive (VS) cells in the lobula plate of *Drosophila* providing the experimental foundation for the future neurogenetic interference with the function of the network. Currently we analyze the receptive field properties of VS cells and their electrical coupling by neurobiotin injections. Our results demonstrate the close proximity of *Drosophila* to its bigger cousin *Calliphora* and warrants the interactive analysis of both species to investigate elementary motion detection.

# Robust information processing in motion vision

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The degree to which information encoding is degraded by spike timing variability is fundamental to understand the underlying principles of sensory information encoding.
We are interested in how external and internal noise conditions affect the coding efficiency on sensory systems. We address this question by measuring the response of a motion sensitive neuron in the fly's visual system to a time-varying stimulus at several light levels and temperatures.
We quantified the coding efficiency, response latency and spike-timing variability at six different temperatures (from 15 to 27ºC) and at seven different luminance levels (from 0.1 to 84 cd/m2) each. A coding efficiency of ~ 30-35% is maintained in a wide range of the experimental conditions. Spike time variability and bandwidth of the response did not depend on temperature perturbations. Latency, however, is reduced in approximately 20% for a ten-degree increase on temperature, independent of the luminance level. These results suggest that motion information processing in the fly's visual system is relatively robust against internal and external perturbations, within the range of conditions tested.

# Motion sensitive premotor neurons of the blowfly *Calliphora vicina*

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In the blowfly, panoramic and small-field visual stimuli elicit optomotor movements of the head and body which attempt to stabilize the visual input on the retina. Large field motion is processed in the lobula plate, where approximately 60 tangential cells receive local motion information from retinotopical arranged elementary motion detectors. These lobula plate tangential cells (LPTCs) have characteristical visual response properties; for example tangential cells of the vertical system (VS-cells) are excited by downward motion in distinct areas of the receptive field and inhibited by motion in the opposite direction [1]. However, much less is known about the neurons postsynaptic to the LPTCs, which are involved in processing and conveying this motion information to motor neurons in the thoracic ganglion.

Here we describe the physiological response characteristics of a subset of 3 descending neurons projecting into the thoracic ganglion. These neurons were anatomically described by Strausfeld and Bassemir [2] and were named Descending Neurons of the Ocellar and Vertical cell System-1-3 (DNOVS-1-3). In DNOVS-1 we found graded shift in membrane potential when stimulated by moving gratings, whereas DNOVS-2, 3 showed spiking responses. Presenting different combinations of motion stimuli in two areas of the receptive field, we found highly specific responses in DNOVS-3 to rotational stimuli indicating an influence of the contralateral eye. This specific response of DNOVS-3 could not be explained by a linear summation of the cell's responses to single components of the rotational stimuli.

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[2] Strausfeld N.J. and Bassemir U.K. (1985): Lobula plate and ocellar interneurons converge onto a cluster of descending neurons leading to neck and leg motor neuropil in Calliphora erytrocephala. Cell Tissue Res. 240:617-640

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# Lateral interactions between VS cells of the fly visual system give rise to two distinct receptive fields in single VS cells

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In the lobula plate of the blowfly, visual interneurons integrate input from retinotopic arrays of local motion detectors (LMDs) giving rise to receptive fields (RFs) that match certain optic flow-fields as generated by the fly's ego-motion. For neurons of the vertical system (VS cells), these RFs resemble flow-fields that arise from rotation of the fly around different preferred axes distributed along the equator of the fly's visual field. Previous work has shown that neighboring VS cells share their receptive fields[1] through lateral interactions putatively mediated by gap junctions in the axon terminals[2]. These interactions act to broaden the RFs of these cells relative to the RF expected from the extent of their dendritic arborizations.

In this work, we further characterize the effect of lateral interactions on the RFs of VS cells. Using calcium imaging, we show that the RF broadening happens between the different compartments of single VS cells, resulting in two distinct RFs: a narrow dendritic RF corresponding to local LMD input, and a broader, axon-terminal RF which includes the effects of the interactions with neighboring VS cells. By voltage-clamping synaptic excitation arising in the dendrite, we show that the input from lateral interactions enters the cells via the axon terminal.

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[2] Haag J, Borst A (2004) Neural mechanism underlying complex receptive field properties of motion-sensitive interneurons. Nat Neurosci 7: 628-634.

# Light dependent modulation of Kv -Currents in Photoreceptors of *Drosophila*

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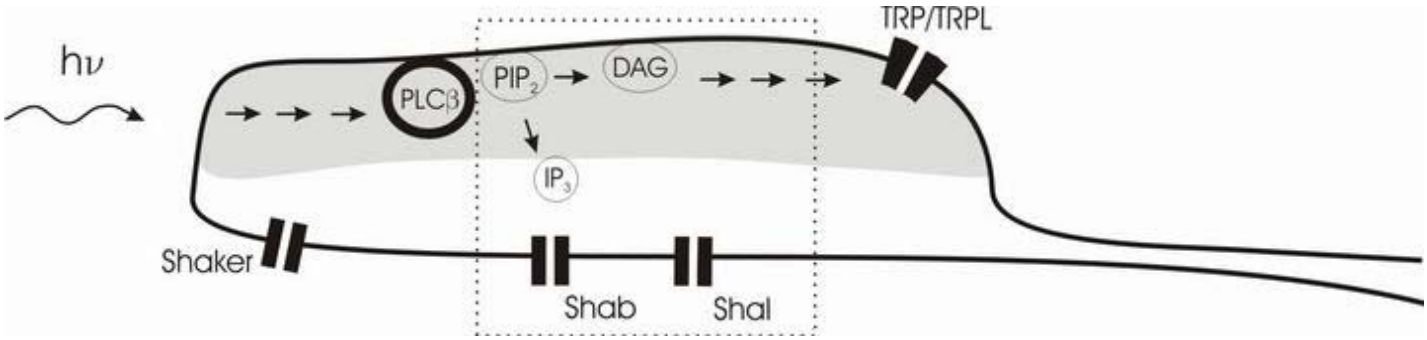
The initial process in vision is the generation of an electrical response by light energy. In invertebrates, this process is accomplished by a phototransduction cascade in the microvillar part of the photoreceptors. In *Drosophila*, phototransduction eventually leads to activation of TRP and TRPL - channels (encoded by *trp* and *trpl* genes), where diacylglycerol (DAG) or one of its metabolites is believed to be the activating agent. DAG itself originates from the hydrolysis of phosphatidylinositol-4,5-biphosphate ($PIP_2$) by PLCβ (encoded by *norpA* gene) into the second messengers DAG and inositol -1,4,5,-triphosphate ($InsP_3$). The role, if any, of $InsP_3$ in the photoreceptor's light response, is unknown. Here, we investigate the light dependent regulation of voltage-activated potassium channels (Kv-channels; coded by *shaker*, *shab* and *shal* genes ) in isolated photoreceptors of *Drosophila* with the whole-cell patch-clamp technique. The Shaker current seems to be unaffected whereas one or more of the other Kv-currents are regulated by light stimulation. Upon light illumination, we typically find a shift of activation of about 10 mV to hyperpolarized potentials, resulting in a ~25% increase in outward currents elicited by a standard voltage step (from -100 mV to +10 mV), recovering with a time course of ca 100 s in the dark. Intriguingly, experiments with *trpl;trp* double mutants, which do not exhibit an electrical light response, show even stronger Kv-channel regulation than wild type flies. On the other hand, in *norpA;trp* double mutants, which do not exhibit an electrical light response, Kv-channels are not affected by illumination, indicating that the observed Kv-channel regulation is mediated by PLC. Future experiments will test which of the 3 products of PLC activity (DAG, $InsP_3$ or a reduction in $PIP_2$) are responsible for the Kv-channel regulation. Preliminary experiments show that the light dependent Kv-channel regulation could be at least partially mimicked by application of a membrane permeable $InsP_3$-ester. The final result of this work may help to answer the unsolved question of the role of $InsP_3$ in the phototransduction cascade and to better understand the function of Kv-channels in *Drosophila* photoreceptors.



# Functional properties of UV photoreceptors in the compound eye of the cockroach

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The visual system of the cockroach *Periplaneta americana* is supposed to have evolved for a mainly crepuscular lifestyle. The large compound eyes contain photoreceptors sensitive to green and UV light, with spectral peaks at 507 nm and 365 nm. Three out of the total of eight photoreceptors in an ommatidium are of the UV sensitive type [1].

Earlier it has been shown that there is a large variability in the function of the green sensitive photoreceptors and they also produce action potentials in their axons [2, 3]. So far, little has been known about the functional properties of UV photoreceptors although their relative number out of the total amount of photoreceptors is quite high.

To study the functional properties of UV cells, a stimulus apparatus was built using commercially available LED technology. Responses to current and light stimulation were recorded intracellularly from UV photoreceptors. The functional properties of the UV cells are characterized by membrane impedance, light-to-voltage transfer function, light step response and signal-to-noise-ratio. These properties are also compared to those of green sensitive photoreceptors.

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# Is there a common genetic program to specify polarization-sensitive photoreceptors in insects?

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The ability to exploit skylight polarization is widespread among insects. Photoreceptors in a specific region of the compound eye, the so-called dorsal rim area (DRA), are modified to be sensitive to the electric vector of linearly polarized light. In the developing retina of the fruit fly (*Drosophila*), the diffusible morphogen Wingless (Wg) induces expression of the homeodomain transcription factor Homothorax (Hth) in a subset of photoreceptors located in the dorsal periphery of the eye, thereby switching their fate from a color-sensitive default state to a polarization-sensitive DRA fate[1]. So far, almost nothing is known about the regulatory mechanisms underlying the morphological and molecular specification of the DRA in other insect species. We are therefore investigating whether Hth plays a similar role during the development of the DRA in crickets (*Gryllus*).

A comparison between *Drosophila* and *Gryllus* is particularly interesting because of the following reasons: (1) Group-specific fine-structural disparities in the design of the dorsal rim ommatidia suggest that polarization vision may have arisen independently in several insect orders including the Diptera (e.g. *Drosophila*) and the Orthoptera (e.g. *Gryllus*)[2]. (2) The DRA varies in shape among insect species. Compared to *Drosophila*, where polarization-sensitive photoreceptors are located in only one, maximally two rows of ommatidia along the entire dorsal eye margin[1], the DRA in *Gryllus* is much wider and shorter, being restricted to the dorsalmost part of the eye[3]. (3) Between holometabolous insects, such as fruit flies, and hemimetabolous insects, such as crickets, there are fundamental differences in the timing of eye development. In *Drosophila*, compound eyes do not form until metamorphosis and they are fully developed when the adult fly emerges from the pupa[4]. Cricket larvae, on the other hand, hatch with small compound eyes already equipped with a DRA[5]. Throughout larval stages additional rows of ommatidia are recruited from a budding zone at the anterior margin of the eye[6].

These points suggest important differences regarding the control of DRA differentiation in *Drosophila* and *Gryllus*. It is therefore particularly interesting to investigate whether homologous genes (*hth*) or signaling pathways (Wg) are required to induce the development of the DRA both in flies and crickets. Importantly, homologous groups of transcription factors have previously been shown to be responsible for the formation of independently evolved and therefore un-related eye structures. Our study will thus provide crucial insight into the evolution of retinal patterning.

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**6** Pohl R (1988) Zool Jb Anat 117: 353-393.

# The optics of the polarizing tapetum in the postero-median eyes of the gnaphosid spider *Drassodes cupreus*

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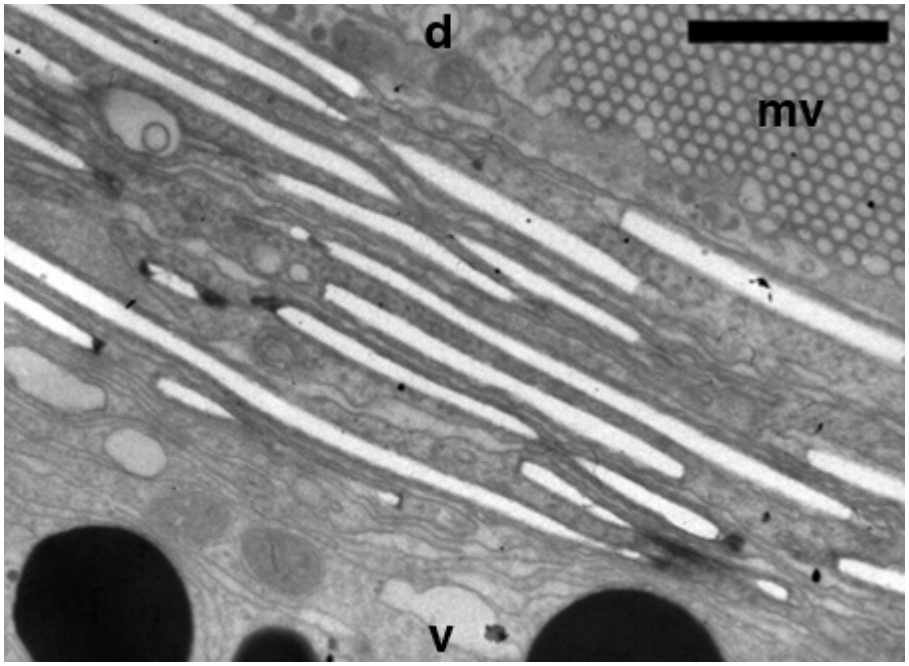
Apart from insects, some spiders also seem to exploit skylight polarization for navigation (1). In the spider *Drassodes cupreus* (Araneae, Gnaphosidae) the postero-median eyes have previously been shown to be highly specialized for polarization vision (2). In these eyes the polarization sensitivity of the photoreceptors is enhanced by a special tapetum that serves as a polarizer, i.e. if the eyes are illuminated with unpolarized light, the light reflected by the tapetum is partially plane-polarized (2). Here we study the optical mechanisms leading to this polarizing effect.

The tapetum of the postero-median eyes of *Drassodes* belongs to the canoe-shaped type, which is common in spiders (3). Histological examinations revealed that the tapetum consists of two multilayer interference reflectors forming a V-shaped groove with an angle of approximately 90° between them. Histochemical examinations and HPLC/MS analyses indicate, that the high refractive index layers of the multilayer stacks consist of guanine crystals. By micro-reflectometry the intensity and the degree of polarization of reflected light were measured in intact eyes of *D. cupreus* for different wavelengths and angles of incidence. The optical data were compared with theoretical values calculated for a modeled tapetum, which was based on our histological and histochemical findings and included different assumptions about the orientation of the guanine molecules within the birefringent crystals. This comparison indicates that the observed polarization of light returned by the tapetum results from a double reflection of incident light at the two reflectors of the V-shaped tapetum. Thus, the polarizing effect of the tapetum is based on the mechanism of reflection polarization, and to explain the observed high degrees of polarization, no additional mechanisms must be invoked. In the agelenid spider *Agelena labyrinthica* the postero-median eyes are also equipped with a canoe-shaped tapetum, but the reflected light is only weakly polarized. This may be due to both a less regular arrangement of the tapetal crystals and concave instead of plane reflectors.

(1) Dacke et al. (2001) J Exp Biol 204, 2481-2490; (2) Dacke et al. (1999) Nature 401:470-472; (3) Homann H (1971) Z Morph Tiere 69:201-272.

Fig.: Cross section through a postero-median eye of *D. cupreus*, showing the multilayer structure of the tapetum. **d** dorsal, **v** ventral, **mv** microvilli of photoreceptors, scale bar: 1 µm



# Poster Topic

## T15: Visual system II: Retinal circuits

**T15-1A** the visual code: neuronal pattern representation
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**T15-5A** Tapping retinal ganglion cell activity in humans with the multifocal pattern electro-retinogram
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**T15-4B** The role of horizontal cell coupling in the formation of antagonistic receptive fields in mouse retinal ganglion cells
*K. Dedek, C. Pandarinath, NM. Alam, K. Wellershaus, K. Willecke, GT. Prusky, S. Nirenberg and R. Weiler, Oldenburg, New York (USA), Lethbridge (Canada) and Bonn*

**T15-5B** Identification and localisation of cpCx43.4 and cpCx47.6, two homologs of mmCx45 expressed in the carp retina
*G. Hilgen, S. Beermann, P. Dirks, R. Weiler and U. Janssen-Bienhold, Oldenburg*

**T15-1C** Connexins expressed in horizontal cells of the fish retina
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**T15-2C** Connexin30.2 is Expressed by Ganglion Cells of the Mouse Retina
*L. Pérez de Sevilla Müller, M. M. Kreuzberg, S. Maxeiner, S. Lorenz, K. Willecke, R. Weiler and U. Janssen-Bienhold, Oldenburg and Bonn*

**T15-3C** HCN Channels Expressed by Rod Bipolar Cells of the Mouse Retina, Confer a Band-Pass Response to Input Signals
*L. Cangiano, C. Gargini, GC. Demontis, L. Della Santina and L. Cervetto, Pisa (I)*

**T15-4C** Can chicken retinal ganglion cells distinguish image defocus from a drop in image contrast?
*E. Diedrich and F. Schaeffel, Tübingen*

**T15-5C** Functional characteristics of retinal ganglion cells in the blind P23H rat as a perspective model for testing retinal prosthesis
*B. Kolomiets, E. Dubus, JA. Sahel and S. Picaud, Paris (F)*

# the visual code: neuronal pattern representation

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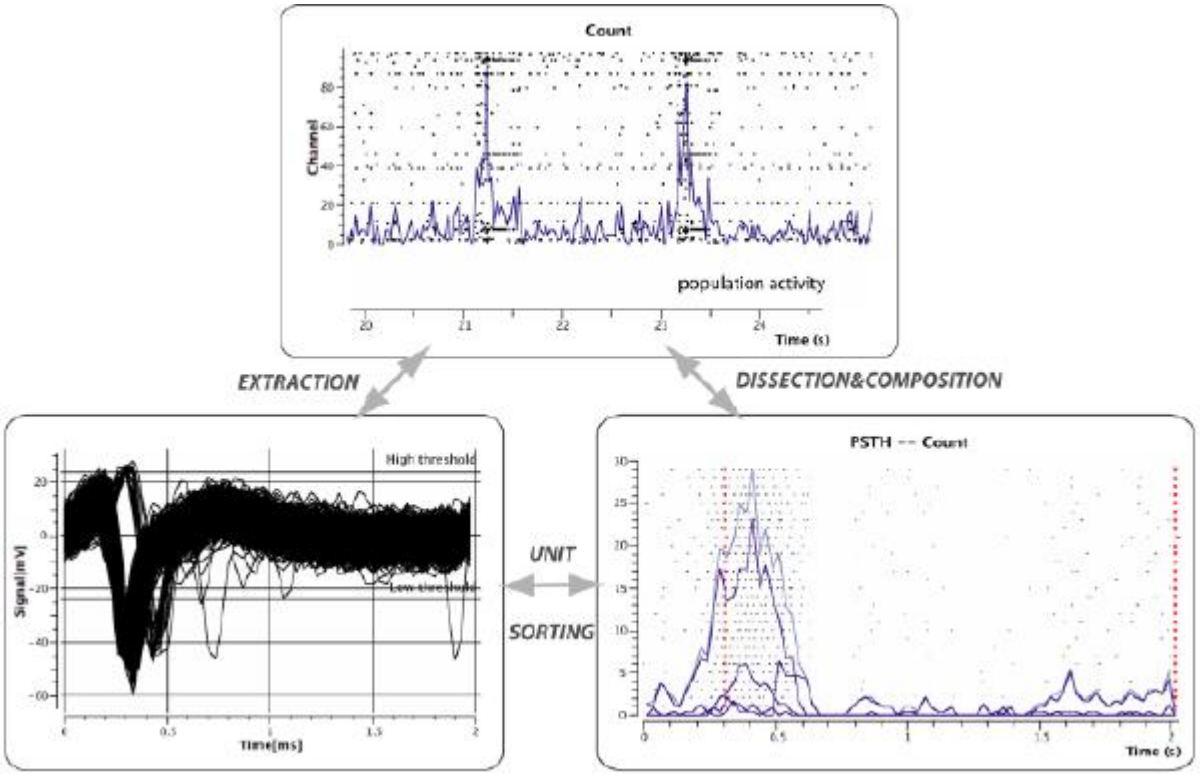
The concerted firing patterns of neuronal populations in the vertebrate retina represents the encoded visual information transmitted of the brain. The underlying spike trains are composed of different structural elements which represent the distributed visual code. By identifying and analyzing the individual-, cell ensemble- and population-activity, and the emergence of unitary events, as well as the identification of distributed firing patterns we are able to reveal a general distributed coding scheme of the retinal output.

Neuronal responses of retinal ganglion cell populations were recorded using the braingate[tm] multi-electrode array in rabbits. Using this approach we were able to record from up to 400 neuronal units in an indivisual experiment at the same time. The registered spikes were sorted in a first step into "classical" units and then classified according to the temporal course of the individual unit response. Synchronicity in the recorded spike trains was approacched by detecting and analyzing coincident spikes in a firing window of 1-5kHz. Using a Markov-chain like approach all existing firing patterns were extracted, identified and further analyzed to identify performance limits of the identified distributed code.

Based on this approach we are able to identify a possible general distributed coding scheme for a selected set of visual stimuli. The combinatorial neuronal firing pattern can sucessfully be decomposed into sub-ensembles distributing to the code. Sub-ensembles are composed of a temporal firing sequence of a discrete number of of up to 15 neuronal units.
The distributed coding scheme observed in the retinal ganglion cell populations recorded, and the emerging synchronicity on small time-window scale, gives further evidence that their target neurons in primary visual cortex could partly act as coincidence detectors. Population- and ensemble activity vectors, as well the neuronal firing sequences on a time-scale of up to seconds allow to approximated how the visual information is coded in the vertebrate retina.



# Glycine Receptors of Narrow-Field Amacrine Cells of the Mouse Retina

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Purpose: Glycine and GABA are the major inhibitory transmitters of the mammalian retina, and approximately half of the amacrine cells are glycinergic. They comprise between 10 and 20 different morphological types and synapse onto bipolar, ganglion and amacrine cells. The purpose was to reveal the expression of glycine receptors in amacrine cells.

Methods: Patch-clamp recordings were performed from retinal slices of wildtype, Glrα1(-/-) and Glra3(-/-) mice. Whole cell currents following glycine application and glycinergic inhibitory postsynaptic currents(IPSCs) were measured.
During the recordings the cells were filled with neurobiotin and Alexa488 in order to identify the cell types. Results from AII amacrine cells and other narrow-field amacrine cells are compared.

Results: All amacrine cells showed prominent responses to the exogenous application of glycine. Hence, they all express functional glycine receptors (GlyRs). Spontaneous or potassium-induced inhibitory postsynaptic currents could be recorded from 61 amacrine cells. AII amacrine cells receive faster synaptic input (mean decay time constant: $\tau w \sim 10$ ms) than other narrow-field amacrine cells (mean $\tau w \sim 25$ ms). The decay time constants of AII amacrine cells and other narrow-field amacrine cells in Glrα1(-/-) mice were not significantly different from those of wildtype mice.

Conclusions: GlyRs are expressed in all amacrine cells recorded. AII amacrine cells and narrow-field amacrine cells differ significantly in the decay time constants of their glycinergic IPSCs, with faster IPSCs recorded from AII cells. The IPSCs of Glrα1(-/-) mice were not significantly different. GlyRs of amacrine cells are probably composed of several different GlyR subunits.

# Light-induced alterations of S cone opsin expression in the albino rat retina

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**Purpose:**
Constant ambient light induces photoreceptor death in the normal rat retina, with the extent of damage depending on the intensity, duration, and spectral characteristics of radiation exposure. In this study we aim to assess the effects of a modest light increase under cyclic lighting conditions on shortwave-sensitive (S) cone visual pigment expression in the retina of pigmented and albino rats.

**Methods:**
Pigmented (Brown Norway) and albino (Wistar) rats were raised under cyclic dim light (12 h 5 lx light, 12 h dark). Young adult individuals of both strains were exposed to bright (160 lx) or dim (5 lx, control group) cyclic white light for 21 days. Retinae were isolated and assessed for changes in S cone opsin expression using immunohistochemistry on retinal wholemounts and on transverse cryostat sections. S cone photoreceptor structure was evaluated using S cone opsin immunostaining. PKC-alpha and recoverin immunostaining served to identify bipolar cell classes.

**Results:**
After cyclic exposure to 160 lx for 21 days, no apparent morphologic changes were identified in the retina of Brown Norway rats, whereas in Wistar rats S cones displayed various stages of degeneration. Structural changes included reduction of outer and inner segments, sprouting of axon collaterals, deteriorating nuclei, and total disintegration of S cones. No structural alterations of middle-to-longwave-sensitive (M) cone photoreceptors were observed. A set of bipolar cells located adjacent to pedicles of degenerating S cones stained positive for N-and C-terminus specific S cone opsin markers. These cells were of uniform morphology, with narrow dendritic fields apparently exclusively contacting the adjacent S cone pedicle. Their somata were located near the outer border of the inner nuclear layer and their axon terminals ramified in the inner lamina of the inner plexiform layer (ON bipolar cell morphology). S opsin positive bipolar cells were negative for PKC-alpha and recoverin.

**Conclusions:**
Different rat strains differ in their susceptibility to S cone photoreceptor damage by modest increases of ambient light intensity. In Wistar rats, S cones are more susceptible to light-induced degeneration than M cones. Degenerating S cones may either trigger expression of S cone opsin in adjacent bipolar cells or transfer membrane-bound S opsin to adjacent bipolar cells in the course of disintegration. Further analysis will have to show whether the above bipolar cells are a novel type of 'blue cone bipolar cell' that has escaped previous classification studies, or whether their distinct morphology reflects secondary degeneration.

# Light-evoked $Ca^{2+}$ signals in starburst amacrine cell dendrites: Are internal $Ca^{2+}$-stores involved?

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**Introduction:** The detection of motion and its direction is a major function of the visual system. More than 40 years after the first studies on retinal direction selectivity (DS) the underlying biophysical mechanisms are still not completely understood. Because synaptic input to retinal DS ganglion cells is already directionally-tuned, motion direction is computed (at least in part) presynaptically, likely in starburst amacrine cells (SAC), which generate DS $Ca^{2+}$ signals in their dendrites (Euler et al., 2002, Nature 418:845-852). In these experiments SACs were filled with $Ca^{2+}$ indicator via sharp microelectrodes, which were immediately withdrawn. When studying dendritic DS in SACs with whole-cell patch-clamp recordings (Hausselt et al., 2004, SfN #299.4; manuscript submitted) it was found that in most cases visually-evoked (but not voltage step-evoked) $Ca^{2+}$ signals disappeared while the cells still displayed DS electrical responses to visual stimulation. In many cases visually-evoked $Ca^{2+}$ signals could be recovered after patch electrode removal. These results suggest that visually-evoked $Ca^{2+}$ responses in SACs rely on dialysis-sensitive intracellular signaling, possibly $Ca^{2+}$ release from internal stores, which have been shown to participate in transmitter release from cultured retinal amacrine cells (Warrier et al., 2005, J Neurophysiol 94:4196-4208).

**Aim:** To determine the distribution of visually-evoked $Ca^{2+}$ signals along SACs dendritic branches and whether internal $Ca^{2+}$ stores are involved.

**Methods:** Displaced (ON) SACs in whole-mounted rabbit retina were filled with $Ca^{2+}$ indicator via sharp microelectrodes, which were quickly retracted. Dendritic $Ca^{2+}$ responses to visual stimuli were recorded using 2-photon imaging. Inhibitors of intracellular $Ca^{2+}$ signaling pathways were applied with the perfusion medium or locally via pipettes.

**Results:** We mapped visually-evoked $Ca^{2+}$ signals along SAC dendrites and found - in accordance with earlier observations - the strongest signals in the distal dendrites, corresponding to the SAC's output region, and localized to the varicosities. The distribution of signals was heterogeneous: some branchlets ('hotspots') responded strongly to the stimuli, while neighboring branchlets displayed much weaker or no signals. Moreover, some hotspots were DS while neighboring ones were not. The occurrence of 'hotspots' is similar to what has been described in isolated amacrine cells (Warrier et al., 2005). In responsive hotspots $Ca^{2+}$ signals were often very persistent and could be observed during the entire recording time (more than 3 hours). Application of a combination of intracellular-$Ca^{2+}$-signaling-pathways inhibitors (cyclopiazonic acid, 2-APB, and Ryanodine, which inhibit SERCA pumps, $IP_3$ receptors, and Ryanodine receptors, respectively) strongly reduced or abolished visually evoked $Ca^{2+}$ signals in SACs.

**Conclusions:** Our preliminary pharmacological data suggest the involvement of internal $Ca^{2+}$- stores in the generation of visually-evoked $Ca^{2+}$ responses in SACs. Internal $Ca^{2+}$- stores could support a functional compartmentalization of dendritic $Ca^{2+}$ signals; which may be reflected by the $Ca^{2+}$ activity 'hotspots' we observed in SAC dendrites.

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# Tapping retinal ganglion cell activity in humans with the multifocal pattern electro-retinogram

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Purpose: Multifocal visual evoked cortical potentials (mfVEPs) enable us to identify visual field defects objectively [1]. Multifocal pattern electro-retinogram recordings (mfPERG) might allow one to test the association of such visual field defects with retinal ganglion cell damage [2]. However, mfPERG-recordings are hampered by small response amplitudes. Here we tested whether mfVEP and mfPERG responses are boosted by slowing down the multifocal stimulation sequence. Further we compared the efficacy of two stimulation modi, namely pattern-reversal and pattern-onset.

Methods: Using VERIS Science 5.1.10X (EDI, Ca, USA) we simultaneously recorded mfVEP and mfPERG-responses to pattern-reversal (PR) and to pattern-onset (PO) stimulation from 8 controls during binocular stimulation at 52 locations comprising a visual field of 44° diameter (mean luminance: 47 cd/$m^2$; contrast: 96%). Three different PR and two different PO stimulus conditions, which differed in their maximal stimulation frequency, were compared: For PR stimulation, a contrast reversal occurred with 50% probability for a particular stimulus location after one (1f-PR, the standard setting for mf-PR stimulation), two (2f-PR), or eight frames (8f-PR), for PO stimulation, a pattern onset occurred after two (2f-PO), or eight frames (8f-PO).

Results: (1) mfVEPs: Both, PR- and PO-stimulation evoke sizable mfVEPs. Responses were greatest for slow pattern-onset stimulation (responses are boosted by approximately factor two for the 8f-PO compared to the standard setting, 1f-PR). (2) mfPERG: PR-stimulation was clearly more effective than PO-stimulation. Responses were greatest for slow pattern-reversal (responses are boosted by approximately factor 1.6 for the 8f-PR compared to 1f-PR).

Conclusion: (1) Retinal and cortical responses can be boosted by slowing down the multifocal stimulation sequence, which indicates adaptation or low-pass properties of the underlying neuronal substrate. (2) While PO-stimulation is effective for mfVEPs recordings, it is clearly inferior to PR-stimulation for mfPERGs recordings. This might be related to different contrast transfer functions of PERG and VEP responses. (3) Simultaneous spatially resolved testing of retinal ganglion cell and cortical responses might be facilitated using slow PR multifocal stimulation.

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# Recording of retinal ganglion cell activity with a high density multi-transistor-array (MTA)

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The retinal ganglion cells are the final relay station of visual information before it is transmitted to higher brain areas. It is currently assumed that the mammalian retina deconstructs the visual world using about a dozen neural channels, embodied in the morphological and physiological types of ganglion cells [1].

Fundamental questions regarding retinal encoding are thus addressed using multi-electrode array technology. In this technique, many ganglion cells from an isolated retina are simultaneously recoded by an array of extracellular microelectrodes, while visual stimuli are projected from a computer screen onto the photoreceptor layer. However, conventional multi-electrode arrays technology currently allows for either the sampling of ganglion cell at low spatial resolution (i.e. recording ~10% of the cells in a retinal patch) or for the recording of most cells in a small patch of 0.04 $mm^2$ [2, 3].

Here we report the recording of ganglion cell activity in the rabbit retina by a multi-transistor-array (MTA) with 16,000 electrodes. The electrodes cover an area of 1 $mm^2$ with the spatial resolution of 7.8 µm [4]. The current sampling rate is 4 kHz and can be increased by skipping recording traces of pre-selected electrodes.

The electrode density of the multi-transistor array is higher than the ganglion cell density in the rabbit retina (an in all mammalian retinas). Every spike is picked up simultaneously on several transistor electrodes producing for each ganglion cell a unique spatial activity pattern. Our spike sorting approach- the identification of spike trains of individual cells - takes advantage of this unique spatial pattern. We thus do not rely on the poorly defined extracellular shape of the waveform.

In summary, our preliminary results show that a high density multi-transistor-array is well suited to record retinal ganglion cell activity at high spatial and temporal resolution over an extended area of 1 $mm^2$ and the time of many hours.

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# Effects of GSM 900 electromagnetic field exposure on retinal ganglion cell responses.

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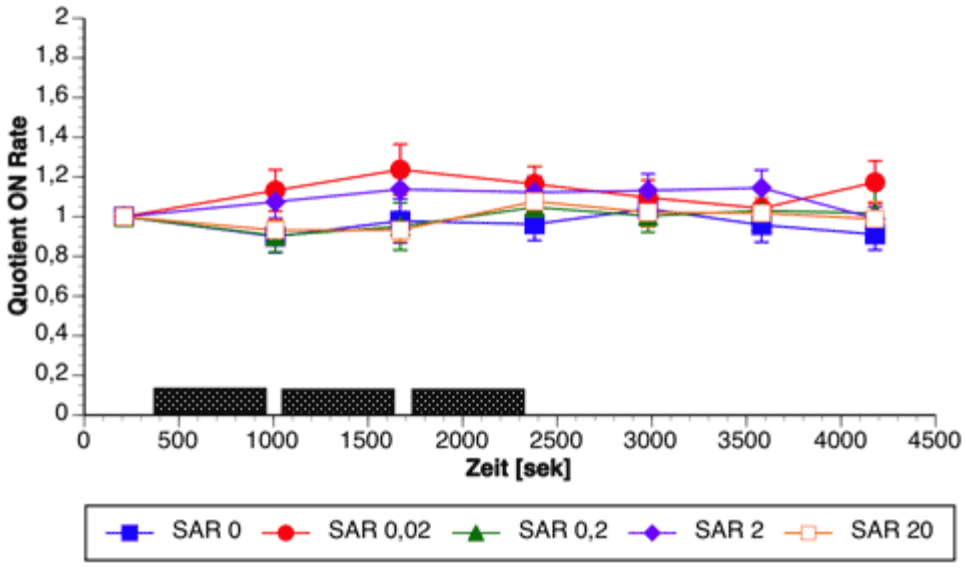
In the literature, there exist a lot of conflicting results about the effects of mobile phone radiation onto the nervous system. In particular, it is unclear whether putative effects are a by-product of general thermal warming or whether direct electromagnetic effects exist.
In this double-blind study we measured the light responses of retinal ganglion cells to various light intensities before, during, and after 30 minutes exposure of the retina to GSM 900 electromagnetic radiation. Responses were recorded extracellularly in the isolated mouse retina inside a calibrated exposure system. SAR exposure levels of 0.02 W/kg, 0.2 W/kg, 2 W/kg, and 20 W/kg were used. Sham exposure (0 W/kg) served as control. In order to minimize thermal effects the temperature of the superfusate surrounding the retinae was regulated to remain at a constant value. ON and OFF response rates and latencies of single ganglion cells were determined for analysis. Separate experiments were performed to estimate the effects of temperature alone onto response rates and latencies by varying the temperature of the superfusate without exposure.
For any tested intensity, response rates remained fairly constant during and after exposure in all exposure groups (SAR 0.02 W/kg - 20 W/kg), compared to the values before exposure. This is plotted as ON response ratio in figure 1 below for an intensity of 16 lx retinal illumination. Grey areas indicate exposure periods. Response latencies had a slight tendency to increase during the course of the experiments. Very similar results were obtained for the control group. Preliminary statistical analysis indicates that responses of the exposure groups did not differ from the control group. In the experiments with temperature variation alone, increasing temperature increased rate and decreased latencies. These results indicate that putative effects of GSM 900 radiation result from thermal warming and not from electromagnetic field effects per se.

Supported by Bundesamt für Strahlenschutz: StSch 4429



# Effects of Complexin III and IV depletion on the mouse electroretinogram

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Complexin (CPX) III and IV are unique complexins at retinal ribbon synapses. They are differentially expressed in the inner (IPL) and outer (OPL) plexiform layer of the mouse retina. In the OPL, CPX III was found mainly in cone and to a lesser extent in rod synaptic endings, whereas CPX IV was restricted to rod endings. In the IPL, CPX III was found in rod bipolar terminals and putative glycinergic amacrine cells, in contrast to CPX IV, which was found in cone bipolar terminals (Reim et al. 2005, J Cell Biology). To study the effects of CPX III and/or IV depletion on retinal function, ERGs were measured in CPX III, CPX IV single knock-out mice and in CPX III / IV double knock-out mice and the results compared with their wild-type littermates.

Depletion of CPX III resulted in a reduction of the oscillatory potentials riding on the ERG b-wave and a tendency towards lower peak frequency components as determined with Fourier analysis. In addition, b-wave implicit time was prolonged. Implicit time and amplitude of the a-wave were unchanged. Interestingly, CPX IV depletion had no significant effect on the ERG compared to the wild-type littermates. Depletion of both CPX III and IV, on the other hand, changed virtually all ERG components. Only a-wave amplitude and peak frequency of the oscillatory potentials remained unchanged. B-wave amplitudes and oscillatory potential amplitudes were significantly reduced. B-wave implicit times were prolonged at all intensities, as were a-wave implicit times at medium intensities. The latter was mainly due to a previously never observed secondary peak in the a-wave which was very pronounced at these intensities.

Contrary to the expectation that CPX IV depletion should strongly reduce the scotopic b-wave amplitude due to reduced transmission to rod bipolar cells, its amplitude remained unchanged. Depletion of both, CPX III and IV, on the other hand, severely reduced the b-wave amplitude despite the fact that CPX III depletion alone had also no effect on b-wave amplitude. In addition, effects on response components that probably reflect timing in synaptic transmission like implicit times and oscillatory potentials were more severely affected in CPX III / IV double knock-out mice than in CPX III single knock-out mice, despite the fact that depletion of CPX IV again had no effect on these ERG components.

In conclusion, the effects on the mouse ERG in the CPX III / IV double knock-out mice, compared to the single knock-out mice, indicate a cooperative action of the two complexins. For the ERG response components analyzed, CPX IV alone does not seem to play an essential role. It seems to be important, however, in combination with CPX III for transmission from rods to rod bipolar cells and for certain aspects of retinal timing reflected in the oscillatory potentials, which probably originate from interactions in the IPL.

# The role of horizontal cell coupling in the formation of antagonistic receptive fields in mouse retinal ganglion cells

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Most retinal ganglion cells possess antagonistic receptive fields with an excitatory center and an inhibitory surround. In the outer retina, horizontal cells are thought to contribute to the surround formation in ganglion cells since they form an extensive network by gap junctional coupling and feed back onto the photoreceptors. Here, we analyzed the role of horizontal cell coupling in ganglion cell surround generation. Therefore, we used a transgenic mouse line in which horizontal cell coupling was abolished (Hombach *et al*., 2004) and receptive field size was reduced (Shelley *et al*., 2006). In this mouse line, the gene for *connexin57* (*Cx57*) was deleted and replaced with a *lacZ* reporter gene (Hombach *et al*., 2004). Using multi-electrode recordings, we evaluated the receptive field properties of ganglion cells from wild-type and Cx57-deficient retinas with drifting sine wave gratings. Receptive fields of wild-type ganglion cells showed small or no surrounds while adapted to scotopic light. Adaptation to photopic light led to an increase in surround size for most cells and thus to a tuning towards higher spatial frequencies. Ganglion cells from Cx57-deficient mice showed essentially the same behavior. Moreover, contrast sensitivity and acuity, measured *in vivo* using a virtual optomotor system, were almost identical for wild-type and Cx57-deficient mice. These results indicate that the coupling of horizontal cells does not contribute to spatial tuning of ganglion cells. To further study the role of horizontal cells in spatial tuning, we impaired horizontal cell feedback to photoreceptors with cobalt. Compared to controls, ganglion cells from wild-type and Cx57-deficient mice were tuned towards lower spatial frequencies when cobalt was present. This suggests that feedback of horizontal cells contributes to the spatial tuning in ganglion cells. The coupling of horizontal cells, however, is not necessary in this process.

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# Identification and localisation of cpCx43.4 and cpCx47.6, two homologs of mmCx45 expressed in the carp retina

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Gap junctions represent the most ubiquitous form of intercellular communication in vertebrates. In a recent genomic screen 37 putative zebrafish (zf) connexin genes have been identified (Eastman et al., 2005), almost twice the number found in rodents. Several of these connexins (zfCx27.5, zfCx35, zfCx43, zfCx52.6, zfCx55.5) were first identified in the retina. Due to its characteristics as "the nature's brain slice", and the fact that almost all subtypes of retinal neurons are coupled through gap junctions, the vertebrate retina is an excellent model system to study the expression patterns of connexins. Here, we report the identification of two new carp retinal connexins showing high homology to mmCx45 and analysed their expression patterns by multiple tissue RT-PCR and fluorescence in situ hybridisation (FISH) in the carp retina.

First, we used degenerate primers to search for unknown connexins expressed in the carp retina. Using RACE RT-PCR, we then cloned two new connexins designated cpCx43.4 and cpCx47.6. BLASTn search results and nucleotide sequence alignments identified these retinal connexins as the carp orthologs of zfCx43.4 (92% identity) and zfCx47.1 (87% identity). Alignments of the complete nucleotide sequences of the two cpCxs revealed only 55% identity overall, but higher identity scores (up to 78%) in the N-terminal and transmembrane regions. The cytoplasmic loop and the C-terminal regions showed the lowest levels of identity and similarity, as is well known for connexins. Alignments of cpCx43.4 and cpCx47.6 nucleotide sequences with mmCx45 characterised both as a homologue of mmCx45, with 57% and 53% identity and 80% and 78% similarity, respectively.

Expression analysis using multiple tissue RT-PCR revealed the highest level of cpCx43.4 expression in carp brain, kidney and heart, only weak expression in retina, liver and muscle and no expression in lens. In comparison, cpCx47.6 showed the highest level of expression in carp retina and brain, low expression in kidney and heart, but no expression in lens, liver, and muscle.

The analysis of the distribution patterns of both connexin transcripts within the carp retina using conventional, alkaline phosphatase-based in situ hybridisation and FISH revealed a restricted expression pattern for cpCx47.6. Labelled cells were found exclusively in the proximal part of the inner nuclear layer, at the border of the inner plexiform layer, where somata of amacrine cells are located. In contrast, the distribution pattern of cpCx43.4 mRNA could not be clarified by in situ hybridisation. This is probably due to the low level expression of this connexin in the retina.

A subsequent experiment to characterise the cpCx47.6-expressing amacrine cells by combining FISH and immunofluorescence showed that these amacrine cells are not glycinergic, and express neither the calcium-binding protein calbindin nor caldendrin.

In summary, we have cloned two new connexins from carp retina, designated cpCx43.4 and cpCx47.6. The latter is expressed exclusively in a distinct type of putative GABAergic amacrine cell, which do not show anti-calbindin or anti-caldendrin immunoreactivity.

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# Connexins expressed in horizontal cells of the fish retina

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Horizontal cells are second-order neurons of the vertebrate retina mediating the lateral feedback on photoreceptors. They exhibit strong electrical coupling via gap junctions and recently the functional expression of mmCx57 within mouse horizontal cells has been described (Hombach et al, 2004). While the murine retina possesses only one type of horizontal cell, within the teleost retina up to four distinct types (H1-H4) have been identified. Horizontal cells of the same subtype form autonomous networks, and it is tempting to speculate that gap junctions of independent circuits are composed of different connexins.

In order to search out connexins expressed in different types of horizontal cells of the fish retina, we used RACE RT-PCR and cloned four connexins with close relationships to mmCx57. The distribution of connexin transcripts within the carp retina was investigated by means of in situ hybridization, and expression of a newly identified connexin (cpCx53.8) was analyzed by using newly developed antibodies, specifically designed to the C-terminal end region.

BLAST search results identified two of the cloned connexins as the carp orthologs of the zebrafish Cx52.6 and Cx55.5. The third connexin (cpCx53.8) revealed highest homology to cpCx52.6 and 80% identity with mmCx57 at the C-terminal region (final 20 amino acids (aa)). The fourth connexin, designated as cpCx49.5, exhibited only partial homology to published connexins. Alignments of amino acid sequences revealed most similarities between cpCx55.5 and cpCx49.5 (65 % identity) and between cpCx52.6 and cpCx53.8 (92 %). Similarities between cpCx55.5/cpCx49.5 and cpCx52.6/cpCx53.8 were restricted to aa sequences at the N-terminal region, the transmembrane domains, the extracellular loops and parts of the cytoplasmic loop of each connexin. The C-terminal regions appeared as most divergent. When aligned with mmCx57, cpCx52.6 and cpCx53.8 showed a slightly higher homology to this mouse horizontal cell connexin than cpCx49.5 and cpCx55.5.

Fluorescence in situ hybridization (FISH) with riboprobes encoding the C-terminal region of cpCx52.6 and cpCx55.5 revealed highly restricted expression patterns for both connexins. Labelled cells were exclusively found in the distal part of the inner nuclear layer, where somata of horizontal cells are located. This unique expression of both connexins in horizontal cells was confirmed by FISH in dissociated retinal cells, where both transcripts were localized in H1-, H2- and H3-type horizontal cells.

Immunocytochemistry, using the highly specific anti-cpCx53.8 antibodies (rabbit), identified cpCx53.8 as the major connexin participating in the formation of the relatively large dendro-dendritic, soma-somatic and axo-axonic gap junctions between all types of horizontal cells. Large patches of Cx53.8-immunoreactivity were localized in all types of fish horizontal cells and their axon terminals. No other retinal cells were labelled by these antibodies.

Taken together, our data provide the first evidence for the unique expression of three different connexins (cpCx52.6, cpCx53.8 and cpCx55.5) in horizontal cells of the fish retina.

Supported by the Deutsche Forschungsgemeinschaft (JA 854/1-1, 1-2).

# Connexin30.2 is Expressed by Ganglion Cells of the Mouse Retina

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Gap junctions are clusters of intercellular channels connecting the cytoplasms of two cells providing a direct intercellular communication. They are formed by proteins termed connexins (Cxs) which form a hexameric hemichannel or connexon (for review see Söhl et al., 2005). During the past years, several studies revealed an unexpected high density of gap junctions in the retina, composed of different Cxs. In this work we present a new retinal Cx using a transgenic mouse line allowing the identification of retinal neurons expressing this protein. In this mouse line, Cx30.2 coding region was replaced by the LacZ gene. The LacZ signal was expressed in the nucleus of the cells and could be observed in cell bodies located in the ganglion cell layer (GCL) and in the inner nuclear layer (INL). Previously, this connexin, and its putative human orthologue Cx31.9, have been described in brain, vascular smooth muscles, testis and heart (Nielsen et al., 2002; Nielsen et al., 2003; Kreuzberg et al., 2005).

We have concentrated our study on the ganglion cell layer (GCL) using immunocytochemistry and tracer experiments to characterize the morphology of the cells expressing Cx30.2. One of the ganglion cell types expressing Cx30.2 corresponds to the RGA1 neurons according to the classification made by Sun et al. (2002), or giant cells. RGA1 neurons have a polygonal soma and 3 to 5 primary dendrites leave the soma in a radiate pattern of branching. The dendrites radiate primarily in stratum 5 of the inner plexiform layer, making it likely that these ganglion cells are ON-ganglion cells. They are coupled to numerous amacrine cells having their somas in the GCL. These displaced amacrine cells do not express Cx30.2 and we conclude that the connecting gap junctions are composed of heterotypic channels involving Cx30.2 and a yet unidentified connexin. In order to find an immunocytohistological marker for these neurons, injected giant cells were incubated with different antibodies directed against calcium-binding proteins. These studies revealed a positive immunostaining to parvalbumin, whereas labeling to calretinin and calbindin was negative.

In this study we show that giant ganglion cells express a new retinal connexin, Cx30.2, and are coupled to displaced amacrine cells by heterotypic gap junctions.

This study was supported by Deutsche Forschungsgemeinschaft (International Graduate School "Neurosensory Systems" (R.W.); JA 854/1-1,2 to U.J.B & R.W. and WI 270/22-5,6 to K.W)

# HCN Channels Expressed by Rod Bipolar Cells of the Mouse Retina, Confer a Band-Pass Response to Input Signals

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The functional role of retinal cyclic nucleotide-gated hyperpolarization-activated channels (HCN), which mediate $I_h$, is not fully understood. Recent evidence on the expression and properties of HCN in retinal rods as well as the consequences that an $I_h$-blockade has on visual responses suggest a role for HCN in the temporal processing of visual information. Here we characterize the expression pattern and functional role of HCN channels in second order neurons of the rod pathway, in mice.

We performed immunohistochemistry targeting subunit isoforms HCN1/2, in combination with markers of rod bipolar cells (RBCs) and rod-RBCs synaptic contacts. We found a marked, dot-like expression of the HCN2 subunit in the outer plexiform layer. HCN2 colocalized with the dendritic tips of RBCs (PKC), with a similar distribution to that of postsynaptic glutamate receptors (mGluR6), but adjacent to the presynaptic terminal machinery (bassoon). RBCs thus appear to express HCN2. In agreement with previous reports, HCN1 was found in the inner segments of rods but not in RBCs.

We examined the functional role of HCN channels in individual RBCs by perforated-patch recordings obtained in the inner nuclear layer, followed by morphological identification with Lucifer Yellow. Active currents of recorded neurons were characterized by voltage-clamp protocols. The frequency-dependence of input impedance was explored by sinusoidal frequency-modulated current stimuli (0.1-30 Hz) delivered in current-clamp mode at different potentials. RBCs were found to display $I_h$ having a relatively slow time constant (up to 400 ms, RT). Impedance analysis showed that, in the range of activation of $I_h$, RBCs display a band-pass transfer function (peak ~2 Hz). To confirm that the observed frequency tuning could be directly ascribed to $I_h$, we built for each cell a simplified model (single isopotential compartment) incorporating passive properties and $I_h$ as the only active current, with parameter values taken from the actual records. The theoretical impedance function derived from the model matched closely the experimental one.

Finally, a pharmacological blockade of $I_h$ converted the band-pass response of RBCs to a low-pass one.

We conclude that the ability of $I_h$ to act as a negative-feedback mechanism in RBCs, dampening voltage responses to slow synaptic current inputs, may help tune the visual system to brisker, behaviorally relevant visual signals.

# Can chicken retinal ganglion cells distinguish image defocus from a drop in image contrast?

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The chicken is the most frequently used animal model in myopia research. We used a Micro-Electrode-Array (MEA) to record ganglion cell responses to phase-reversing checkerboards of different angular field sizes, contrast and defocus. The final goal of these studies is to find out whether ganglion cells can distinguish optical defocus from a global drop in image contrast and whether they can distinguish positive from negative defocus.

The frequency of the spikes following a phase reversal of the checkerboard (referred to as "responses" below) were dependent on both spatial frequency and contrast of the fields. Responses declined with increasing spatial frequency. Three selected ganglion cells showed responses up to 7 cyc/deg of the checkerboard which matches the behavioural optomotor acuity (around 7cyc/deg, e.g. Schmid & Wildsoet 1998, Vision Res 38, 2629-2634). To evaluate the average contrast sensitivity of the ganglion cells, spike frequencies of all 60 ganglion cells were added while the contrast of the checkerboard was varied. The average threshold contrast was around 0.3. Examples of three individual cells are shown in figure 1.
The most interesting result, however, was that at least three ganglion cells showed response inhibition at best focus ("0D" defocus, figure 2) at 2.6 cyc/deg, and enhanced responses for small amounts of defocus in either direction (between +/- 0.5D to +/- 1.0D). It could have been that they just preferred a certain sub-maximal contrast. However, their responses for the same checkerboards presented at different contrasts (Figure 1) did not show such an optimum function: the highest responses occurred at the highest contrast. The drop of responses to defocused stimuli beyond 1 D could be predicted from the modulation transfer function of a defocused optical system (the first Bessel function).

*In vitro* recordings from ganglion cells showed a similar spatial resolution as in behavioural studies. This suggests that the ganglion cell responses were "representative".
Most interestingly, ganglion cells did not respond equally if the contrast was reduced by defocus, or directly on the presentation screen. Apparently, these cells do distinguish between both, although there is no indication of a distinction of the sign of defocus. These experiments should provide better insight into the retinal image processor that controls eye growth and myopia.

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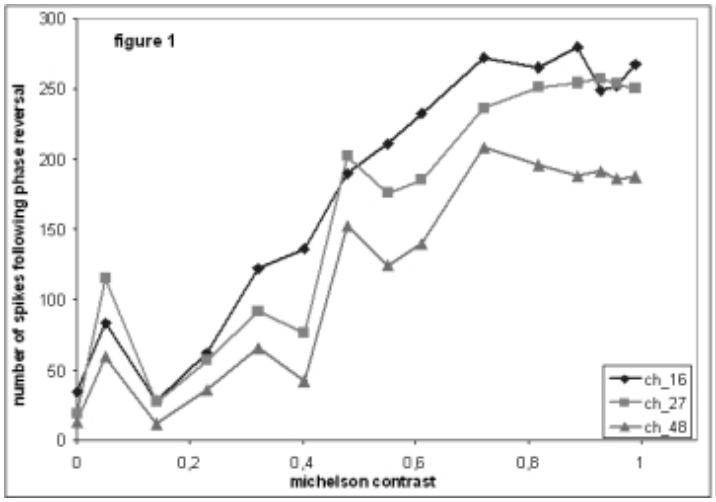


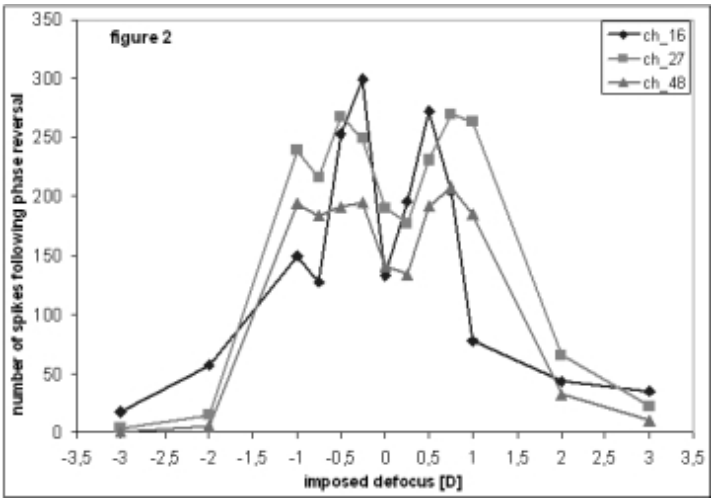

# Functional characteristics of retinal ganglion cells in the blind P23H rat as a perspective model for testing retinal prosthesis

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**Purpose:** After photoreceptor degeneration it remains unclear what visual signal is provided to the brain. This signal is provided by retinal ganglion cells (RGCs) which generate action potentials. In the present study, we have applied the multielectrode recording technique (MEA60, Multi Channel Systems MCS GmbH, Germany) permitting to record simultaneously extracellular activity from large populations of RGCs in the isolated retina of P23 H transgenic rats, a model of retinitis pigmentosa with photoreceptor degeneration.
**Results:** To assess the functional state of the RGCs in the blind aged (>1, 5 years) transgenic rats carrying the P23H opsin mutation, we evaluated the functional properties of RGCs in *ex vivo* isolated retina: ability to generate action potentials, to respond to light and to electrical stimulation. Preliminary behavior testing proved absence of visual-guided reflexes at age 18 months. Light microscopic examination of retina morphology revealed the nearly complete loss of the photoreceptor cells while the normal morphology of retinal ganglion cells was preserved. In line with these findings the electrophysiological recordings confirmed that RGCs in blind rat capable to generate typical biphasic somatic and triphasic presumably axonal action potentials. Spontaneous activity rates varied in the same range frequencies typically encountered in the wild type rats. Patterns of spontaneous activity also did not show any significant abnormalities in RGCs of mutated animals. In none of recorded RGCs presentation of the light stimuli of different intensities (scotopic - photopic) , colors (green, white) and durations (500 ms - 5 s), elicited any light responses. At the same time, applying the electrical stimulation to the one of MEA array electrodes (single biphasic and monophasic square pulses), and recording the activity of RGCs at distant electrodes (200 - 1000 µM), affected the firing rate of about 3- 5 % of RGCs producing only long-latency (>100 ms) tonic excitatory responses at chosen current intensities. The stimulation threshold for these delayed responses was approximately 10 µA.
**Conclusion:** We present original results showing that in the blind mutant rats P23H which is considered a good model of retinitis pigmentosa, the retinal ganglion cells remain functionally active in old age, and capable to produce typical action potentials, maintain the spontaneous firing even in the absence of visual input, as well as modulate their activity in response to local electrical stimulation within the retinal ganglion cell layer, thereby permitting transmission of visual images into the brain.

# Poster Topic

## T16: Visual system III: Central processing

**T16-1A** A midbrain feedback loop in a slice preparation: electrophysiology of relevant elements
*U. Netzel, R. Wessel and H. Luksch, Aachen and Saint Louis (USA)*

**T16-2A** Operating regimes for cortical computation
*M. Stimberg, K. Wimmer, R. Martin, J. Mariño, J. Schummers, DC. Lyon, M. Sur and K. Obermayer, Berlin, A Coruña (E), Cambridge (USA) and La Jolla (USA)*

**T16-3A** Influence of recurrent excitation and inhibition on the response dynamics in a network model of orientation tuning
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**T16-8C** Functional architecture of horizontal connections in the cat primary visual cortex
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**T16-9C** Lateral connections in cat's area 17
*CC. Girardin and KAC. Martin, Zurich (CH)*

# A midbrain feedback loop in a slice preparation: electrophysiology of relevant elements

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Feedback loops play an important role for neuronal responses all over the brain. Since elements are usually scattered, the cellular circuitries are difficult to study. Here we investigated the reciprocal connection between the optic tectum and the isthmic nuclei in birds. Its function might be a "winner-takes-all-circuit", providing increased excitability of an activated locus and decreased excitability in the surrounding.
One type of tectal output neuron projects upon three isthmic nuclei, each of which projects back to the tectum with specialized efferents. Nucleus isthmi pars parvocellularis (Ipc) and nucleus isthmi pars semilunaris (Slu) have cholinergic efferents and project back to the tectum in a homotopic fashion. Thus, they project upon the same location in the tectum where their input comes from. Contrary, nucleus isthmi pars magnocellularis (Imc) has GABAergic efferents projecting upon the tectum in a diffuse manner, thus in a heterotopic fashion. All elements, including the retinal afferents are preserved in a 500 µm slice.
We investigated the electrophysiology of the circuitry's elements by whole-cell patch recordings. Each neuronal type has characteristic electrophysiological properties. In order to analyze the integration of each element within the circuitry, retinal afferents were electrically stimulated with up to 8 bipolar electrodes. Responses of nucleus isthmi pars magnocellularis and nucleus isthmi pars parvocellularis were both excitatory. We found a two-peak distribution for the latencies in both nuclei: In the cases where Shepherd's crook neurons were stimulated directly and one synapse was involved, latencies were around 6 ms. In the cases, where afferents in the upper layers of the tectum were stimulated and two synapses were involved, latencies were around 14 ms. Shepherd's crook cells responded with long-lasting excitations; latencies were around 7 ms.
To assess the latency from isthmic activation to tectal output, Ipc and Imc were also stimulated. Extracellular recordings were made in the deep tectal layers. Latencies were 1 to 2 ms in both cases, corroborating results obtained in intact animals.

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# Operating regimes for cortical computation

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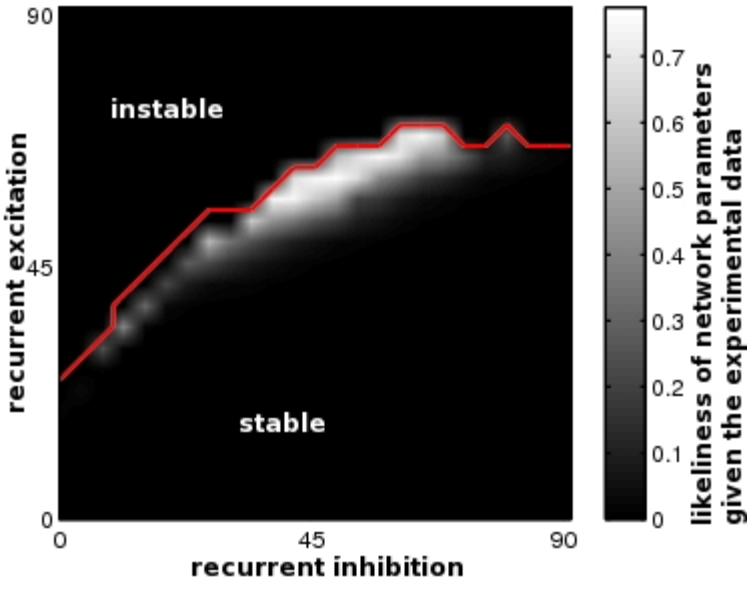
In the primary visual cortex (V1), information processing in local neuronal circuits is influenced by the spatial location in the orientation preference map (ranging from orientation domain to pinwheel regions). A signature of these influences is the dependence of the orientation tuning of a cell's conductance input, its membrane potential and its spike output on the map location. Here we use a firing rate network model and a physiologically more realistic Hodgkin-Huxley based network model to analyze how much evidence recent intracellular measurements from cat V1 [1] provide for the different cortical operating regimes and whether the available data allows to single out the most likely operating point. Using data of a neuron´s spike output, its membrane potential, its total excitatory, and its total inhibitory input conductance, we find that the experimental data most strongly support a regime where the afferent input is well tuned and where the local recurrent synaptic network provides significant excitatory and inhibitory inputs when compared to the feedforward drive (see Figure). This result is highly robust against changes in basic model assumptions, because neither Mexican-hat type interactions nor a particular spatial range of the lateral excitatory vs. inhibitory connections have to be invoked to draw this conclusion. The analysis also shows that the tuning properties of the total excitatory conductance and the membrane potential are most informative about the relative strengths of feedforward vs. recurrent inputs. Location invariant spike tuning, however, can be achieved for a fairly wide range of model parameters. Furthermore, our analysis predicts that - due to the strong recurrency - the most likely operating point is close to a "line of instability" across which the cortical network becomes unstable and the neural activity increases dramatically.

[1] Marino, J. et al., Nat Neurosci 8, 194ff (2005).



# Influence of recurrent excitation and inhibition on the response dynamics in a network model of orientation tuning

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The relative contribution of afferent, recurrent inhibitory and recurrent excitatory inputs and their relevance for cortical computations have long been a matter of controversy. Spike tuning to orientation in V1 has been simulated successfully in artificial neural networks with varying contributions of these components. Using intracellular measurements to constrain the model space, the most likely operating regime for a network model composed of Hodgkin-Huxley neurons in an artificial orientation map was previously shown to be recurrent with both, significant excitation and inhibition, and with parameters close to the unstable region of network operation [1, 2].

Here we extend the analysis from the steady state response of the network to its temporal dynamics. The analysis of the network response kernel for simulated reverse correlation input shows that feed forward dominated and balanced recurrent regimes closely track the afferent input, showing sharp onsets and offsets in their responses. Inhibition dominated networks show a shortened response while excitation dominated networks have a prolonged time course; both are incompatible with data from reverse correlation experiments. A high-firing-rate recurrent regime with strong excitation and moderate inhibition lacks the sharp offset but it is marked by critical slowing down near the phase transition to the unstable region. When considering the time course for neurons located either in the orientation domain or near a pin wheel center separately, only the balanced and feed forward regimes show location invariant responses to preferred orientation. Further analyses to constrain the operating point for cortical computations, such as an analysis of the response variability and the difference between the network's impulse response and the response kernel, estimated through ongoing stimulation, are also presented.

In sum, the results for the balanced recurrent and for the feed forward regimes are best compatible with existing experimental results. Thus the evidence from the steady state simulations and from the temporal responses for different orientation map locations converges. It points towards a balanced regime with significant recurrent contributions that processes moderately tuned afferent input as the likely operating point of cortical networks processing sensory stimuli.

[1] Mariño J et al., Nat Neurosci 8 (2005)
[2] Wiesing P, et al., Soc Neurosci Abstr (2005)

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# Closing the Loop: The Dynamical Performance of Oculomotor System and Perception

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When we see a moving stimulus, our eyes are able to follow the motion very accurately. In addition, we have a sensory impression of stimulus motion. The question arises to what extent the perceived stimulus velocity corresponds to the velocity of the moving eyes and how well both the perception and the oculomotor system perform. During the open-loop period of oculomotor following responses after the onset of stimulus motion the overall retinal image motion induced by the self-motion of the eye has not yet been processed by the visual system, in contrast to the closed-loop period.
It turns out that the oculomotor system performs much worse during the open-loop period than the perceptive system, and that the performances of both systems only become approximately equal in the closed-loop period. In addition, the ocular responses are only weakly correlated with the perceptual responses. Our findings indicate 1) that the precision of the ocular following system is strongly based on the feedback loop between sensation and action, and 2) that the variability of the ocular responses cannot be explained by noise in the sensory system alone.

# Effects of High- and Low-Frequency rTMS on the Metabolic Activity and the Inhibitory Systems in Adult Rat Cortex

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Repetitive TMS, a non-invasive and painless method, is widely used to modulate the cortical excitability in specific regions of the human brain in treatment of disorders, which are related to abnormalities of cortical excitability. Recently, rTMS has been reported to be effective in treating depression and other neuropsychiatric disorders. Findings to date suggest that the modulatory effects of rTMS depend on the frequency, intensity and duration of the stimulus. High frequency rTMS leads primarily to facilitation, whereas low frequency rTMS leads to inhibition of the treated cortex. However, knowledge is limited concerning the cellular mechanisms and brain regions affected by rTMS. Therefore, in this study, we investigated whether different stimulus frequencies of rTMS affect the expression of metabolic activity markers (zif-268 and c-fos) similarly in different cortical regions of adult rat brain. In addition, we studied the effects of high (10 Hz) and low (1 Hz) frequency rTMS on the expression of the inhibitory system protein GAD65, that synthesizes preferentially GABA for vesicular release, and GAD67 that synthesizes preferentially cytoplasmatic GABA. Rats received 1 hr 10 Hz (high) or 1 Hz (low) frequency rTMS under urethane anaesthesia. For 10 Hz rTMS treatment we applied 2400 pulses (4 blocks of 12 trains of 1 s repeated every 5 s, blocks repeated every 2 minutes, intertrain interval 20 min) total 70 min. For 1 Hz we applied 3600 pulses (3 blocks of 20 min continuous stimulus, interval 25 min) in 70 min. Stimuli were applied to the occipital pole of rat forebrain (primary visual cortex) as biphasic pulses via a 70 mm figure-of-eight coil (MagStim rapid) with induced current directed from the right to the left hemisphere. For immunohistochemical studies, animals were perfused transcardially immediately after rTMS treatment. Immunohistochemisty was performed on free-floating coronal sections of frontal, motor, somatosensory and visual cortex for c-fos, zif-268, GAD65 and GAD67. So far, our preliminary results show that high and low frequency rTMS induced immediate early gene (IEG) products differently. 10 Hz rTMS enhanced zif-268 expression, while 1 Hz increased c-fos expression. Counting of GAD67 immunoreactive cells implies that GAD67 was influenced by both low and high frequency rTMS. In conclusion, our results suggest that neuronal activity/plasticity markers are differently induced by rTMS depending on stimulus frequency. rTMS might activate different cell populations in the cortex depending on the stimulus parameters (frequency and pattern). The effects of rTMS on the expression, concentration and distribution (cytoplasmatic vs dendritic) of GAD65, GAD67 are still under investigation. Supported by the DFG (SFB 509 TP C12).

# KCC2 and NKCC1 in the visual cortex of pigmented and albino rats: a molecular and immunohistochemical approach

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In the Wistar albino rat albinism is caused by a mutation in the tyrosinase gene (Blaszczyk et al., 2005) that results in a lack of pigmentation of hair, skin and eye. Additionally, albinism results in anatomical abnormalities like differences in retinal cell number, misrouting of visual pathways and abnormal representations of the visual field in higher areas of visual processing.

Besides these anatomical changes, electrophysiological alterations are also evident in the albino visual cortex of the rat: *in vitro* electrophysiological recordings imply an altered chloride reversal potential in the albino visual cortex (Barmashenko et al., 2005). This shift of the intracellular chloride concentration might lead to alterations in the $GABA_A$ mediated signalling and information processing in albinism.
How can a shift in intracellular chloride concentration be explained?
In the albino visual cortex this shift was suggested to be due to an altered expression of the inwardly directed cation-chloride cotransporter (CCC) NKCC1 or the outwardly directed CCC KCC2, or both (Barmashenko et al., 2005).

To study the molecular basis of CCC expression the RNA as well as protein levels are examined. Quantitative real-time RT-PCR shows no differences at RNA expression levels in the visual cortex of pigmented Long Evans and albino Wistar rats aged P28 of both transporters. Quantitative protein expression examined with the Western blot technique mirrors the RNA expression data, where no differences in expression levels are evident between pigmented and albino rats in the visual cortex. Additionally, preliminary results indicate neither any differences in KCC2 oligomerization nor in KCC2 or NKCC1 phosphorylation between both strains.
Not only the amount but also the allocation of CCC protein might explain differences in physiology, therefore immunohistochemical stainings are performed. At a glance, no qualitative differences in the staining pattern of both transporters in the visual cortex can be detected confirming the molecular data. Further analysis of prelabelled pyramidal cells in layer V of the visual cortex will reveal if any cell specific differences in allocation of both CCCs does occur. Such differences in the celll specific distribution of both transporters might explain the physiological differences present in the visual cortex of albino rats.

# *GABAergic projections of the visual system in the rat. An in vivo and in vitro study*

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The superior colliculus (SC), the dorsal and ventral lateral geniculate nucleus (dLGN and vLGN) and the pretectal nuclear complex (PNC) are major parts of the subcortical visual system. We already showed that the superficial grey layer (SGS) in the SC and the dLGN receive GABAergic inhibitory input from the PNC. Here we ask if the reciprocal connection between the vLGN and PNC is also GABAergic and focused on the postnatal development of all four projections. Another question is, if individual cells in the PNC project to both, the SGS and dLGN. For the first part of this study we used whole-cell patch-clamp recordings from single vLGN, PNC, SGS and dLGN neurons. We found that all projections are functional at P0 and that in all cases $GABA_A$ is the mediating receptor. To study whether single PNC cells project to SC and dLGN we performed double labelling experiments in adult Long Evans rats using the retrograde tracers Fluorogold and True Blue. After a three day survival time, brains were perfusion fixed and 50 µm thick frontal slices were cut on a vibratome. Photomicrographs of labelled neurons in the PNC were obtained with a microscope using fluorescence optics. So far, it seems that most of the retrogradely labelled neurons in the PNC are single labelled, however there are a few double labelled neurons as well. One reason for those double labelled neurons could be that fibers of passage which originate from the PNC and target the ventral LGN are also labelled. Therefore double stained neurons in the PNC project to the SGS and vLGN but not to the dLGN. So far it seems that the PNC neurons that project to the SGS and dLGN are single labelled and this suggests different functionality of both projections. The pretcto-tectal pathway may play a role in the postsaccadic supression whereas the pretectal projection to the dLGN may be involved in the postsaccadic facilitation. Less is known about the function of the reciprocal connection between the PNC and vLGN, we suggest a role for visuomotor responses.

# Effects of albinism on direction selectivity in the retina and the accessory optic system of rats

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A subclass of retinal ON-type ganglion cells is direction selective and shows strong responses during a stimulus movement in a preferred direction but less during a movement in the opposite direction. These ganglion cells project to the accessory optic system, which consists of three distinct nuclei: the medial terminal (MTN), the lateral terminal and the dorsal terminal (DTN) nucleus, which is associated with the nucleus of the optic tract (NOT). Earlier *in-vivo* studies revealed that direction selectivity is disturbed in albinotic ferrets and rats in a way that animals were unable to generate an optokinetic nystagmus. Electrophysiological experiments in albinotic ferrets indicated that neurons in the NOT-DTN show a reduced responsiveness to moving visual stimuli and lack direction selectivity. So far it is unknown if this disfunction is a problem of perception of the stimulus movement or the result of a disturbed integration of the movement signal at higher levels of the visual pathway. To answer this question it would be neccessary to clarify (a) if the ON-type direction selective ganglion cells in albino animals are functional and (b) if there are functional abnormalities of cells in the accessory optic system.

In the present study we performed *in-vitro* electrophysiological analysis of retinal ganglion cells and cells of the NOT-DTN in albinotic and pigmented rats. Extracelullar recordings from retinal ganglion cells were taken to analyse spiking acitivity in response to moving light stimuli. A comparison of physiological parameters using patch clamp techniques revealed no differences in resting potentials and input resistances of measured cells. Measurements of NOT-DTN cells in the gramicidin perforated patch configuration indicate a significant ($p<0.001$) shift of the reversal potential of GABA related currents in albinos (-45.2 mV, n=17) compared to pigmented rats (-63.8 mV, n=23). We conclude that unspecific response patterns and the accompanied loss of direction selectivity in the albino DTN neurons are a result of a disinhibition due to their shift in reversal potential of GABA related currents.

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# Electrophysiological recordings in the pretectum of the small-spottet dogfish (Scyliorhinus canicula)

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During ego and external motion the accessory optic system (AOS) together with the nucleus of the optic tract (NOT) ensure retinal image stabilisation. The neurons which belong to the AOS and the NOT are highly-direction selective and can therefore process signals about direction and speed of the retinal slip. In mammals a strict segregation occurs, the NOT and dorsal terminal nucleus (DTN) code for horizontal movements and the lateral terminal (LTN) and medial terminal nucleus (MTN) code for vertical directions. In osteichtyes the central pretecal nucleus (CPN) takes the part of image stabilisation during self and environmental movements. In contrast to mammals this nucleus contains neurons which code for all directions of motion [Klar and Hoffmann, 2002]. So the question arises what kind of visuo-motor organisation is realised in chondrichtyans? We investigated a retinorecipient pretectal area in Scyliorhinus canicula. Electrophysiological recordings in the left pretectum of four S. canicula were made. The visual stimulation consisted of a random-dot-pattern projected by a planetarium. The following stimulus movements were presented: 1. horizontal rotation around the vertical axis of the fish´s head (YAW), 2. vertical rotation around the longitudinal axis of the fish (ROLL), 3.vertical rotation around the left anterior right posterior axis (LARP) and 4. vertical rotation around the right anterior left posterior axis (RALP). In every axis the stimulus turned in clockwise (CW) and counterclockwise (CCW) direction. We recorded twenty-four direction selective cells, which coded in all axes of rotation. However the neurons seem to prefer movements, around the RALP and LARP axes, i.e. axes of the semicircular canals. Therefore we suggest that the pretectum codes self-motion in a reference-frame that is similar to the semicircular canals.

# Spatial and Temporal Regulation of Intracellular Protein Tyrosine Phosphatases in the Developing Mouse Retinocollicular System

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The developing mammalian retinocollicular system presents an appropriate model to investigate the molecular mechanisms of axonal growth and guidance as well as topographic map formation. These processes rely on several molecules such as eph receptors and their ephrin ligands, semaphorins, cell adhesion molecules as well as netrins. The signaling of many of these diverse molecules is regulated by tyrosine phosphorylation. The level of phosphotyrosine on substrate proteins is controlled by the balance between protein tyrosine kinases (PTKs) and their opponents, protein tyrosine phosphatases (PTPs). However, the mechanisms by which PTPs regulate axonal growth and pathfinding of retinal ganglion cell axons still remain unknown.

In the previous study different receptor- and intracellular-PTPs were identified in the embryonic E13 mouse retina and colliculus superior using a degenerate primer-PCR strategy. To investigate the spatio-temporal expression of five identified intracellular PTPs (TCPTP, SHP1, SHP2, MEG2 and PEST) in the developing mouse retinocollicular system we performed quantitative real-time-PCR, Western Blot and immunhistochemical analysis. In the retina, when compared with E13, TCPTP, SHP1, SHP2 and PEST were downregulated in the embryonic (E15, E18) and early postnatal stages (P0, P4), whereas at the late postnatal stages (P8, P20) an upregulation was observed. In contrast, MEG2 showed a downregulation in all investigated developmental stages. In the colliculus superior, SHP1 mRNA and protein were downregulated in the embryonic and early postnatal stages, whereas the expression increased in the late postnatal stages. TCPTP, SHP2, MEG2 and PEST revealed constant downregulation in all developmental stages. Immunhistochemical analysis in the embryonic retina confirmed that TCPTP, SHP2, MEG2 and PEST but not SHP1 were expressed in retinal precursor as well as in postmitotic neurons. Postnatally, the expression of these PTPs was restricted to defined retinal layers. SHP1 showed unique expression in microglial cells. In the embryonic colliculus superior, the immunoreactivities of all investigated PTPs could be observed in the upper visual layers. In the optic nerve, TCPTP, SHP2 and PEST were expressed in axons, SHP1 labelled microglial cells, whereas no immunoreactivity could be detected for MEG2. Taken together, these results suggest a potential role of intracellular PTPs in the regulation of a variety of developmental processes such as retinal cell proliferation and differentiation as well as axonal growth and guidance.

# Contour integration modulates neuronal activity in monkey primary visual cortex

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Contour integration is the process which links aligned, orientated elements (e.g. Gabor patches) to the percept of a coherent contour. Anatomical evidence suggests that the primary visual cortex (V1) builds up a suitable neuronal substrate for this process [1]. Accordingly, electrophysiological studies found contour-specific effects in monkey area V1 starting with a latency of about 90 to 160 ms [1, 2]. However, monkey psychophysics suggests that contours can be integrated in much shorter time [3]. Therefore, we investigated whether contour integration also modulates early neuronal responses in V1.

A monkey (*M. mulatta*) was trained to a contour-identification task, in which S- or U-shaped contours made of aligned Gabor elements were embedded in a background of randomly oriented Gabor elements (mean distance 1.6°). The contour stimulus was followed by a mask with a stimulus onset asynchrony (SOA) of 50 or 1000 ms. Then the monkey had to report by means of a saccade the location (left / right) and shape of the contour (U / S) (4AFC-task). While the monkey was performing the task, spikes and local field potentials were recorded extracellulary in V1. To investigate contour-specific effects, two stimulus conditions were compared: The element in the receptive field of the recorded unit was either part of the contour (contour condition) or the same element was part of the background (background condition).

By comparing the contour condition with the background condition at a SOA of 1000 ms, we found a contour-specific increase, starting 30-40 ms after response onset and reaching a plateau 50 ms later. In a time window of 35-635 ms after response onset, the increase in firing rate (FR) was 13 % and the increase of γ power spectral density (γPSD) was 16 %. At 50 ms SOA, the contour-specific increase in neuronal activity starts at the same latency and with the same time course. It was interrupted by the onset of the mask and was therefore much smaller (FR: 7 %, γPSD: 1 %). Interestingly, we found comparable modulations also for Gabor stimuli, which did not match the orientation preference of the recorded unit. At both SOAs the monkey had a high performance of approx. 90 % correct responses.

Since the contour-specific effects became fairly small at the short SOA, whereas the behavioural performance stayed at high level, the question arises, whether the observed effects represent the primary neuronal correlate of contour integration. Alternatively, they could result from contour information processed at higher stages which is then fed back to V1. In line with this argument is the finding that the contour effects are independent from the match between the orientation preference and the orientation of the stimulus in the receptive field. This challenges the view that such modulations arise from long-range projections in V1 connecting orientation columns of the same orientation preference. Instead they could result from top-down influences enhancing the region representing the behaviourally relevant object in a more unspecific way.

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# Single cell responses from monkey area MT reveal a strong influence of selective visual attention on the neuronal representation of feature changes.

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Neuronal processing of visual information strongly depends on selective attention. Attention modulates neuronal firing patterns, thereby influencing basic response characteristics like average firing rate, temporal structure of responses, response variability, and stimulus selectivity. Recent results on the effect of attention on neuronal processing were mostly achieved by training awake animals on tasks requiring detection of a feature change of a target object, e.g. a change in color, orientation, form, velocity, or the like. Such changes may occur within or outside the attentional focus, and by measuring neuronal activity during visual stimulation it is possible to compare responses to visual objects depending on whether they are attended or not. For such analyses, usually neuronal activity recorded before occurrence of the relevant feature change is considered. However, from a behavioral point of view, it is the feature change itself that is most crucial for solving the task. Therefore, the questions arises how this feature change is represented in neuronal activity and whether it is influenced by attention in a similar, or different manner as during the continuous representation of the object prior to the change.

Here, we report results from a study in macaque area MT that demonstrate a strong influence of attention on neuronal activity shortly after a behaviorally relevant feature change of a moving object. Two monkeys were trained on a motion discrimination task, in which two bars were presented, each in one of the visual hemifields. Both bars could undergo a change in velocity at pseudo-random points in time, but only the change of the bar that was cued at the beginning of a trial was relevant to perform the task. PSTHs of responses to both behaviorally relevant and irrelevant objects show an increase of firing rate shortly after speed-up of the bar by approximately equal strength, reaching a maximum at about 250ms after acceleration. However, for non-attended bars activity then falls down again to roughly the value before acceleration whereas for attended bars the enhanced firing rates sustain. During this ongoing period the attention-dependent difference in firing rate reached values considerably larger then before acceleration - for both monkeys we found an increase in the Attention Index by about 40%. Moreover, an error analysis revealed attention-dependent differences in response to feature changes only for those trials, in which the monkeys succeeded in detecting the change, whereas for trials in which the change was missed no difference in the average neuronal response between attended and non-attended trials was found.

These results indicate a specific attentional mechanism involved in the task-related configuration of the neural network for optimal representation of the behaviorally relevant stimulus change.

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# Systematic latency gradients of evoked and induced cortical oscillations reflect multiple parallel bottom up and top down processes

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The dynamics of cortical gamma oscillations have been shown to be closely related to neuronal processes of sensory coding, attention and memory. As oscillations occur simultaneously in widely distributed areas of the cortex their fine spatiotemporal structure may reveal information about the coordination of multiple processing levels involved in solving a behavioural task. Stimulus evoked and stimulus induced oscillations have been shown to occur at different latencies in various areas and modalities. As visually evoked oscillations (VEO) are phase-locked and therefore more tightly coupled to the stimulus than visually induced oscillations (VIO), one would predict that VEO are more likely to reflect feed-forward processing than VIO. In contrast, VIO have longer latencies and often last longer which suggests that feedback signals might be involved. In order to test whether task-related oscillations occur in spatiotemporal patterns supporting feed-forward or feedback interactions, we examined latency gradients across simultaneously recorded transcortical field potentials from up 21 implanted microelectrodes in visual, parietal, sensorimotor and premotor areas of three macaques. The monkeys performed a visuo-motor task in which a moving stimulus had to be transformed into a trained lever position. Visual stimuli consisted of moving sine wave gratings instructing arm movements to four different target positions at which a lever had to be stabilized against the force of springs. Signal analysis was based on forward and backward band pass filtering the signals in 15 Hz bands and thresholding them with a three sigma baseline criterion. The first threshold crossing was accepted as onset latency when the threshold was crossed three times in a time window of four cycles. We observed latency gradients of VEOs in the high gamma range (60 - 95 Hz) from occipital to frontal areas while in the low gamma range (20 - 55 Hz) parietal areas led and frontal as well as occipital areas lagged. The onset of VIO was shorter in visual than in premotor / motor areas. However, a similar frequency split was observed, which divided an early bottom-up process from a later one, onset latencies differing by 20 - 30 ms.

These findings imply that VIOs are involved in processes being expressed earlier in visual than in parietal and motor areas, suggesting underlying feed-forward processes that might be a signature of transmitting visual information required for guiding the well learnt arm movements. As the latencies of VEOs in the high gamma range are shorter in visual than motor areas they might be part of early bottom up processing. In contrast, VEOs at lower gamma frequencies had shorter latencies in motor than in visual areas, suggesting that stimulus-locked oscillations in the beta and lower gamma frequency range might represent processes originating in parietal cortex which coordinate sensory and motor areas. We conclude that the complex spatiotemporal pattern of cortical oscillations appears to leave room for multiple parallel processes serving the coordination of large networks.

| | **VEO** | **VIO** |
|---|---|---|
| 20 - 55 Hz | (top down) p-m-v | 20-30ms later |
| 60 - 95 Hz | bottom up | bottom up |

# Timescale-dependent Criticality of Repeating Spatiotemporal Spike Patterns

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The concept of self-organized criticality implies that systems of interacting nonlinear elements evolve over time into a critical state in which event sizes are scale-free and can be characterized by a power-law. Recently, this concept has been applied to neural networks, and circumscribed spatiotemporal patterns of local field potentials have been shown to meet the criteria of self-organized criticality [1] and to fulfill many of the requirements expected of a substrate for information transmission and storage like high temporal precision and long-term stability [2]. To which extent these findings may also be valid for the organization of spiking activity has not been investigated yet.

We approached these issues by analyzing single unit activity from acute slices of rat visual cortex. Recordings were performed with a 32-channel extracellular electrode matrix (spacing 400 microns) yielding on average close to 100 isolated single units per session. Spatiotemporal firing sequences were detected using sliding windows with lengths of 5 to 50 milliseconds and checked for significance by common Monte Carlo methods. Additionally, longer sequences composed of temporally non-overlapping multineuronal spike patterns were assessed that lasted up to many seconds. In this way, we were able to collect thousands of statistically significant spatiotemporal firing sequences from ten slices, occurring on different timescales that span several orders of magnitude. The distributions of their spatial and temporal dimensions were then searched for signs of criticality.

The number of participating neurons rarely exceeded 7 in time-restricted patterns and never exceeded 9 in temporally unrestricted patterns, although on average nearly 100 single units had been recorded in parallel. In addition, distributions of pattern complexities clearly do not follow a power-law. The same was true for the spatial spread of activity patterns, involving rarely more than 7 electrodes and never more than 9 electrodes, respectively. The finding that pattern sizes peak around a few neurons and electrodes suggests that the neural system is far from being in a critical state concerning the spatial arrangement of repeating firing sequences, rather favoring well defined small cell assemblies, irrespective of the particular durations of the patterns.

This view changes when it comes to the temporal organization of patterns. Short sequences that had been restricted to 50 milliseconds at maximum show approximately uniform distributions of durations, covering the whole time window, with the exception of a prominent peak around 2.5 milliseconds. Longer sequences, however, display distributions of pattern durations that can be characterized by a power-law with a unique exponent of -3/2, suggesting the existence of a critical branching process operating in the temporal dynamics of living neural networks on timescales well above 50 milliseconds.

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# Changes in neuronal dynamics in AE3-deficient mice

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To study the influence of neuronal hyperexcitability on synchronization and on oscillations in stimulus induced neuronal discharges, AE3-deficient knockout mice were recorded simultaneously from multiple cortical sites with a 16-channel four-shank Michigan-probe. AE3-knockout mice are deficient for the neuronal sodium-independent electroneutral anion exchanger AE3, which substitutes intracellular bicarbonate for extracellular chloride and thereby lowers the intracellular pH. The disruption of the anion exchanger leads to neuronal hyperexcitability, which has also been associated with idiopathic generalized epilepsy in humans. To investigate neuronal assembly dynamics in response to controlled visual stimulation, multi- and single-unit activity as well as local field potentials were recorded from the primary visual cortex of isoflurane anesthetized mice. Responses to moving gratings and random dot patterns were analyzed with respect to neuronal synchrony and temporal patterning. Oscillation strength, incidence of oscillations, and strength of synchronization across separate recording sites were quantified for both types of stimuli. Both gratings and random-dot stimuli induced sustained high-frequency oscillations in local field potentials and multi-unit activity in AE3 deficient animals as well as in the control group. Compared to control animals, the knockout mice showed a higher incidence of oscillations although firing rates were, on average, slightly decreased. The oscillatory activity of the AE3 knockouts was shifted towards a higher frequency compared to controls, but the strength of oscillatory modulation did not differ between the two groups. The quantification of horizontal and vertical neuronal interactions with correlation techniques showed a dependence of synchrony on cortical depth and horizontal electrode separation. Synchrony between spatially distributed sites was significantly stronger in AE3-deficient mice than in the control group. The results show enhancement in oscillatory dynamics and synchronization in AE3-deficient mice, and support the hypothesis that AE3 plays a role in modulating neuronal excitability, thus affecting both neuronal synchronization and local generation of oscillatory responses.

# Brain activity in human albinism during a visuo-motor integration task

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Purpose: In albinism, part of the temporal retina projects abnormally to its contralateral visual cortex. This abnormal input is organised as an orderly retinotopic map of the ipsilateral visual hemi-field in the early visual areas [1] and is behaviourally relevant [2]. As this indicates that the abnormal representation is made available for visuo-motor integration tasks, we investigated whether the projection abnormality is also evident in higher-tier areas involved in such tasks.

Methods: In an event-related fMRI-paradigm we determined the brain activity (3T, Siemens TRIO; data analysis with SPM5) for a visuo-motor memory task in a subject with albinism and an age- and sex-matched control subject: While the subjects fixated a central point monocularly, a coloured target (blue/red; size: 0.5°) embedded in an array of grey distractors (element size: 0.5°; 30 elements; repositioned every 83 ms; array size 6.5°x 6.5°; array centred at 5.5° left or right from fixation) was presented for 250 ms either in the left or right visual hemi-field. Subjects were instructed to press one of four response buttons, when the colour of the fixation target changed: Left thumb, upper and lower button, for blue targets that appear in the upper and lower part of the visual field, respectively. Right thumb, upper and lower button, for red targets that appear in the upper and lower visual field, respectively. This paradigm allows one a) to localise the visual activity and thus to assess whether a misprojection of the optic nerves is present, b) to localise the motor activity and thus to test whether there are abnormalities in the motor cortex associated with the misprojection of the optic nerves, c) to tap the parietal network involved in the visuo-motor integration.

Results: In the control subject the visual activity was always dominant in the visual cortex contralateral to the stimulated hemi-field. The motor activity was dominant in the motor cortex contralateral to the responding thumb. Both bilateral activations and activations contralateral to the visual stimulation were evident in the parietal cortex. While, in the subject with albinism, there was an abnormal ipsilateral representation of the right hemi-field in the early visual areas, the lateralisation of the motor activity resembled that of the control. Evidence hints at both a normal and an abnormal representation in the parietal cortex.
Conclusions: This case-study indicates that in albinism no gross deviations from the normal lateralisation of the motor activity appear to be induced by the representation abnormality of the visual cortex. For a sound assessment of lateralisation abnormalities in the parietal cortex a greater sample of subjects has to be investigated.
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# Control of saccades towards stationary and moving targets: the role of the macaque lateral intraparietal area (LIP)

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Primates perform saccadic eye movements in order to bring the image of an interesting target onto the fovea; this target might be either stationary or moving. Yet, saccades towards moving targets are computationally more demanding since the oculomotor system must use speed and direction information as well as internal knowledge about its processing latency to program an adequate saccade. In non-human primates different brain regions have been implicated in the control of voluntary saccades. One of these regions is the lateral intraparietal area (LIP). While the tuning of LIP neurons for the saccade metric (length and direction) has been described before (e.g. Barash et al., 1991), it is not known whether LIP neurons show differential activation related to the control of saccades towards stationary as compared to moving targets.

We recorded single unit activity in area LIP of two monkeys (Macaca mulatta). First we determined the preferred saccade direction. In the following experiments saccades were always along this preferred direction. Then monkeys performed in pseudo-randomized order either (i) visually guided saccades towards different stationary targets or (ii) saccades to a target moving in or against the direction of the saccade (step-ramp-paradigm). Stationary saccade targets were located in such a way that the metrics of saccades towards moving and stationary targets were identical.

Confirming previous results, many LIP neurons showed saccade related discharges that were tuned for amplitude and direction. Yet, given identical saccade metrics, in about one third of these neurons discharge varied depending on whether saccades were followed by stable fixation or smooth pursuit. We conclude that area LIP is involved in the control of saccades towards stationary and moving targets.

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# Coupling of local field potentials in monkey primary visual cortex during a relative disparity judgment task

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Introduction. The temporal binding model states that coupling of neuronal signals on a millisecond time scale is used to flexibly bind groups of neurons into assemblies. Within this framework neurons can, for identical local stimuli, be part of different assemblies depending on the global percept. Alternatively, we might suspect that for identical local stimuli, strength of signal coupling is unaffected by the global percept and merely reflects the underlying anatomical cortical connectivity. We examined temporal binding of neuronal signals in a globally unambiguous scene-segmentation task that locally, on the spatial scale of neurons in primary visual cortex (V1), could either be ambiguous or not.

Methods. Two overlapping rectangles (6.5 deg x 2 deg) forming a cross were presented binocularly with a Wheatstone stereoscope. The horizontal rectangle was presented with either crossed or uncrossed disparity to mimic stimulus configurations with either the horizontal or the vertical rectangle in front. In the locally unambiguous condition the surfaces consisted of random-dot patterns. In the locally ambiguous condition the surfaces of the rectangles were homogeneous, i.e., task-relevant disparity information was restricted to the vertical borders of the rectangles. A macaque monkey performed a relative disparity judgment task, while local field potentials (LFP) were recorded with a 2x8 microelectrode array in the upper layers of V1. Stimuli were positioned so that half of the cRFs were located on the overlapping part of the rectangles, while the other half was located on the horizontal rectangle. The overlapping part of the rectangles and consequently the cRFs located on it could be either part of the horizontal or the vertical rectangle, depending on the relative disparity of the two rectangles. Coherence of mean free LFP was calculated for all pairs of simultaneously recorded units.

Results. In the locally unambiguous situation (unique local disparity cues) coupling strength between recording sites was reduced by 38% +- 2% (mean +- standard error, $p<.0001$, Wilcoxon Signed rank test) in the alpha- and beta-band when their cRFs were located on different rectangles (vertical rectangle in front) compared to when lying on the same rectangle (horizontal rectangle in front). This reduction was drastically attenuated (3% +- 2%) but still significantly different from zero ($p<.01$) in the locally ambiguous situation. Contrary to our expectations based on a previous study, modulation of coupling strength in the gamma-band was not observed in any case.

Conclusions. A marked influence of the local stimulus configuration on signal coupling was observed whereas coupling strength hardly covaried with the global disparity cues in cases with locally ambiguous stimuli. The observed coupling behavior could therefore be explained by selectivity of lateral connections to absolute disparity preference. Thus, our results suggest that signal coupling might facilitate the formation of certain percepts in situations with locally unambiguous stimuli, while different global percepts do not influence signal coupling in V1 when locally identical stimuli are presented.

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# Responses to orientation and color in macaque primary visual cortex in fixation and saccade tasks

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Studies investigating neural representation in visual cortex in awake primates often use stimulation paradigms where stimuli are presented in the region of the classical receptive field while the animal is fixating. This situation contrasts with natural viewing, where stimulation of visual receptive fields changes mainly due to eye and body movements of the observer. We investigated whether the responses elicited by the appearance of a stimulus in the receptive field during fixation relate to the responses to a stimulus brought into the receptive field by a saccade.

We recorded multi-unit activity from primary visual cortex of an awake behaving macaque. Stimuli were 2 degree circular square grating patches of different orientations and colors. In each trial, the monkey either had to hold fixation while a stimulus was flashed for 600 ms within the receptive field (passive viewing), or had to make a saccade to a target location, such that a stationary stimulus was brought into the receptive field by the saccade (saccade condition).

Qualitatively, responses were similar in the two viewing conditions but there were systematic differences in the dynamics and magnitude of the responses. In the saccade condition, peak firing rates were lower than in the passive viewing condition, which may be attributable to the differences in dynamics of the retinal image. However, mean firing rates in a 300 ms time interval after response onset were significantly higher ($p<0.01$, Wilcoxon test) for the saccade condition. Response latencies were similar if measured with respect to the onset of the eye movement in the saccade condition.
For most recording sites, tuning for orientation or color was similar under both viewing conditions, but selectivities for both orientation and color were slightly lower in the saccade condition compared to passive viewing.
More than one third of the recording sites showed a significant activation preceding a saccade. This activation occurred within 100 ms before saccade onset and was on average 5% of the postsaccadic response to the stimulus.

Our results demonstrate that responses to visual features such as orientation and color in primary visual cortex are qualitatively similar in fixation and saccade tasks, but there are systematic quantitative differences that indicate an influence of eye movements on the representation of visual objects.

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# Examining structural changes in mouse visual cortex underlying functional recovery after retinal lesions using chronic two-photon imaging

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The restricted removal of retinal input results in reorganization of functional topography within the deprived region of the visual cortex. Following a retinal lesion, functionally deafferented neurons become responsive to novel positions in visual space during subsequent weeks and months. In cat visual cortex, this functional reorganization is accompanied by terminal sprouting of horizontal connections in the upper cortical layers (Darian-Smith and Gilbert, Nature 368:737, 1994); however, this information has been obtained from fixed tissue. Time lapse imaging, which provides much richer information about the morphological remodeling of pre- and postsynaptic elements during this functional reorganization, has not yet been done.

To investigate the structural alterations following retinal lesions, adult mice, expressing GFP in a subset of cortical neurons, were implanted with a cranial window to allow for chronic two-photon imaging. After induction of retinal lesions by laser photo-coagulation, optical imaging of intrinsic signals was used to localize the deprived region of the visual cortex. This permitted targeted two-photon imaging to measure the dynamics of pre- and postsynaptic structural changes in both the deprived region and the surrounding visual cortex. Animals were imaged repeatedly over several weeks in order to correlate changes in morphology with the degree of functional recovery. Within the deprived region of the visual cortex, we found an increase in spine turnover. These changes were apparent within days following the lesion and they lasted for many weeks. In the subsequent months, spine turnover rate decreased back to baseline levels. In the non-deprived visual cortex, we observed turnover rates significantly smaller than in the deprived region and similar to those reported in the literature. On the presynaptic side, we found that, immediately following the lesion, turnover rates of axonal boutons inside the deprived visual cortex did not increase relative to turnover rates measured in the non-deprived visual cortex. At timepoints months after the lesion, we found an increase in bouton turnover rate in the deprived region. Our data indicate that sensory deprivation leads to changes of pre- as well as postsynaptic structures in the cortex on different time scales.

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# Firing rate and local field potential provide complementary information regarding spatial summation and centre-surround influences in primary visual cortex of the awake macaque monkey

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Neurons in the macaque primary visual cortex (V1) integrate over a limited portion of the visual field. They generally increase their firing rate if a stimulus extends beyond the classical RF (CRF). The increase in firing rate occurs until the stimulus covers the so called spatial summation field (SSF) and is due to facilitatory inputs from beyond the CRF. Stimuli extending beyond the SSF often reduce the firing rate, due to inhibitory influences from beyond the CRF. The spatially overlapping facilitatory and inhibitory regions constitute the non-classical receptive field (nCRF), which is responsible for the characteristics of spatial integration and centre-surround (CS) modulation. The features of spatial integration and CS modulation are well studied in terms of neuronal firing rates. Firing rates reflect the thresholded local computation within a single neuron. In contrast, the local field potential (LFP) provides information about incoming (feedforward, lateral, and feedback) information, as well as about local recurrent processing. It could thus yield insight into the processes that contribute to spatial integration and centre surround modulation, beyond those that can be extracted from spike rates.

To study these we simultaneously recorded LFP and spiking activity from 30 locations in V1 of macaque monkeys. Monkeys were fixating a small spot on a monitor while we presented visual stimuli in the centre and in the surround of the neurons CRF. Stimuli were stationary sinusoidal gratings optimised for the preferred orientation, spatial frequency and phase of each neuron. The SSF was estimated with circular patches of gratings of variable diameter (0.1º-4º). The extent of the surround was estimated with a circular grating of a constant diameter (6º) but with different annuli sizes (0.1º-4º). To test CS interactions we presented grating annuli surrounding a central grating patch. We analysed the power of the LFP spectra in the alpha (8-13Hz), beta (14-30Hz) and gamma (30-70Hz) frequency band. We found that large high contrast (60%) grating stimuli generally increased the LFP power in the beta and gamma frequency band in the late part of the response (250-500 ms after stimulus onset) while the alpha band was unaffected. The increase in beta power was much smaller than in gamma power. Alpha power also increased monotonically with stimulus size. Gamma power showed a sigmoidal increase with increasing stimulus size and saturated for large stimuli. Firing rates and LFP gamma power changed differently for stimuli extending the CRF. Firing rates decreased, showing the well known effect of surround suppression, which was not observed for gamma power. Importantly gamma power did not increase relative to baseline when the surround was stimulated alone. Thus the increase of gamma power with increasing stimulus size (and centre surround configurations) reflects a mutual interaction between local CRF and nCRF processes, and cannot be accounted by activation from beyond the CRF alone. (supported by Wellcome Trust and BBSRC).

# Long-Range Interactions in Cat Visual Cortex during Natural Stimuli Processing Investigated by Voltage-Sensitive Dye Imaging

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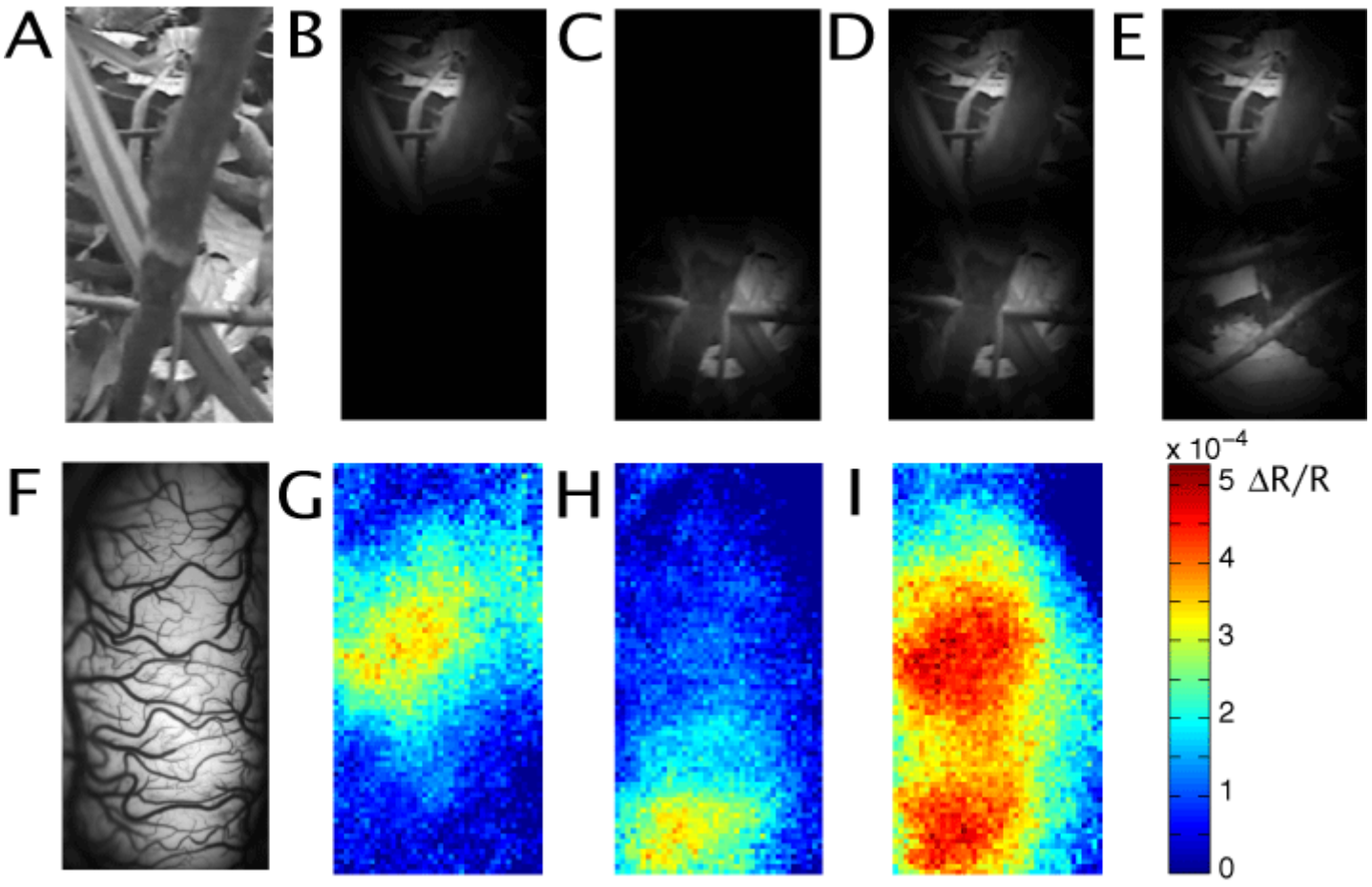
To understand processing of natural stimuli it is essential to unravel neuronal interactions within cortical areas. Here we investigated the dynamics of long-range cortical activation by using voltage-sensitive dye imaging in cat visual cortex upon presenting natural environments.

Movies recorded with a camera mounted on the head of a freely moving cat served as complex visual stimuli (Fig. A). To systematically study space-time dependent activation profiles, movies were presented either through one or two local gaussian apertures (Fig. B-E) that largely exceeded typical receptive field sizes. Through the two apertures either the same video (congruent, Fig. D) or two different videos (incongruent, Fig. E) were visible. Neuronal activity was recorded by imaging the signal of voltage sensitive dye applied to primary visual cortex under anesthesia (Fig. F).

Our results show local cortical activation patterns induced by each stimulus segment (Fig. G-H). When presenting both apertures simultaneously, a facilitatory effect on neuronal activity was observed (Fig. I). Furthermore, compared to incongruent conditions this effect was slightly larger for congruent segments in which the movies had similar spatio-temporal characteristics.

In summary, the modulatory effect of complex natural stimulation outside classical receptive fields is facilitatory and dependent on integration of specific spatio-temporal context, i.e. stimulus congruence.



# Orientation tuning of the local field potential and multi-unit activity in the primary visual cortex of the macaque

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Oscillations in the local field potential (LFP) are abundant across species and brain regions. The possible relationship of these low-frequency extracelluar voltage fluctuations with the activity of the underlying local population of neurons remains largely elusive. To study this relationship, we used an array of chronically implanted tetrodes spanning a distance of 700 µm and simultaneously recorded action potentials from multiple well-isolated single units, multi unit activity (MUA) and LFP from area V1 of the awake, behaving macaque. Moving and static gratings of different orientations were used for visual stimulation.

In agreement with previous studies we find that the increase of LFP gamma-band power is a function of the orientation of the stimulus. However, the power of the gamma-band contains much less information about the orientation of the stimulus than the MUA and SUA recorded at the same site (Figure 1A). The average discriminability d' between preferred and orthogonal orientation was 2.46 for MUA, 2.45 for SUA and 1.01 for the LFP. Moreover, in contrast to recent results from area MT (Liu and Newsome, 2006) we find only a weak correlation between the preferred orientation of the MUA tuning function and that of the LFP (Figure 1B, different colors indicate different animals). Interestingly, all nearby LFP recording sites in our array were tuned to a similar orientation while the preferred orientations of MUA tuning functions were widely scattered. These results suggest that the power of LFP signals does not capture local population activity at the scale of orientation columns in area V1.

Literature:
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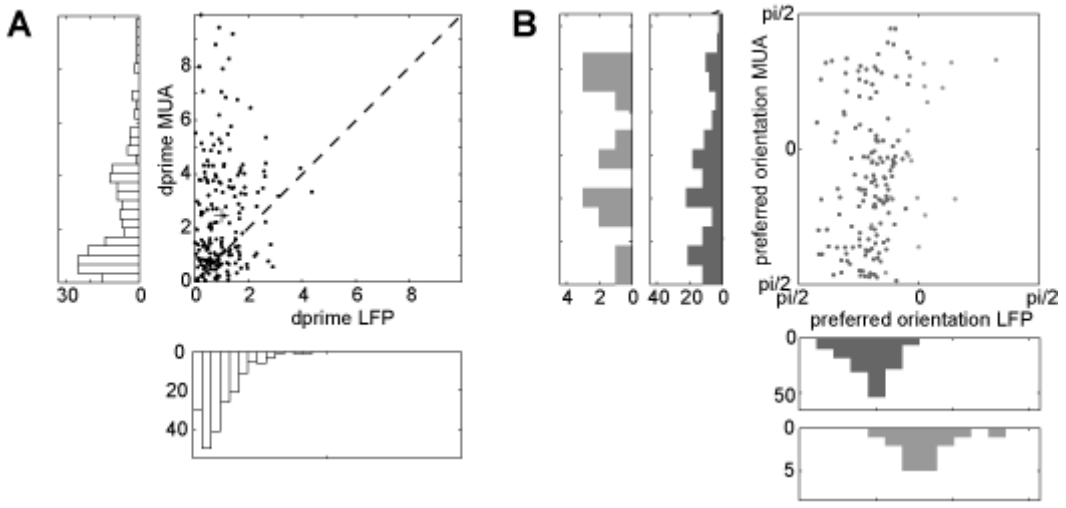

# Topological tree-analysis of the microvascular system in macaque visual cortex

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For a profound understanding of functional brain imaging in research and in clinical applications, investigations of neurovascular coupling are mandatory. Three-dimensional tree-analysis of cortical vasculature elucidates the structural aspects of neurovascular coupling such as the organization of the cortical vasculature and network topology. Here we report a technique to obtain high resolution tomographic images of the cerebral vasculature, accurate reconstructions of the whole vasculature and extraction of vessel attributes to reliably quantitate large vascular networks. Non-human primate (Macaca mulatta) brains were collected and processed. Samples were punched from the primary visual cortex and scanned at the material science beamline of the Swiss Light Source to yield X-ray tomographic images for 3D reconstruction of the vasculature. Key vessel parameters have been evaluated for different levels of analysis (from single samples to grouped data). The diameter and length distributions of the cortical vessels indicated a high percentage of capillaries. Layer 4cβ had the highest density of capillary and noncapillary vessels in comparison to the other cortical layers. Mean volume fraction was 2.5% for cortical gray matter. Extravascular distance measure yielded an average mesh size of 56 μm. Branching pattern analyses have been performed for single vessels extracted from whole networks for investigation of network geometry. In conclusion, these results indicate the reliability of the technique in studying cortical vasculature. The results were in good agreement with histological data as well as with data from the literature. Quantitative three-dimensional morphometry of vascular networks is critical for future blood flow modeling in the cerebral cortex.

# Retinotopic activation of macaque area V2 without input from primary visual cortex.

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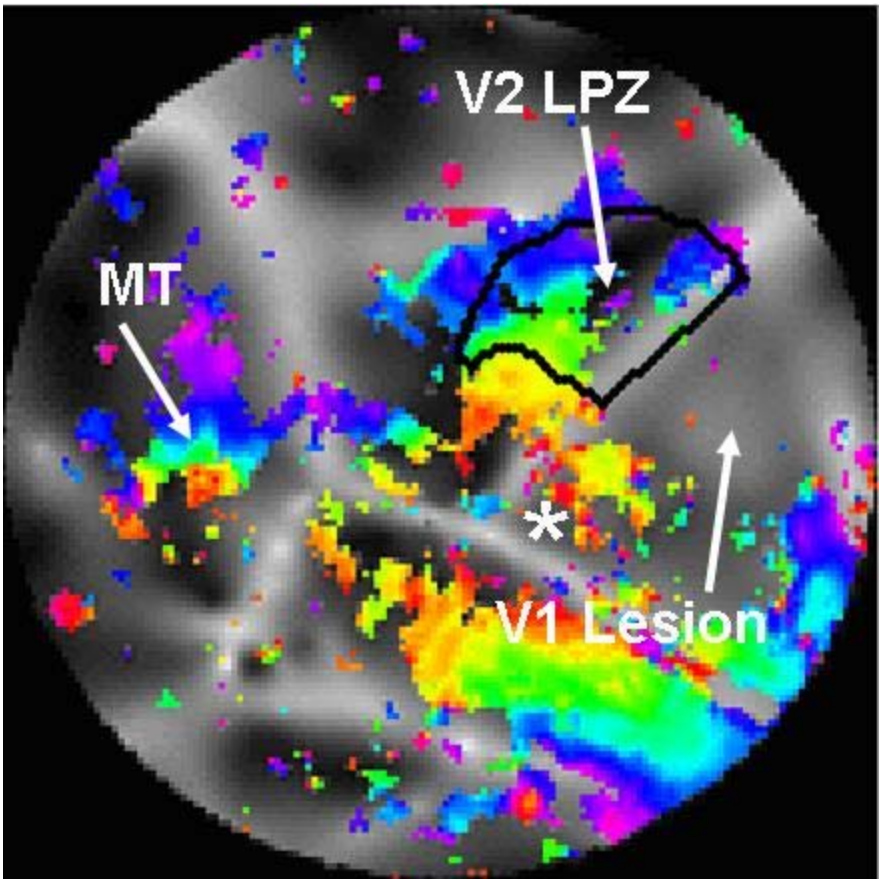
The presence of focal lesions in primary visual cortex (V1) provides the opportunity to study the role of extra-geniculo-striate pathways for activating extra-striate visual cortex. Previous studies in the macaque have shown that cells in area V2 stop firing after reversibly cooling V1 (Girard and Bullier, 1989; Schiller et al., 1974). However no studies on long term recovery after V1 lesions have been reported in the macaque. Here we use fMRI of the macaque monkey brain to study the organization of V2 from baseline levels up to 16 months post-lesioning. We find that BOLD responses in the lesion projection zone (LPZ) of area V2 are reduced by 80 % compared to pre-lesion levels. Surprisingly the retinotopic organization inside the area V2 LPZ is similar before and after inducing the V1 lesion, suggesting that V2 activation is not the result of input arising from nearby non-lesioned V1 cortex. Monitoring of the activity over time after the lesion did not reveal systematic changes in signal amplitude near the LPZ border. We conclude that visually driven activation of extra-striate area V2 as revealed by the BOLD signal is 1) significantly reduced, but still present after depriving it of V1 input, 2) the area V2 LPZ largely retains its original retinotopic organization, and 3) the strength of visual modulation inside the LPZ does not seem to increase significantly up to 16 months post-lesioning. We discuss our findings in the context of parallel pathways in the brain which can activate V2 in the absence of V1 input and may contribute to the behavioral phenomenon of blindsight.



# The role of intracortical connections and thalamocortical synaptic depression in generating responses to masking stimuli in cat primary visual cortex

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The goal of this study was to investigate the mechanisms by which neuronal responses to a preferred stimulus are altered by the addition of a second 'masking' stimulus. Determining the source of this response nonlinearity can give insight into how subcortical input and local intracortical connectivity interact to shape the output of cortical neurons. One candidate mechanism is depression of the thalamocortical synapse, which models have shown can reproduce some key features of masking suppression (Carandini et al. (2002) J. Neurosci. 22:10053-65). Since neurons in the lateral geniculate nucleus of the thalamus (LGN) are not orientation selective, suppression with this mechanism should be observable for all orientations of the masking stimulus, including the optimal orientation for the recorded cortical neuron. We have tested this prediction by performing experiments to compare responses when the mask and test stimulus are both at the preferred orientation of the neuron (iso-oriented masks) and when the masking stimulus is at 90 degrees from the preferred orientation (cross-oriented masks).

We made extracellular recordings from 61 neurons in the primary visual cortex and 32 neurons in the LGN of anesthetised cats. Iso-oriented test and mask stimuli elicited responses ranging from suppression to facilitation in cortical neurons. However, the majority of these neurons also showed significant response suppression when cross-oriented masking stimuli were presented. As expected, responses in LGN neurons were suppressed or not affected by superimposition of masking gratings at either orientation. This argues against a sub-cortical origin for response enhancement seen with iso-oriented masks.

We then investigated whether the simple feedforward model of Carandini et al. could describe the results observed with iso-oriented masks. In simulations performed using this model, we could not obtain the facilitation seen with iso-oriented masks and suppression for cross-oriented masks observed experimentally. We expanded on this model by including cortical inhibitory input and recurrent cortical excitatory input to the model V1 neuron. This recurrent model produced enhancement of responses as seen with iso-oriented masks in V1 neurons, as well as the suppression observed in responses to cross-oriented masks. In the recurrent model thalamocortical synaptic depression still produces suppression with masking gratings, but intracortical excitation and inhibition provide a stable amplification of response for iso-oriented, but not cross-oriented masking stimuli. These results show that intracortical mechanisms play an important role in explaining responses to masking gratings. Supported by FP6-2005-015803.

# Functional architecture of horizontal connections in the cat primary visual cortex

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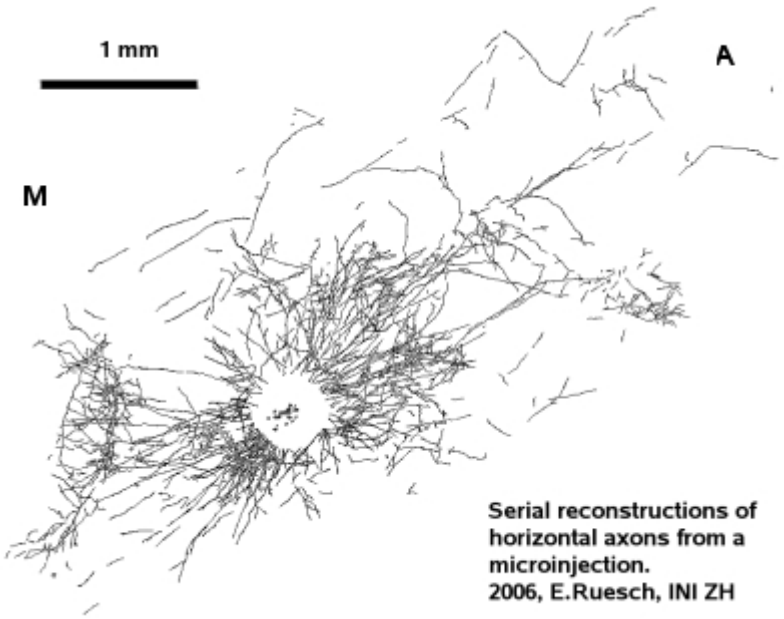
The neocortex of higher mammals like primates and cats has a lattice-like network of local circuits embedded within inter-areal connections, which form the "daisy architecture" (DA). Our hypothesis is that the DA supports self-organized, context-dependent processing in all cortical areas. In this study we examine the relation of the DA to key features of the functional architecture of the cat primary visual cortex. The functional architecture of the map of orientation selectivity in the primary visual cortex was revealed by high spatial resolution optical imaging of the intrinsic signal associated with cortical activity. Small clusters of neurons and single cells in different parts of the orientation map were labeled by micro-injections of the tracer biocytin and intracellularly filled with HRP respectively. The pattern of axonal projections and distribution of boutons of the labeled cells were revealed and correlated with the functional map of orientation selectivity. Our results indicate that the DA formed by these axonal projections are less specific than it would have been expected from other studies using not micro-injections.



# Lateral connections in cat's area 17

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In the cortex excitatory neurons form clusters of connections within their own layer. These lateral connections represent the vast majority (almost 40 percent) of excitatory synapses in layer 3 (Binzegger et al., 2004). In layer 4 the recurrent cortical connections also dominate the feedforward thalamic input, which represents no more than 5 percent of the excitatory synapses in layer 4. Thus, the cortical lateral connections play an important role in the cell's response to visual stimuli. In this study we report that silencing even a small fraction of the input a cell receives can shift the orientation tuning of the cell. We recorded extracellularly from 120 cells located throughout the depth of area 17 in 22 anaesthetized and paralyzed cats. Orientation tuning curves were plotted quantitatively before and during gama-aminobutyric acid (GABA) inactivation of a cluster of cells lying approximately 0.5 mm lateral to the recorded cells.

About 17% of the cells (n=21) tested showed shifts in a portion of their tuning curve when part of their lateral input was silenced with GABA. The tuning width was usually preserved. The median shift magnitude was 7.5 degree (21 cells). The preferred orientation at the inactivation site was similar (always <65 degree and <40 degree for 12/15 (80%) cells) to the preferred orientation of the recorded cell. The effect was observed when the inactivation and the recording were done in the same layer only. The present results show that the orientation preference is strongly influenced by lateral connections in all layers tested. These connections offer the means by which the sensitivity of neurons can be changed dynamically. It will be interesting to discover whether similar effects can be detected in optically imaged orientation maps. Supported by FP6-2005-015803.

# Poster Topic

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**T17-2A** Statistics of eye movements of monkey freely viewing natural scenes.
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**T17-3C** Properties of the pursuit-related activity recorded from primate frontal eye field
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**T17-5C** Using SE-EPI to Measure Visual Responses in the Awake Macaque at 7Tesla
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**T17-6C** The role of color in natural images to recognition performance and neural activity in extrastriate and prefrontal cortex
*S. Liebe, N. Logothetis and G. Rainer, Tuebingen*

**T17-7C** Neural encoding of species dependent face-categories in the macaque temporal cortex.
*GR. Sigala Alanis, K. Nielsen, N. Logothetis and G. Rainer, Tuebingen*

**T17-8C** OKR assay and fundoscopy: new tools for adult zebrafish vision research
*M. Tschopp, O. Biehlmaier and S. Neuhauss, Zürich (CH)*

# Optical aberrations in barn owl eyes

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The visual system of barn owls is in several ways special compared to other birds and animals. The visual performance of barn owls is hyperacute in the binocular and monocular domain, both demonstrated in behavioural acuity tasks, i.e. stereo and vernier acuity. We were interested to find out to what extent spatial resolution in this bird is limited by the optical properties of their eyes. For that purpose eight eyes from 4 adult American barn owls (*Tyto alba pratincola*) were studied. Ocular aberrations were measured to the $6^{th}$ - order Zernike polynomials using a Schwind Hartmann-Shack system. Owls were awake and slightly retained for measurements, their pupils were normal. Preliminary results indicate mild hyperopia and few higher order aberrations.

# Statistics of eye movements of monkey freely viewing natural scenes.

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Eye movements have been extensively studied under experimental conditions where the task required precise eye movements to defined target locations. More recently, attention has been drawn to eye movements during the exploration of complex visual scenes [1]. To identify possible mechanisms underlying the choice of the positions of fixations, we investigated the eye movements of a freely viewing monkey during the exploration of different images of natural scenes [2,3,4]. We extracted the eye fixations during 3s of image presentations (on average 7±1.4 per image) and determined the respective fixation positions within the images.

Interested in the image features that correlate with fixation positions, we analyzed their relation with (1) saliency maps of these images and (2) conspicuous objects present in the natural scenes. To quantify the correlation of the distribution of fixation positions with low level features in the images as measured by saliency maps, we calculated the Kullback-Leibler Distance (KLD) of the two. In 7/11 images the KLD was signifcantly smaller than expected for random viewing suggesting a tendency for feature guided fixations. However, the correlation analysis of fixation positions with objects present in the images showed that in 10/11 images the number of fixation positions within objects was significantly higher than expected by chance. This rather suggests a dominant cognitive component guiding the eye movements.

In a next step, we took into account the spatial sequence of eye movements. Using a mean shift algorithm we first determined clusters with a high density of fixation positions. This resulted in 3 to 5 significant clusters per image, potentially indicating regions of interest in the individual images. Then we investigated the scan path by applying a Markov chain analysis by assigning to each cluster of fixations one Markov state. Our analysis revealed a non-random processing of the images. The animal was primarily making saccades within a cluster (local exploration), and with lower probability changes to a different cluster (global exploration).

Future research will be directed to the investigation of the neuronal activity recorded in these experiments, with focus on the various LFP components in relation to object exploration in the free viewing condition.

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[2] Maldonado, Babul, Singer. Soc. Neurosci. Abstr.: 558.8, 2002.
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[4] Flores, Berger, Gruen, Maldonado. Soc. Neurosci. Abstr.: 165.7, 2005.

Acknowlegements: Supported in part by the BMBF (grant 01GQ0413 to BCCN Berlin), the Volkswagen Foundation, the Iniciativa Cientifica Milenio, the Stifterverband fuer die deutsche Wissenschaft and the Honda Research Institute, Offenbach, Germany.

# Neuronal responses of the nucleus of the basal optic root (nBOR) and visually elicited head nystagmus during horizontal optokinetic stimulation in the zebra finch (Taeniopygia guttata castanotis)

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Detection of moving objects in the environment is one of the key features for visually guided behaviour. Therefore, it is essential for animals and humans to discriminate between external motion and visual changes resulting from self-movement. In the present study, the head nystagmus and the neuronal properties of the nucleus of the basal optic root (nBOR) within the accessory optic system (AOS) have been observed seperately while pretending a self-motion situation. Electrophysiological responses of neurons of the nucleus of the basal optic root (nBOR) of the accessory optic system (AOS) were examined during horizontal visual large-field stimulation. BOR neurons were excited by slow moving stimuli (14 °/s) of a particular direction and were inhibited by the opposite direction. Most neurons (24, n=34) were driven monocular by the contralateral eye, whereas just a small amount (10, n=34) was innervated from both eyes. In most cases, monocular cells responded to stimuli moving in the nasal to temporal direction in the contralateral visual field (19, n=24). These observations are in accordance to earlier studies performed in other avian species but have not been shown in zebra finches so far.

Maximum head nystagmus frequencies during horizontal optokinetic stimulation showed strong asymmetries under monocular conditions with significantly higher maximum frequencies ($p<0,05$, n=12) for a temporal to nasal direction (109 °/s) compared to the opposite direction (49 °/s) of stimulus movement. This leads to a higher temporal resolution for objects appearing into the visual field in the horizontal plane. Thus, this mechanism might play an essential role in faster predator detection, which is especially important for the zebra finch as a bird of prey.

# How to read a pigeon's mind: Pecking density as an indicator for relevance of visual features

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Pigeons are able to discriminate a vast variety of visual stimuli in operant conditioning experiments. However, in most cases it is unclear which strategy they use to solve a certain visual task, as in many experiments stimuli can be discriminated unambiguously by several different features. In search of a tool to identify the relevant features that the animals use in such tasks, we examined the pecking locations of 11 pigeons in two different forced-choice-experiments.

Both experiments have been designed in a way that only one small, well defined area can be used to identify a rewarded stimulus. However, pecking anywhere on this stimulus was reinforced. The two experiments differed in terms of the nature of the used stimuli: five pigeons were trained to discriminate between abstract geometric shapes, six pigeons were trained to discriminate photographs of humans from photographs without humans. Pecking locations were determined using a touch screen monitor. In both experiments the pecks started to cluster around the critical area as soon as the pigeons performed above chance level. This pecking behaviour may serve to identify a relevant feature also in stimuli in which this feature is not predefined. Therefore it may provide a highly valuable tool for the investigation of the pigeon's recognition system.

# What ERPs tell us about the perception of a figure defined by multiple visual cues

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A central aspect in the processing of a visual scene is the separation of a figure from its background. In most cases this separation is based on multiple cues. While psychophysical studies have demonstrated that both detection and identification of a figure are enhanced by an additional cue, little is known about the underlying neural mechanisms. In the present study we investigated the relationship between psychophysical data and event-related potentials (ERPs) in a figure identification task. We tested how ERPs are influenced by (1) the perceptibility of a figure and (2) the combination of visual cues. Stimuli consisted of a matrix of Gabors, where the Gabor elements of the figure differed from the background either in orientation, spatial frequency, or both (cue combination). Subjects had to distinguish between two mirror-symmetrical figures, which was complicated by randomization of the figures' position and orientation. Three levels of difficulty were used in each condition. As expected, a better perceptibility is reflected by a negative shift in the ERP influencing the posterior P2 and N2 components. Cue combination results in a further negative shift of the P2 component as well as a positive shift after 400 ms most prominent over parietal and parieto-occipital electrodes (see figure). Although all subjects had the highest identification-benefit from cue combination in the most difficult perceptibility condition, the negative shift is most prominent in the easiest level. In general, we find a good correspondence between the subjects' performance and our electrophysiological results.

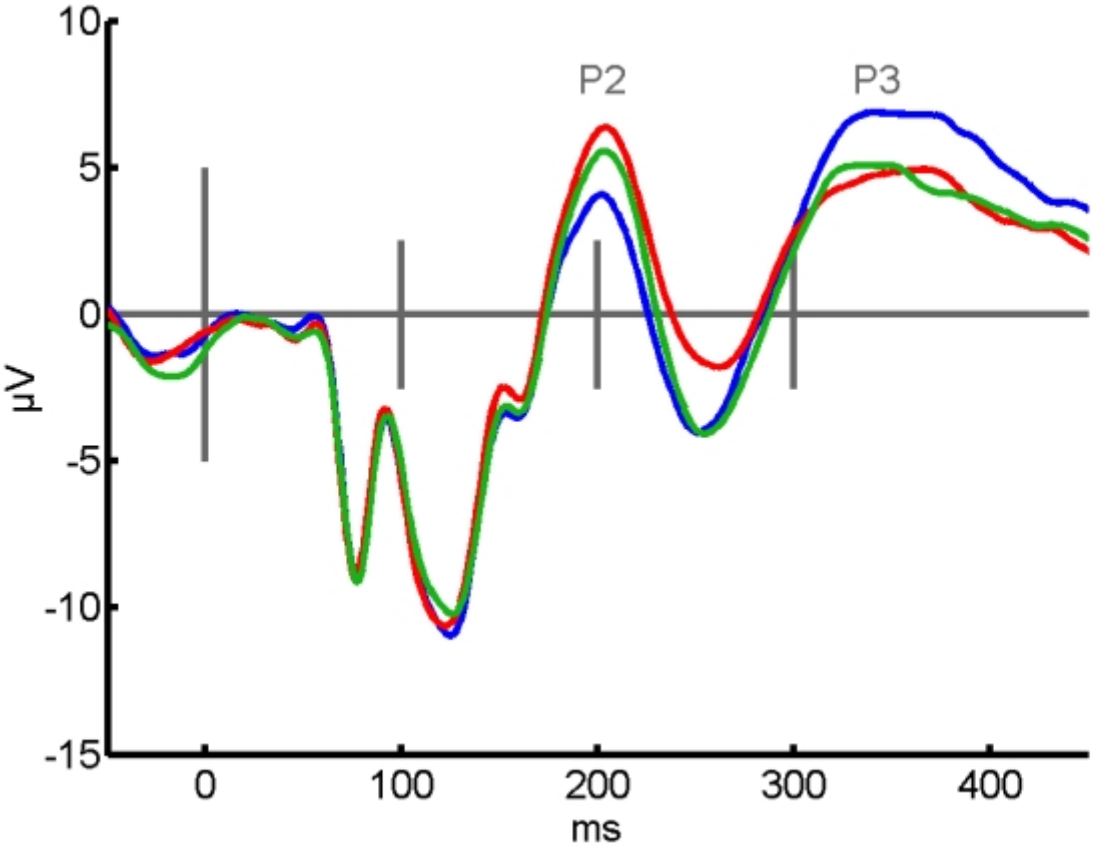


**Figure: Grand Average (n=5) ERPs at the POz electrode in the following conditions: spatial frequency (green), orientation (red) and cue combination (blue). Cue combination shifts the P2 component negatively and P3 component positively**

# Prism adaptation in a patient with damage to the right parietal cortex - A case study

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Prism adaptation demonstrates that sensorimotor representations in the brain can be altered rapidly in an experience-dependent way to ensure high flexibility of human motor behavior. In the present study, we examined the ability to adapt to a prism-induced shift in sensorimotor control in a patient (TB) with extensive damage to the right posterior parietal cortex, a neural structure involved in sensorimotor integration. The task was to perform ballistic pointing movements towards a visual target using the right arm. The precision of movements was assessed using a high-resolution ultrasound device. The prisms applied shifted the visual image horizontally by 17 deg either to the left or to the right. TB's general pointing accuracy (baseline condition, prisms off) was not impaired. However, we found striking differences in the adaptation process and the magnitude of the aftereffect when comparing leftward versus rightward shifting prisms. Whereas the initial prism effect was rather small for the leftward shifting prisms, a marked prism effect was observed with the rightward shifting prisms. Conversely, while an aftereffect emerged upon removal of the leftward shifting prisms, no aftereffect occurred after adaptation to the rightward shifting prisms. When we tested spatial generalization of adaptation from one target location to another, TB showed complete transfer for the leftward shifting prisms but had to adapt anew when wearing rightward shifting prisms. The same pattern of results was observed when testing transfer of adaptation from one eye to the other. Our results indicate that while rapid trial-by-trial error correction was observed for both directions of optical displacement, an adaptive spatial realignment did not take place for the rightward shifting prisms since no aftereffect occurred here. The asymmetry of effects suggests a specific contribution of the right posterior parietal cortex to the realignment of sensorimotor maps to rightward displaced vision.

# The effect of adaptation duration on facial aftereffects

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Adaptation processes in human early visual cortical areas are sensitive to the exposure time of the adaptor stimulus. The goal of the present study was to explore short- and long-term adaptation effects at the higher, shape-specific stages of visual processing using facial adaptation. It was shown that long-term (5 sec) adaptation to a female face evokes aftereffects consisting a translation-invariant (TI) as well as a position-specific (PS) component: the test faces were judged more masculine when they were displayed in the same location as the female adaptor face (overlapping - OL condition) as compared to that when they were presented in the opposite visual hemifield (non-overlapping, non OL condition). On the other hand, when the adaptation duration was 500 ms the resulting facial aftereffect was found to be completely TI and no PS adaptation effects were observed. In accordance with these behavioral results, we found that the adaptation effects, measured on the amplitude of the N170 ERP component consisted of a PS component only after long-term, but not after short-term adaptation conditions.

These results suggest that both short- and long-term exposure to a face stimulus leads to adaptation of position invariant face-selective processes, whereas adaptation of position-specific neural mechanisms of face processing requires long term adaptation. Our findings imply that manipulating adaptation duration provides an opportunity to specifically adapt different neural processes of shape-specific coding and to investigate their stimulus selectivity.

# Stimulus-dependent gamma oscillations in monkey V1 and its modulation by expectancy.

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Synchronous gamma oscillations have been associated with cognitive processes demanding selective attention such as, figure-ground discrimination, visual search and recognition of shapes. Most of the evidence obtained so far comes from studies in area V4. Here we have applied a cueing paradigm while recording activity at multiple sites in V1 of the behaving monkey.

Initially, we have used drifting gratings and plaids to determine the dependence of gamma oscillations on stimulus characteristics, such as spatial frequency, speed and direction of movement. Simultaneous recordings were obtained from V1 in four monkeys trained to respond to a change in fixation point color, while the stimulus was presented over the receptive fields. Responses to different stimuli were compared using sequences of single and superimposed gratings (plaids) within the same trial. We analyzed the power spectrum and coherence of the spiking activity and the local field potential. Strong oscillatory activity was evoked by the gratings, both at the single cell and population level. Adding a second component (plaids configuration) led to a redistribution of frequencies with the appearance of components at higher frequencies.

In our paradigm, the duration of the trials and the stimulus sequence were fixed. This allowed the monkey to estimate the timing of fixation point change, for which a motor response was required. We observed that gamma power increased strongly for a stimulus presented at the end rather than at the beginning of the trial. Interestingly, the observed increase in strength was specific to the main oscillation frequency component induced by a particular stimulus. In a second series of experiments, the stimulus was kept constant and a cue was introduced to signal the timing of fixation point change. This was accomplished by incrementing slightly the luminance of the fixation point 800 ms prior to its change in color at different epochs of the trial. Our cueing paradigm led to a precisely timed increase in stimulus induced gamma power, suggesting that gamma activity is involved in tasks for which expectancy and attention are relevant.

# Sensory responses in different layers of the neocortex in vivo

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Laminar processing of information in the neocortex is believed to contribute to the exceptional information processing capability of this part of the brain. Neuro-anatomical data suggest that information flow is directed from the input layer IV to layers II and III, where most of the intra-area processing takes place. The processed signals are then most likely transferred to layer V and VI, where cells with long-range connections to other brain areas are commonly found. Electrophysiological studies have revealed increasing levels of complexity of optimal stimulus features along this putative information processing path (Hirsch and Martinez 2006). Direct observations of activity spreading through the layers of the neocortex, however, have not yet been reported. To date, the information about differences in the activity in different layers is limited to single cell recordings or relatively course estimations of the in vivo firing rates in different layers (Gur et al. 2005; Haupt et al. 2005).

In the present study, we investigated the spatio-temporal structure of activity spreading in the rat visual cortex in vivo. We recorded from a 3 x 4 array of extracellular electrodes arranged in a plane perpendicular to the cortical surface and used optical stimulation to elicit sensory responses. To reveal the exact positions of the recording sites, silver was deposited from the electrode tips after the recording session and stained post mortem by means of a silver enhancement staining.

Analysis of multi-unit spike signals confirmed previous findings about layer-specific mean firing rates in the neocortex. More importantly, the response latencies to visual stimulation differed between layers, as well as the frequency of rate oscillations after stimuli suitable to elicit oscillatory activity. Our data suggest that differences of signal processing in different layers may be reflected in different firing rates and the spatio-temporal pattern of stimulus-evoked activity in primary sensory areas of the brain. Studying the activity dynamics with respect to cortical layering will help to better define the roles of different layers for information processing in the brain.

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Haupt SS, Spengler F, Husemann R, Dinse HR (2004) Exp Brain Res. 155(4):485-99.
Hirsch JA, Martinez LM (2006) Curr.Opin.Neurobiol. 16, 377-384.

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# Visual evoked potentials (VEP) stimulated by contrast and flash modulation from awake, freely moving rats

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Purpose: The assessment of visual pathways can be achieved over time by VEP recordings. The aim of the study was to establish a chronic implant model, which enables us to measure VEP from awake, freely moving rats. Stimulating the entire visual field has the advantage of being independent on refractive and attention. In addition we wanted to test influences of different anaesthetics and the effect of optic nerve crush and retinal ischemia.

Methods: Potentials were recorded in Brown-Norway rats by screw electrodes implanted epidurally over the visual cortex. Reference electrodes were placed over the frontal brain. Fourier analysis was used to extract the VEP from background EEG activity to the calculate vertical noise free amplitudes. Stimulation modalities comprised series of flashes with a frequency range of 1.8-38 Hz with contrast 100% and series of 7.5 Hz-flicker with a modulation depth from 1%-80%, stimulating the entire visual field of the rat with a ganzfeld bowl. To evaluate anaesthetics, Isoflurane was applied vaporized in oxygen in concentrations of 0.2% to 1.0%. Choralhydrate 200 and 400 mg/kg and the combination of Ketamine 65 or 130 mg/kg and Xyaline at 7 or 14 mg/kg respectively were given intraperitoneally. If recordings were performed for each eye separately, one eye was blinded by aluminium foil and eye ointment containing coal particles. To evaluate effects of retinal ganglion cell damage the optic nerve was crushed approximately 2 mm behind the eye for 10 s after performing a partial orbitotomy. Retinal ischemia was achieved by elevating the intraocular pressure for 60 minutes above systolic blood pressure.

Results: VEPs with responses up to 16.1±1,1 µV (mean±SEM) depending on stimulation parameters were detectable over at least 10 days. With decreasing modulation depth the VEP amplitudes decreased; the frequence tuning curve showed a bimodal shape with an maximum amplitude at 18.75 Hz. When one eye was blinded, the potentials recorded from the contralateral side were not affected, and the potentials of the ipsilateral side were markedly reduced. Isoflurane depressed the VEP with increasing concentrations. Chloralhydrate and Ketamine/Xyaline acted differently depending on concentrations. After optic nerve crush neither flashes of different frequency, nor flicker stimulation of different modulation depth evoked significant potentials. After 60 minutes of retinal ischemia the potentials remain detectable, but markedly decreased in comparison to the control.

Conclusions: This new VEP methology allows to monitor the visual system of rats over an extended period. Compared to checkerboard stimuli our paradigm has the advantage of being independent on the refraction of the eye. Narcotics change VEP amplitudes and were not necessary. VEP after retinal ischemia is able to quantify functional damage of retinal ganglion cells. Hence, this new technique allows the evaluation neuroprotective substances or treatments on visual function.

# Neural correlates of speed illusions in area MT of the macaque monkey

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The speed of visual motion is not always perceived veridically. For instance, a particular motion presented within a small aperture often appears faster than the same motion presented within a large aperture. Little is known about the neural basis of such misperceptions.

We performed extra-cellular single unit recordings from direction-selective neurons in the middle temporal area (MT) of two macaque monkeys. The animals were trained to fixate a small dot on a computer monitor until it changed its luminance while a behaviorally irrelevant random dot pattern (RDP) was moving within a stationary virtual aperture placed in the receptive field (eccentricity 5 to 20 deg) in the preferred or null direction of the neuron under study.

We first determined the tuning curve for speed of each neuron and then selected a stimulus speed from the steeply sloped part of the tuning curve. Here small changes of speed cause large changes in neuronal responses and therefore the neuron is particularly well suited to contribute to the perception of such speeds. To test for an effect of stimulus size on the neuron's responses, we used RDPs of three different sizes (25, 50 and 100% of the receptive field diameter) moving at the selected speed.

We used an ROC analysis to determine differences in the firing rate distributions in the three size conditions. We observed that responses to smaller stimuli were significantly higher in cells where the selected speed lied on the ascending portion of the speed-tuning curve (i.e. where higher speeds caused higher responses), and responses decreased significantly when the selected speed lied on the descending portion of the speed tuning curve. In other words, choice probability was significantly different from 0.5, indicating a choice bias in favor of the smaller stimulus when determining the faster of two stimuli.

We have previously reported that for smaller stimulus sizes preferred speeds of MT neurons tend to be lower (Society for Neuroscience Meeting, USA, 2005) which similarly agrees with the predictions based on the psychophysically observed speed illusion. Taken together the results indicate a contribution of MT neurons to the misperception of speed based on stimulus size.

# Categorization of visual scenes based on low-level image statistics

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Humans can correctly categorize visual scenes with presentation times of 100 ms or less. How can this remarkable performance be accomplished? Behavioral evidence suggests that the perceptual speed may be accounted for by the exploitation of low-level differences between man-made and natural scenes. Spatial frequency contents along different orientations present a plausible and easily extractable source of such information to the visual system. Especially the significant difference in orientation energy along the cardinal axes between man-made and natural scenes (Torralba & Oliva, 2003) could provide enough information to a feed-forward system making a first best guess of the depicted environment. To test the contribution of such early visual processes we employed an adaptation and image-filtering paradigm.

We hypothesized that adaptation to an artificial stimulus of spatial frequency contents matched to that of man-made scenes might modify the perception of a subsequently viewed real world image. Subjects viewed grayscale images of natural and man-made scenes. During the adaptation paradigm they were preceded by an adaptation sequence of rectangles approximating the statistical properties of man-made scenes. In a variety of experimental conditions, observers were asked to rapidly decide whether one of two or four simultaneously presented images depicted a man-made or natural scene. These images were presented for 12 ms; followed by neutral mask. Following the adaptation, observers' performance (i.e., reaction time and percent correct) to detect the natural image among distracting images did not change significantly while the detection of man-made images was severely impaired. We suggest that this results from a recalibration of the visual system to the global spatial frequency profile of man-made environments, thus allowing one to make use of statistical deviations from the newly calibrated mean during rapid categorization.

In addition we show that the selective filtering of orientations along the cardinal axes of man-made scenes mimics the adaptation process. Instead of relying on adaptation in a second experiment we directly manipulated information along the cardinal axes of man-made scenes. Images of both categories were filtered reducing the orientation energy along the cardinal axes by 50, 75 or 100%. Observers rated these filtered test image in the absence of adaptation. Performance for correctly categorizing man-made images significantly decreased as a result of degrading information along the cardinal axes via image filtering. The categorization of natural images was unaffected. These observations present more evidence for low-level vision involvement in fast image categorization.

# Modulation of feedfoward response priming by endogenous and exogenous attention

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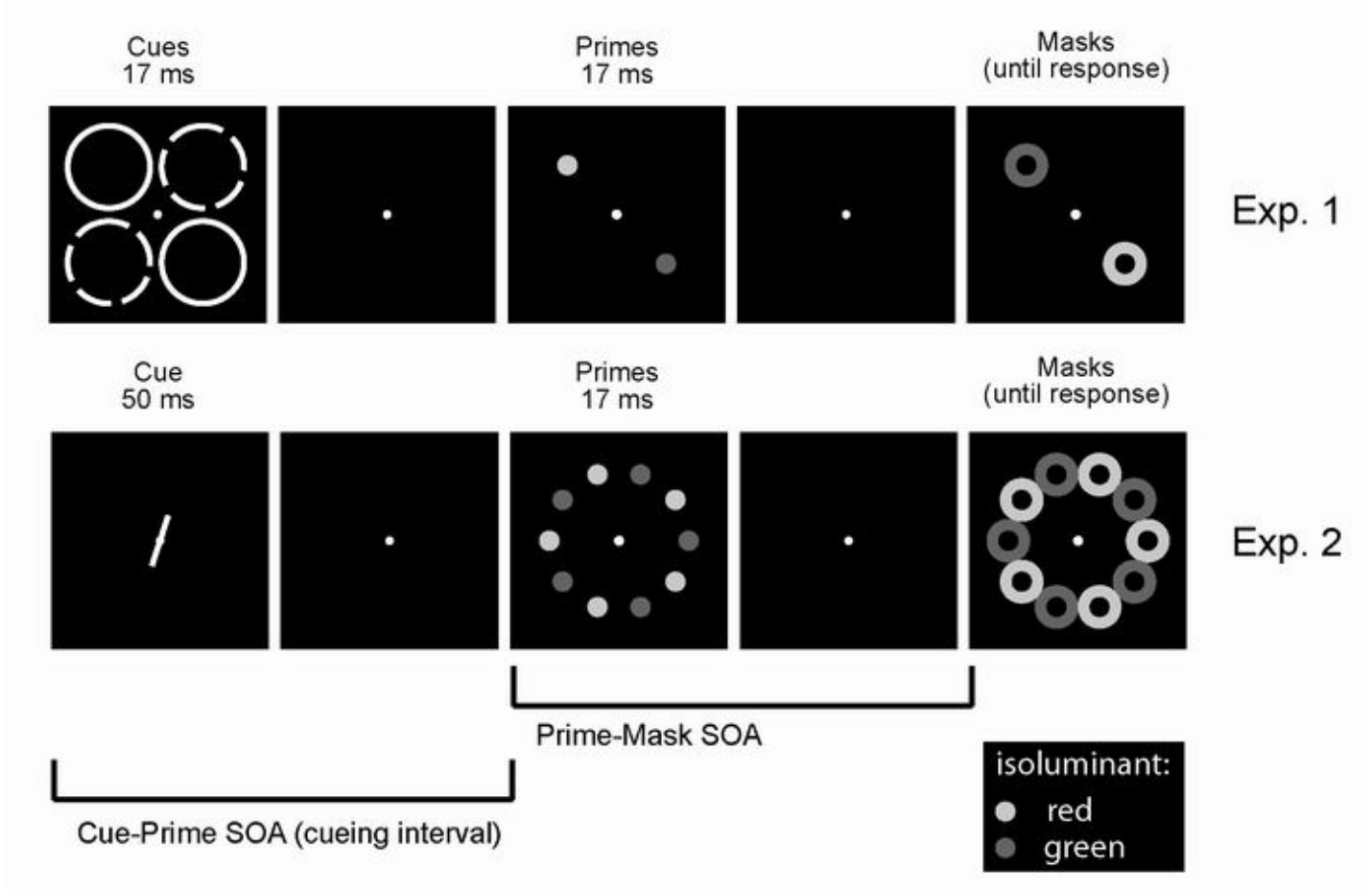
Single-cell recordings indicate that a visual stimulus elicits a wave of rapid neuronal activation that propagates so fast that it might be free of intracortical feedback. We traced the time-course of early feedforward activation by measuring pointing responses to color targets preceded by color stimuli priming either the same or opposite response as the targets. Effects of visual attention at the prime/target locations were studied by giving either an endogenous or an exogenous attentional cue, varying both the cue-prime and the prime-target SOA (stimulus onset asynchrony). Early pointing kinematics were time-locked to prime onset and independent of target onset, indicating that initial responses were controlled exclusively by the feedforward information elicited by the primes. However, early pointing dynamics were clearly modulated by attention at optimal cue-prime SOAs. Results indicate that visual attention modulates cortical feedforward dynamics in advance of critical stimuli.



# Fractal-Like Image Statistics in Visual Art: Similarity to Natural Scenes

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Visual art and natural scenes are generally perceived as esthetically pleasing. It is therefore possible that the two types of stimuli share statistical properties. For example, natural scenes display a Fourier power spectrum that tends to fall with spatial frequency according to a power-law (1). This result indicates that natural scenes have fractal-like, scale-invariant properties. We asked whether visual art displays similar statistical properties and measured Fourier power spectra in examples of graphic art from the Western hemisphere. Graphic art from different countries, artists, centuries and styles was analyzed. Graphic art was compared to images, which display relatively low or no esthetic quality (images of household and laboratory objects, plants and scientific illustrations).

For graphic art, Fourier power tends to fall linearly with spatial frequency according to a power-law, as observed for natural scenes (1). A similar relation was not found for the other image categories. This result indicates that natural scenes and graphic art share scale invariant, fractal-like properties in the Fourier frequency domain. The fractal-like property of the graphic art was independent of cultural variables, such as century or country of origin, technique or subject matter.

An artist creates his works of art by constant feed-back with his own visual system. The resulting stimulus ensemble induces a specific functional state in the visual system ("resonance", 2). We speculate that esthetic perception is linked to the adaptation of the visual system to natural stimuli. Specifically, we propose that esthetic perception is based on the same neuronal mechanisms that underlie sparse (efficient) coding of sensory inputs (3). For example, it is conceivable that visual art can be processed by the visual system with maximum efficiency, or that visual art elicits a pattern of neural activation that is maximally sparse across neurons.

Supporting our model, the following features are shared by sparse coding and esthetic perception: (i) Both relate to the form rather than the content of images. (ii) As shown by our results, both display similar statistical properties, such as fractal-like structure. (iii) Both depend on higher-order statistics of images, i.e. individual image features are perceived in the context of their surround. (iv) Both are universal among human beings, i.e. they do not depend on cultural variables. (v) Both are non-intuitive to cognition and every-day language.

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# Spotlight on Glial Cells: Living Optical Fibers in the Vertebrate Retina

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While cells are mostly transparent they are phase objects that differ in shape and refractive index. Any image that is projected through layers of cells will normally be distorted by refraction, reflection, and scattering. Strangely, the retina of the vertebrate eye is inverted with respect to its optical function and light must pass through several tissue layers before reaching the light-sensitive photoreceptor cells, with each photon having a chance of being scattered. Here we report how nature has optimized this apparently unfavourable situation. We investigated the optical properties of retinal tissue and individual Müller cells, which are radial glial cells that span the entire thickness of the retina. These cells act as optical fibers and guide light that would otherwise be scattered from the retinal surface to the photoreceptor cells. The collective presence of these parallel optical fibers mediates the image transfer through the retina with minimal distortion. This finding explains a fundamental feature of the inverted retina as an optical system and it ascribes a new function to glial cells.

# Posterior parietal lobe control of spatial constancy accross saccades

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The posterior parietal cortex (PPC) is critical for maintaining spatial constancy across saccadic eye movements. This has been shown in previous studies by applying the double-step saccade task. In patients with right posterior parietal lesions the second saccade was dysmetric or performed into the wrong direction, if the first saccade had been directed into the contralesional hemifield and if there was no more post-saccadic visual information about the second target. This could be explained either by assuming a spatiotopic deficit in terms of deficient eye position information for contralesional craniotopic hemispace, or alternatively by a deficient compensation of a contralesional saccadic eye displacement for updating the spatial representation of the second target. These two models predict different deficits, if the double-step task is performed from various initial eye positions, either within the left or totally within the right craniotopic hemispace. In a second lesion study, we investigated 10 patients with right posterior parietal lesions with this paradigm. Results showed that the failure of the second saccade did not depend on the craniotopic hemifield where the task was performed, but on the contralesional direction of the preceding first saccade. Thus it is not an absolute spatiotopic or craniotopic deficit, but a deficit of computing the efference copy signal of a contralesional saccadic eye displacement in order to update the retinal location of the next saccade goal.

The effects of this deficit on perception were investigated in a psychophysical study a group of 11 patients with right parietal lesions, compared to 14 age-matched healthy adults: During a simple visually-guided saccade of 6° or 8° to the right or left, the target was either blanked for 200 ms or not blanked and displaced by 2°, 1° or 0.3° to the right or left. As a measure for the stability of postsaccadic spatial localization, subjects had to indicate the direction of target displacement in a 2-alternative-forced-choice task. Whereas the control subjects were able to localize the target displacement correctly whenever the step of displacement as 1° or 2°, parietal patients performed around chance level for all 3 step sizes. This deficit was independent of the craniotopic hemi-field and independent of saccade direction, although contralesional saccades had increased latencies.

Interestingly patients produced a high rate of corrective saccades in the blanking condition without visual feedback. Data shows that - mostly hypometric - first saccades are followed by a corrective saccade which results in a hypermetric eye position whereas saccades with visual feedback induce metric eye positions.

We conclude that lesions around the right IPS impair transsaccadic spatial localization not only with respect saccadic accuracy but also to perception, independent of saccade direction or craniotopic hemi-fields. Furthermore the fast corrective saccades also suggest a deficient efference copy signal that is misused by the parieto-cerebellar circuit.

# Experiments on size discrimination in goldfish (Carassius auratus)

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Purpose: An important requirement of the visual system is the ability to discriminate between objects of different size. In our investigations of optical illusions of size in goldfish (Müller-Lyer-, Ponzo- and Ebbinghaus-illusion) the question arouse what the limits of size discrimination may be. Therefore we investigated the ability to discriminate objects of different size in behavioural experiments presented at identical distances. Thus the only difference between the stimuli was the visual angel subtended by the objects or the area on the retina.

Methods: Four goldfish were kept separately in tanks. Through two openings of a 'feeding plate' (8 cm diameter) test fields were visible on which two figures (bars or circles, respectively) were show simultaneously on a TFT-display at a distance of 5.5 cm. The fish were trained on a black horizontal bar (50 x 4.5 mm, visual angle 24° x 2°) while a smaller bar (30 x 4.5 mm, visual angle 15° x 2°) was shown for comparison. Second the fish were trained on a black circle (2 cm dia., visual angle 10°) versus a smaller circle (1.6 cm dia., visual angle 8°). In the test, choice frequency was determined for the training stimulus presented together with a test stimulus of varied length (4 - 4.5 cm, visual angle 20-22°) or diameter (1.7 - 1.85 cm, visual angle 8.8-9.5°). In two transfer tests we investigated whether the fish generalized in terms of 'larger' or 'smaller'; first for bars and circles and second for other differently sized objects.

Results: Animals were capable to discriminate between bars of different length down to a length difference of 1 cm. The ratio of visual angles for two bars which could just be discriminated was 1 : 0.83. For circles, discrimination was possible for differences in the diameter of up to 0.2 cm, which equals to a ratio of visual angles of 1 : 0.9. Calculating the area of stimuli the ratio was 1 : 0.80 for bars and 1 : 0.81 for circles, respectively.

In the transfer test animals did neither exhibit a preference for larger circles versus smaller ones nor for a larger object versus a smaller object but showed a relative choice frequency of 50 %. This means, if two circles of different diameters, e.g. 2.5 and 2.2 cm, were shown, animals exhibited no preference for the larger circle. A similar result was obtained if animals were presented with a large triangle and a small triangle or a large and small square, respectively.

Conclusion: Animals were able to discriminate objects which had a visual angle ratio of 1 : 0.83-0.9. However, area ratio was the same (1 : 0.8 and 1 : 0.81). This indicates that the limiting factor for size discrimination, at least within a certain range of visual angels, is the ratio of two object areas.

Animals did not exhibit any transfer in the generalization experiments, i.e. they did neither chose the larger circles nor the larger objects. This means that goldfish do not seem to learn 'larger' is correct, but they prefer the object of trained dimensions.

# Self-Motion Perception in the Elderly: an experimental and theoretical study

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Self-motion through space generates a visual pattern on the retina called optic flow. In a number of psychophysical studies it was shown that this retinal pattern can be used to determine ones direction of self-motion (heading). In our present study we asked whether the capability of using optic flow for heading detection is constantly available throughout life or whether we could find indications for age-related changes.
Psychophysical experiments in normal human subjects were performed in visual virtual reality with a head mounted display. Subjects were separated into two experimental groups: one control group of middle-aged subjects (25+/-5 years) and one test group of older subjects (65+/-10 years). Experimental data were compared with predictions resulting from a modified version of a neural network model of heading detection.
In a first experimental step we examined the case of translational self-motion through a 3D-cloud of random dots. Stimulated observer speed was constant, while presentation time and dot density was varied in blocks of trials. After stimulus presentation a ruler was presented in the visual field and subjects were asked to indicate their perceived self-motion direction on this ruler. In a second set of experiments we induced a random perturbation to the flow field. Dots randomly disappeared from the screen and appeared at a new position with new movement vectors. Only 20% of these movement vectors were in accordance with the simulated heading direction. In a final set of experiments we examined the role of stereoscopic visual information on heading performance.
Results from the first experimental series showed that heading accuracy for elderly subjects was generally worse than for middle-aged observers. Surprisingly, the elderly subjects seemed to be impaired to make use of increasing visual information in the display as provided by longer display durations and larger number of dots in the display. In comparison, the performance of the test group significantly increased with the increasing visual information. The second set of experiments revealed an influence of the perturbation on heading detection only for shorter presentation times. The decline in performance was most prominent for the control as compared to the test group. The last set of experiments showed an overall improvement of heading detection for stereoscopic stimuli. This improvement was found for test and control groups for longer presentation times and larger number of dots but not for short presentation times and a small number of dots.
In a theoretical part we aimed to model our experimental data by modifying an existing neural network on heading detection originally developed by Lappe & Rauschecker (Neural Computation 1993). The model consists of two layers of neuron-like elements which represent the optical flow as input and the computed heading as output. We implemented the process of aging by a biological plausible assumption of age-related cell loss. The network output was in perfect agreement with our experimental results.
In summary, our data clearly show that heading detection decreases with age and that it is mostly the lack of making use of increased visual information content that differs between aged and control groups.

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# How do we allocate attention in different categories of images?

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Low-level stimulus features influence the allocation of visual attention to some degree. In this study, we examined the influence of low-level features on eye-movements in different categories of images.
We recorded eye-movements of human subjects while they were freely viewing color-calibrated images of 7 different categories (Faces, Flowers, Forest, Fractals, Landscapes, Man-Made objects and Rainforest).
The subjects' eye-movements show similar distributions in the different categories, except for faces. In this category the area covered by eye-movements is significantly smaller. We then examined the influence of different stimulus features on eye-movements by comparing the value at fixation locations to subject-specific control locations. Despite similar image statistics, the influence of stimulus features luminance-contrast, red-green and blue-yellow color-contrast is different for different categories (see Figure 1).
In order to examine whether the influence of stimulus features depends on having color information available, we also presented the images in grayscale. The feature values of eye-movements made on grayscale images were calculated on the naturally colored images. Comparing these feature values to those obtained from the eye-movements on colored images, we find no significant differences. Only in images of rainforest we find a significantly higher red-green color-contrast for colored images.
Our results suggest that the influence of different stimulus features on eye-movements strongly depends on the category as well as the chromaticity of images presented.

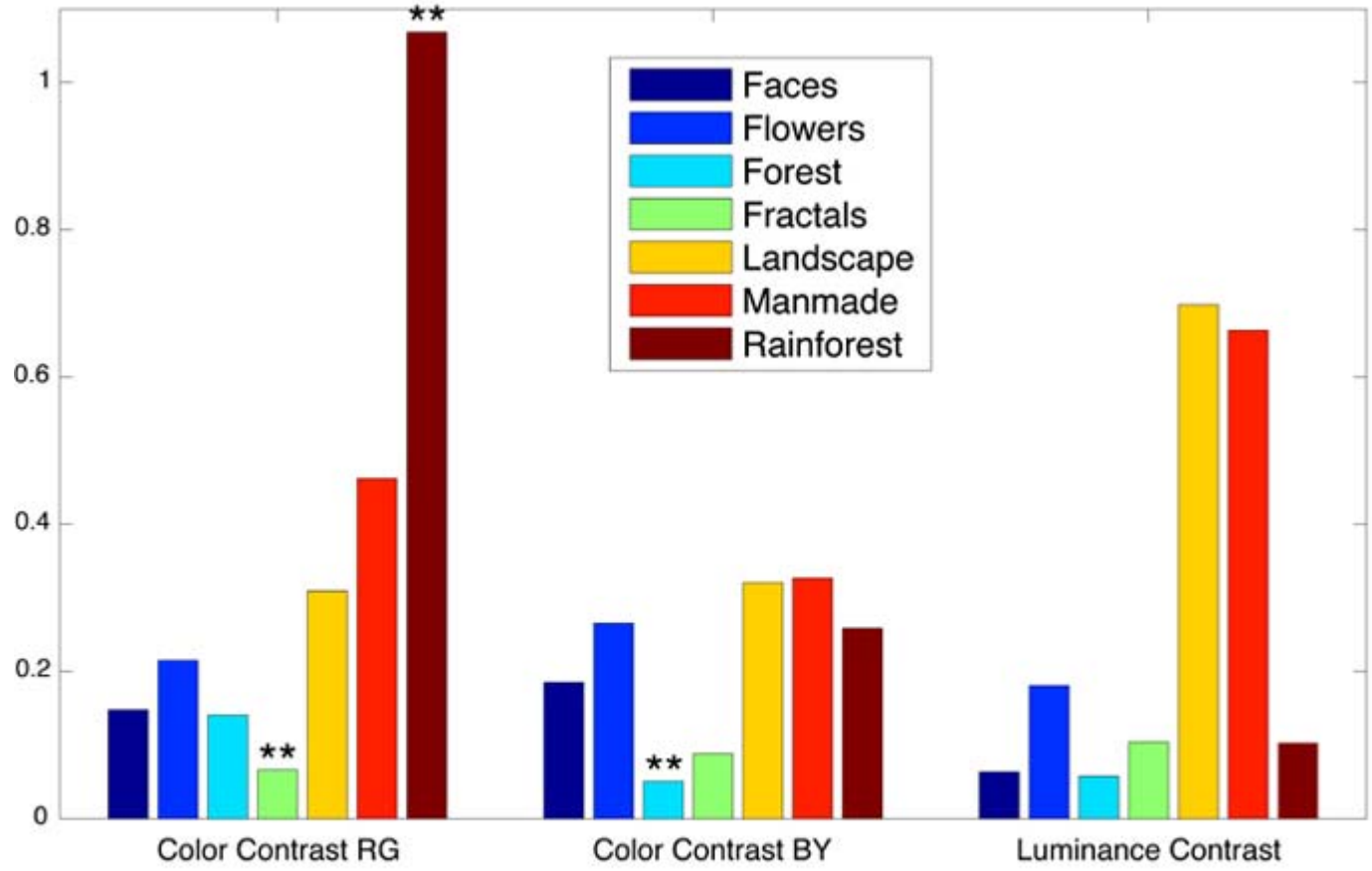


Figure 1: Effect sizes for different features in the 7 categories. Those categories having significantly higher or smaller effect values than all other categories are marked by stars.

# Initial saccadic latencies during tracking of real or illusory contours

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Low-level motion processing is best described by elementary motion detectors of the correlation type. In the case of a moving contour, these detectors are only able to signal motion perpendicular to the contour. Evidently an initial direction error occurs when subjects track a contour tilted with respect to moving direction[1]. Initial saccadic latencies are known to be inversely proportional to target speed. It already has been shown that lines tilted with respect to their moving direction are perceived to move slower than orthogonal ones[2].

Consequently, we ask whether the described 'speed reduction' illusion can be demonstrated behaviourally in the form of prolonged saccadic latencies for real contours tilted with respect to their moving direction compared to orthogonal contours. Does this also hold true for illusory contours? We tried to replicate the findings from the literature regarding the initial direction error, asked whether the directional error during pursuit initiation could be abolished by subjects' prediction and asked whether this error can be observed if illusory contours were tracked.

Horizontal and vertical eye positions of five subjects were recorded at 1kHz using infrared oculography (IRIS Skalar). Stimuli were presented at a viewing distance of 57cm on a 19" CRT-Screen (width:36.2°, height:27°), at a resolution of 1600x1200 pixels and a refresh rate of 104.5Hz. Eye velocity vectors were calculated from the horizontal and vertical velocity profiles of each trial. The direction of these vectors was used to determine the initial tracking error.

The real contour, a white bar on black background, length:20°, width:0.5°, with six possible moving directions, induced an significant initial tracking error (24.24°±4.53°) when tilted 45° with respect to moving direction. To test the effect of prediction, we restricted the directions of target movement to purely horizontal ones. In this condition, the real contour also induced a significant initial tracking error (17.29°±7.30°), showing that prediction of target trajectory is not able to decrease the initial tracking error significantly. The tilted bars yielded significantly longer saccadic latencies than the orthogonal bar (tilted bars:197ms±24ms; orthogonal bar:178ms±23ms, $p<0.05$). Three kinds of illusory contours preserving size of the real contour were tested: i) only the endpoints of the real contour, ii) differing portions of the real bar occluded by a white bar centred on the screen and parallel to moving direction, iii) interleaved line-endings of two line gratings (spatial frequency:2 or 4 cyc/deg) defining the illusory contour. In respect of the initial tracking error, illusory contours turned out to have a smaller effect as real contours (ranging between 0-9°). But interestingly, tilted illusory contours produce saccadic latencies which were prolonged compared to orthogonal contours. Thus, we were able to show the 'speed reduction' behaviourally in the form of prolonged saccadic latencies.

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# Properties of the pursuit-related activity recorded from primate frontal eye field

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Smooth pursuit eye movements (SPEM) were successfully used in studies addressing details of visual motion processing. It is well known that pursuit eye movements can only be executed in the presence of a moving target. This ultimate connection between motion processing and pursuit eye movements makes it very difficult to determine whether neuronal activity which parallels the execution of these eye movements is sensory or motor in origin. To solve this ambiguity problem, we developed the imaginary target paradigm. Here, the monkeys had to pursue either an imaginary figure which is exclusive defined by peripheral visual cues or a real figure. We compared statistically (ANOVA) pursuit-related activity observed during pursuit of an imaginary and a real target, respectively.

To record single-neuron activity from the frontal eye field, we implanted a recording chamber in a frontal plane centred at 17 mm lateral and 24 mm frontal. T2 weighted MRI images confirmed the correct placement of the recording chamber. We used a five-channel TREC MiniMatrix which consists of five independent microelectrodes arranged in a linear array with a spacing of 0.3 mm between electrodes.

So far, we recorded 50 neurons which responded not significantly different during pursuit of the real and imaginary target, respectively. However, inspection of the responses to passive visual stimulations showed that the pursuit-related activity in 30 neurons was caused by stimulation of the peripheral visual field. However, in the remaining 20 neurons, the existence of the response during pursuit of the imaginary target can only be explained by the presence of extra-retinal signals related to the ongoing eye movements.

In addition, 35 out of 50 neurons showed a clear visual directionally selective response to retinal image motion. Note that the preferred direction for retinal image motion was identical to the preferred direction during pursuit.

Similar to our earlier results related to the pursuit-related activity recorded from the middle superior temporal area, we suggest that neurons in the frontal eye field carry information which contains retinal image motion signals as well as eye movement signals.

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# The role of colour in visually guided actions

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Current theories on visual information processing suggest that colour- and action- related information is processed separately in the ventral and the dorsal streams. Each stream codes the information differently, depending on its use. In this experiment we tested the role of colour in guiding action. Both localization accuracy and simple reaction times were measured for pointing at 3 deg sine wave Gabor patches presented at 15 deg retinal eccentricities. The gratings were modulated along the red-green (L-M), blue-yellow (S-[L+M]) and luminance (M+L) axes in the DKL colour space. Their luminance and chromatic contrast were scaled in term of multiples of detection threshold (C=0.04*detection threshold). All observers had normal colour vision as tested by Cambridge Colour Test. Within each session (200 trials) subjects were cued to a different visual attribute. In each trail the grating was presented as either isoluminant colour defined or luminance defined. The observers attended and reacted to colour gratings and rejected the moving ones or vice versa. Pointing errors for all stimuli were less than 0.5 deg visual angle; no significant difference in the reaction times was observed for different stimuli driving the pointing behaviour. These findings suggest that colour information is involved in visuomotor control.

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# Using SE-EPI to Measure Visual Responses in the Awake Macaque at 7Tesla

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In contrast to electrophysiological studies, the advantage of fMRI is that it allows simultaneous mapping of the functional organization of multiple cortical areas. FMRI of awake monkey has benefit of combining behavioral studies with BOLD-measurement to be used to precisely localize functional specific cortical areas for further invasive studies such as detailed electrophysiological single unit recordings. Although high magnetic field offers the benefit of increased signal-to-noise ratios and higher specificity, a drawback is the higher sensitivity to susceptibility gradients caused by the air-tissue interfaces. This can be particularly problematic in the lower floor of temporal lobe because the large macroscopic susceptibility gradients near the ear canal result in distortion and loss of signal when the standard GE-EPI is used. For fMRI of such areas using spin-echo EPI (SE-EPI) is advantageous because it is less sensitive than GE-EPI to susceptibility artifacts, and does not suffer from signal dropout in these regions. Another advantage is that SE-EPI is less affected by frequency-changes in the main magnetic field, which are caused by movement of the animal. In this study, we compared SE-EPI and gradient-echo fMRI in the awake monkey (Macaca mulatta), using a vertical bore 7T MR system. A saddle coil optimized for temporal cortex was used to allow imaging of the major visual areas. The imaging parameters and slice orientation were optimized to minimize susceptibility effects. Resolution was typically 1.5x2x2mm, TE was 40 ms, TR was 1-2 s. In contrast to the GE-EPI images, which showed very large signal dropout in the temporal lobe, SE images showed minimal or no distortion or signal losses. Any remaining distortions were corrected using field-map correction to ensure perfect matching of the functional map to the high-resolution T1-weighted anatomical images. Using movie- stimuli, we confirmed that reliable functional activation could be obtained with SE-EPI at high field, and we show robust activation in the temporal lobe and early visual areas. Using random-dot kinematograms of various coherences we were also able to obtain functional activities in specific visual motion sensitive areas such as MT, MST and an area located within the lower bank of superior temporal sulcus by contract of high coherence (80%) and zero-coherence random dot stimuli. The reliability and specificity of the obtained activations with SE-EPI ensures the application of the method in our on-going visual perception studies.

* Ku S. and Goense J. and contributed equally to this work

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# The role of color in natural images to recognition performance and neural activity in extrastriate and prefrontal cortex

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Although objects can be identified based solely on the information provided by the spatial structure of an image, color adds another perceptual dimension which may facilitate object identification. However, the addition of color in form of colored noise may interfere with object identification. Here we investigate whether color in natural images is associated with changes in visual memory performance. In addition, we examined neural activity in area V4 and dorsolateral prefrontal cortex (PF) and address whether alterations in neural responses are correlated with changes in performance. Additionally we explore whether there is neural evidence of a functional specialization for color processing in natural scenes in either of the two brain areas.

We used a procedure based on Fourier analysis to degrade a unique set of four colored and achromatic natural scenes with increasing amounts of four unique achromatic noise patterns. We also degraded achromatic images with chromatic noise patterns. At a given degradation level, the difference between colored and achromatic images was thus provided only by color: the remaining image specific color or the colored noise. In a delayed matching to sample paradigm a sample stimulus (250ms) at various degradation levels was presented, followed by an undegraded probe stimulus (1s) after a delay period (1500ms). A lever press was required if the sample stimulus matched the probe. We have preliminary results from one monkey. The monkey's recognition performance decreased as a function of noise for all conditions. In addition, we found that the recognition performance was best for the 'natural' color condition and worst for the colored noise condition at the same degradation level (paired t-tests, $p<0.01$). These results suggest that color independent of spatial composition, confers either an advantage or impairment in object identification depending on whether the color is related to the object or not.

Single neuron responses were recorded from a total of 84 neurons in V4 and 76 neurons in PF. In V4 73% of visually responsive cells conveyed less information about image identity with increasing amounts of noise (39/53 units, Signtest $p<0.0001$). In addition, at the same degradation level, the information about image identity was significantly higher for the natural color condition than the colored noise condition (paired ttest, $p<0.05$). In PF 72% of visually responsive neurons conveyed less information about image identity with increasing amounts of noise (42/58 units, Signtest $p<0.001$). In PF no significant differences between the achromatic and chromatic stimulus conditions were found. For both areas, the effect of noise on information conveyed by neural activity about image identity is consistent with the effect of noise on behavioral performance. Furthermore, in V4 the elevated information about image identity for image specific color vs. colored noise is correlated with increased recognition performance in the image specific color condition. These results reveal noise and color specific modulations in neural activity at the level of single neurons in area V4 and PF in object identification.

# Neural encoding of species dependent face-categories in the macaque temporal cortex.

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When perceiving a face, we can easily decide whether it belongs to a human or non-human primate. It is thought that face information is represented by neurons in the macaque temporal cortex. However, the precise encoding mechanisms used by these neurons remain unclear. Here we use face stimuli of humans, monkeys and monkey-human hybrids (morphs) to gain a better understanding of these mechanisms, in particular of the categorization of faces into different species, and how learning affects representation of these stimuli.

We perform single cell and local field potential (LFP) recordings in the inferior-temporal (IT) cortex of the macaque brain during a fixation task. To investigate the perceptual effects of our stimuli and possible relations to the neural data, we conduct in parallel psychophysical experiments with human subjects. On preliminary results of 75 recorded cells in one animal, we found 66 visual responsive neurons. From them, 12 were tuned to faces ('face-cells') and 9 to other test objects (like a hand, clock, fruits, etc.). Six 'face-cells' prefer monkeys while just two prefer humans. Considering the population activity, monkey faces elicited in general higher firing rates on the population of neurons (independent of its category) than human faces. Additionally, these firing rates change gradually according to the human/monkey ratio of the morphed stimuli. After measuring the perceptual category boundary between monkeys and humans faces in our human subjects, we founded that it is shifted to the human side, independent of the method we use to measure it.

Our preliminary cell recordings suggest that neural responses (firing rates) of some cells differentiate between monkey and human faces. Besides, the tuning curves of some neurons and the population correlate with the human-ratio of the morphed stimuli. Our psychophysical experiments confirm, on the one hand, the perceptual effect of our stimuli in which we manipulate the human-monkey ratio and, on the other hand showed a tendency of our subjects to set the category boundary between humans and monkeys closer to the human side. All these findings point to different mechanisms used by the brain to encode human and monkey faces, which seem to be clearly represented by neurons in the inferior-temporal cortex of the money brain.

# OKR assay and fundoscopy: new tools for adult zebrafish vision research

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Zebrafish are widely used in vision research. In contrast to nocturnal animals which possess mainly rods, zebrafish retinae contain many cones and larval retinae are supposed to be cone dominated. Therefore, zebrafish retinae show strong similarites to the cone dominated macula in humans. Furthermore, zebrafish can be easily mutagenized, and thus, several screens for visual mutant zebrafish have been successful throughout the last decade. However, genetic approaches towards the understanding of vision and/or retinal diseases have so far almost exclusively been confined to larval staged animals. This is at least in part due to a lack of robust techniques to assess visual performance in adult fish.

We are currently developing fundoscopy and optokinetic response (OKR) measurements in adult zebrafish, as new techniques to examine the adult zebrafish visual system. To date, the OKR assay could not be applied to adult fish. Larval eyes were easily recognized by our specific software that analyses the OKR-movement. Larval zebrafish are very suitable for such an analysis as their black eyes build a strong contrast to their transparent body, and thus, can be easily detected by our software. In contrast, adult fish are not transparent and the movement of the eyes therefore cannot be traced by the program in the same way. We have reprogramed our computer software and present a new procedure to recognize the eyes of the adult zebrafish and to compute their OKR. A first version of the setup for measuring the OKR of adult fish is now completed and will allow us to dissect the visual performance of adult zebrafish by measuring contrast sensitivity, spatial and temporal frequency.

In ophthalmology, fundoscopy is an indispensable tool for the diagnosis of retinal diseases. With this imaging technique the fundus, the blood vessles, and lens properties can be visualized. This technique now allows a direct comparison between retinal diseases in humans and zebrafish mutants that show similar phenotypes. Furthermore, this technique opens a new possibility for large scale screens in adult mutant zebrafish by using fundoscopy.

# Poster Topic

## T18: Auditory system I: Invertebrates

**T18-1A** Protein expression of voltage gated potassium channels Kv1.1 and Kv3.1 in the development of auditory brain stem neurons in vivo and in vitro
*Y. Sun, T. Kuenzel, H. Luksch, H. Wagner and J. Mey, Aachen*

**T18-2A** Time-warp invariant processing: How do grasshoppers solve this task?
*F. Creutzig, S. Wohlgemuth, J. Benda, A. Stumpner, B. Ronacher and AVM. Herz, Berlin and Göttingen*

**T18-3A** Spike frequency adaptation in an insect auditory pathway
*KJ. Hildebrandt, J. Benda and RM. Hennig, Berlin*

**T18-4A** Parallel processing of binaural inputs: neuronal correlates for summation and contrast enhancement in the auditory pathway of a grasshopper
*O. Kutzki, M. Hennig and B. Ronacher, Berlin*

**T18-5A** The influence of different noise bands on signal recognition in the grasshopper *Chorthippus biguttulus*
*D. Neuhofer, M. Stemmler and B. Ronacher, Berlin*

**T18-6A** Acoustic signal processing in grasshoppers - frequency or time domain?
*A. Schmidt and RM. Hennig, Berlin*

**T18-7A** Intensity dependence of modulation transfer functions in auditory neurons of the locust
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**T18-1B** Comparing the neuronal encoding in two not closely related Grasshopper species: What differs is different?
*S. Wohlgemuth, D. Neuhofer and B. Ronacher, Berlin*

**T18-2B** How do onset cues affect song pattern recognition in grasshoppers in a noisy environment?
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**T18-3B** Neurite Specific Ca2+ Dynamics Underlying Sound Processing in an Auditory Interneurone
*T. Baden and B. Hedwig, Cambridge (UK)*

**T18-4B** Towards a better understanding of the complex auditory behaviour in crickets: Combining a sensitive trackball system with single cell recordings from brain neurones
*M. Zorovic and B. Hedwig, Cambridge (UK)*

**T18-5B** STRUCTURE AND FUNCTION OF *DROSOPHILA* AUDITORY NEURONS
*A. Kamikouchi, K. Ito, A. Fiala and M. Göpfert, Cologne, Tokyo (J) and Wülzburg*

**T18-6B** Tympanal sensilla generate DPOAEs in the locust ear
*D. Möckel, M. Kössl and EA. Seyfarth, Frankfurt/Main*

**T18-1C** Directional processing of crickets differs from that in bush crickets
*M. Brill and A. Stumpner, Göttingen*

**T18-2C** Adaptation of auditory neurons in a bushcricket
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*R. Lakes-Harlan and J. Strauß, Giessen*

**T18-5C** Signal transmission and detection for a public and private mode of communication in a neotropical katydid
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**T18-6C** Sensory bias in the peripheral auditory system of field crickets:
Directional hearing and preference for certain carrier frequencies
*K. Kostarakos and H. Römer, Graz (A)*

# Protein expression of voltage gated potassium channels Kv1.1 and Kv3.1 in the development of auditory brain stem neurons in vivo and in vitro

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Specialized neurons in the auditory brainstem provide the neurophysiological substrate for sound localization. In birds these neurons are localized in the *Nucleus magnocellularis* (NM) and *Nucleus laminaris* (NL) and express the calcium binding protein calretinin. Their ability to produce short action potentials at high frequency is linked to the voltage-gated potassium channels Kv1.1 and Kv3.1b. The physiological and biochemical signals that regulate the specific differentiation of NM and NL neurons are not known.
To address this question we prepared primary cell cultures of auditory brainstem neurons from E7 chick embryos. The protein expression of calretinin, Kv1.1 and Kv3.1b in vitro was analysed with Western blotting, with immunocytochemistry and was compared to their development in vivo. Under serum free culture conditions NM and NL neurons, which were identified by calretinin staining, showed a constant expression of Kv1.1 and a significant increase in expression of Kv3.1 between 3 DIV and 7 DIV. This differentiation corresponded to a similar change in expression between E10 and E14 in vivo. To mimic depolarising synaptic activity cultures prepared at E7 and cultivated for 5 DIV were treated with 20 mM KCl; to investigate neurotrophic effects, cells were treated with 20 ng/ml BDNF. While our preliminary results indicate a positive effect of membrane depolarisation on the protein expression of Kv3.1b the culture data also demonstrate autonomous development of auditory brain stem neurons after E7.

# Time-warp invariant processing: How do grasshoppers solve this task?

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Time-warp invariant pattern recognition is a general task of auditory systems: Temporal sequences, that are stretched or compressed versions of each other such as GOAL! or GGGOOOAAALLL!!!, have to be classified as equal. Communication signals of grasshoppers of the species *Chorthippus biguttulus* portray a particular example: Female grasshoppers respond to male mating songs that consist of specific repetitive syllable-pause sequences. Behavioral experiments with artificial stimuli showed that the female reaction is not determined by the absolute syllable duration but rather by the ratio between syllable and pause duration demonstrating time warp invariant processing. Here, we study the neural basis of processing natural and artificial mating songs focussing on the role of a single auditory ascending neuron (AN12). First, we show that this neuron encodes a) syllable onset by the timing of the first spike within a burst and b) the pause duration preceding a syllable by the spike count within bursts. By this, temporal pattern information is multiplexed into one single spiking event, a burst. Second, a minimal integrate & fire neuron model with two input channels, one instant excitatory and one inhibitory with exponential kernel, replicates this neuronal response. Third, we postulate a plausible decoding mechanism: Integration of the neuron's spike train in response to songs over a fixed time effectively counts total pause duration and, hence, provides a time-warp invariant representation of the song pattern. Forth, we suggest a model based on integration and thresholding that relates the spike train responses of 3 feature detecting ascending neurons to the behavioral response of female grasshoppers.

# Spike frequency adaptation in an insect auditory pathway

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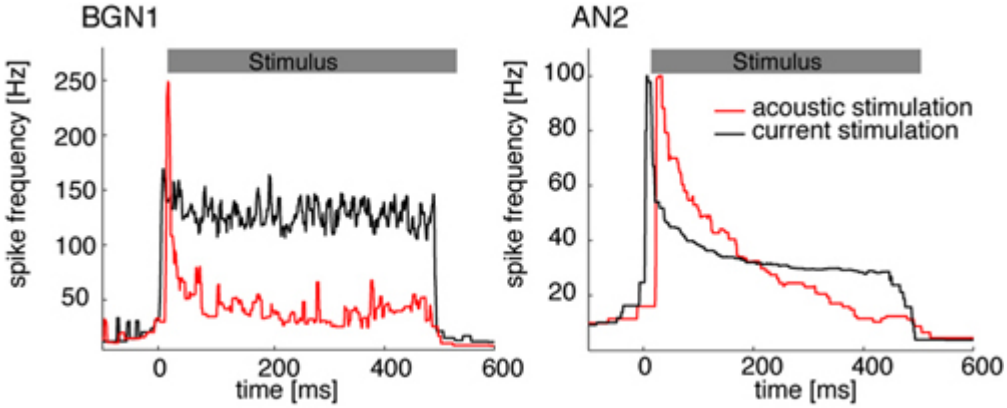
Adaptation is a prominent feature in sensory as well as motor pathways and is known to play a crucial role during sensory processing for signal recognition [1]. In sensory pathways spike frequency adaptation can arise at different processing steps: the transduction process and spike encoding in receptor cells, during synaptic transmission, in dendritic processes or during spike generation in sensory interneurons [2]. Consequently the adaptation observed in recordings from single interneurons can be the result of several adaptation processes along the sensory pathway [1]. Here, we investigate adaptation in the auditory pathway of the locust which exhibits 3 processing levels : receptor cells, local and ascending interneurons. In order to determine the contribution to adaptation currents of individual cells to the overall adaptation accumulated in the auditory pathway, we recorded from auditory neurons at all three processing levels while stimulating either acoustically or with intracellularly applied current pulses. During acoustic stimulation, adaptation arises along the entire auditory path, while current stimuli reveal adaptation in the spike generator of that single neuron only. Using both stimulus types, one can disentangle intrinsic adaptation from adaptation in the entire pathway.

The strength of adaptation during acoustic stimulation ranged from a decrease in spike frequency of 41% (TN1) to 86% (BGN1), while time constants varied between 13ms (BGN1) and 120ms (AN2). The time constants for acoustic and current stimulation were usually in the same range for a given cell type, but the strength of adaptation did not always correlate for both stimulus types (figure). In an ascending neuron (AN2) both time course and strength of adaptation for the two stimulus types showed the same magnitude. However, a local neuron (BGN1) adapted strongly to acoustic stimulation while there was little decrease in spike frequency during current stimulation.

Overall, we observed a remarkable range of adaptation characteristics both to acoustic and current stimuli that were typical for a given cell. In no case could the intrinsic properties of single neurons explain the adaptation to acoustic stimuli entirely. Consequently, adaptation in the auditory pathway of the locust is distributed over several levels of processing and likely contributes to different temporal processing properties of auditory interneurons.

[1] B. Ronacher and R.M. Hennig. Neuronal adaptation improves the recognition of temporal patterns in a grasshopper. J Comp Physiol A, 190(4): 311-319, 2004.

[2] Jan Benda and Andreas V.M. Herz. A universal model for spike-frequency adaptation. Neural Computation, 15(11): 2523-2564, 2003



# Parallel processing of binaural inputs: neuronal correlates for summation and contrast enhancement in the auditory pathway of a grasshopper

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Grasshoppers that use acoustic signals for mate finding have to solve two tasks: to recognize whether a signal is from a potential partner, and to localize the sound source. Behavioural experiments in the grasshopper *Chorthippus biguttulus* indicated that the sound localization cues are processed in parallel to the sound patterns that are crucial for recognition, and that the segregation of the two processing streams occurs already at the level of the metathoracic ganglion, a first important station in the auditory pathway (Stumpner and Ronacher 1994). The conclusion then was that the information from the two ears is summed up in the pattern recognition pathway, while sound localization cues are enhanced by reciprocal inhibition. We examined auditory neurones of the locust, whose auditory system bears great resemblance to that of *C. biguttulus*, with a focus on 2 questions: (1) At which processing level is parallel processing evident and (2) how do phasic onset effects during summation of inputs affect the quality of pattern representation?

In the present study three different neuron types were investigated with intracellular recordings: auditory receptors and two identified first-order interneurons, TN1 and BSN1. Sound patterns that mimicked an attractive song where presented either from one side or simultaneously from both sides. For each speaker the sound pressure level was adjusted to only a few dB above a neuron's threshold in order to achieve largely monaural stimulation (due to attenuation by the body of the animal sound was below threshold on the ear contralateral to the more sensitive side).

TN1 sums the inputs from both ears. This summation of inputs leads to a higher spike rate, reduced latencies and a lower coefficient of variation, and therefore to a higher signal to noise ratio of pattern representation. Due to differential adaptation between both input sides onset effects are minimized. However, the information about the location of the sound source is sacrificed by this summation of both sides. The BSN1 neuron receives a soma-ipsilateral excitation and contralateral inhibition, which leads to larger spike count differences between the two sides. However, at the same time the information about the pattern is degraded. We conclude that the TN1 takes part in the pattern recognition pathway, while BSN1 plays a role in the sound localization pathway. The present data suggest a separation into parallel pathways already at the first synapse of auditory processing.

# The influence of different noise bands on signal recognition in the grasshopper *Chorthippus biguttulus*

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Many insects produce species-specific signals for acoustic communication. The discrimination and correct classification of these signals is impeded by noise acting during propagation through the biotope. A widespread mechanism to increase the signal to noise ratio (SNR) is frequency filtering. Yet no improvement in the SNR is to be expected in the presence of competing conspecific signalers, given that the frequency spectra of the competing, masking sounds and the signal overlap. We tested the effect of noise in different frequency bands on signal recognition in the grasshopper *Chorthippus biguttulus*, in which the recognition of species and sex primarily is based on temporal cues present in the song envelope (D & O von Helversen 1998, Biol Cybern 79: 467). For female songs, the main amplitude modulations lie within a narrow frequency band between 10 and 100 Hz.

The envelope of a recorded female song was progressively degraded by bandpass noise in 3 dB steps relative to the variance of the signal envelope (noise bands 0 - 1000 Hz, 0 - 100 Hz, and 100 - 200 Hz). We conducted a combination of behavioral tests and intracellular recordings of auditory neurons in *C. biguttulus* males. Play-back experiments were performed with males in a sound attenuated room. Whenever the male started to sing, it was answered by envelope degraded model songs in pseudorandom order via a laterally situated loudspeaker. We recorded the number of times the males made turns towards the loudspeaker as a function of the noise level, and determined a critical noise level at which behavioural responses declined to 50 % indicating that recognition failed.

In addition, we conducted intracellular recordings from auditory interneurons in the methatoracic ganglion. Using the same stimuli as in the behavioural tests, we analysed the changes in the neuronal response that are caused by increasing levels of envelope noise. The differences between the spike trains were quantified using a spike train metric introduced by van Rossum (2001, Neural Comp. 13: 751 - 763).

Our preliminary results indicate no differences in how different broadband maskers influenced song recognition. For the broadband noise, the critical noise levels mostly lie between 6 and 12 dB. The 100 - 200 Hz narrow band differed from the broadband envelope noise in that song recognition failed at lower noise levels, mostly between 3 and 6 dB. The 0 - 100 Hz narrow band masker, which was thought to have the greatest influence on song recognition had the opposite effect: most animals always responded, regardless of the noise level.

Raising the noise level led to increasing distortions in the spike patterns as quantified by the metric distance to the unperturbed pattern. Noise in the bands 0 - 100 Hz and 100 - 200 Hz caused similar distortions in the spike train patterns; hence, the distance measure does not directly correlate with the difference in the animals' behavior to these two noise frequency ranges. The 0 - 100 Hz random amplitude modulations were recognized as female songs, even though the spike patterns in response to noise and to true songs are dissimilar.

# Acoustic signal processing in grasshoppers - frequency or time domain?

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The question whether the periodicity of acoustic signals is processed in the frequency or time domain is presently a much debated issue in auditory physiology of vertebrates (Plack, Oxenham, Fay, Popper, 'Pitch-Neuronal Coding and Perception' 2005, Springer Handbook of Auditory Research). A crucial cue to distinguish between these two fundamentally different processing schemes is the role of phase information. If required, this suggests processing in the time domain. Although the auditory system of grasshoppers is by far more limited in processing capacity (i.e. number of neurons) of a signal envelope, frequency components of up to 500 Hz (equivalent to a temporal resolution of less than 2 ms) are known to be resolved by grasshoppers females in behavioural choice experiments. Here we employ behavioural tests in order to determine (1) whether phase information is evaluated at all, (2) to what extent and up to which frequency component altered phase information is detected and (3) whether females accept stimuli without the 'missing fundamental'

The envelope of natural songs of the grasshopper *Chorthippus biguttulus* reveals 5 prominent frequency components (from 10 to 50 Hz, spaced by 10 Hz steps) with decreasing amplitudes. Simple addition of these 5 frequencies thus generates a signal envelope, which is highly attractive for grasshopper females and served as a control for the following behavioural tests: first, the phase of all components was systematically varied. Altered phase information for frequencies up to 40 Hz strongly influenced female responses, only phase information at 50 Hz was irrelevant. The lower the frequency component, the more important phase information was and up to 30 Hz the full range of selectivity was observed (from 0% to 100 % responses). Second, grasshopper females responded well to signal envelopes without the $F_0$-component of 10 Hz i.e. to signals without the 'missing fundamental'. Indeed, in some combinations only 3 of the 5 frequency components were required for signal attractiveness. However, in contrast to vertebrates, females did not tolerate altered phase information in these experiments.
In conclusion, for the frequency components up to 40 Hz both the amplitude *and* the phase information of the envelope determine a signal's attractiveness, thus providing strong evidence that grasshopper females process acoustic signals in the time domain. For envelope frequencies beyond 50 Hz, however, phase information appears to be not relevant any more.

# Intensity dependence of modulation transfer functions in auditory neurons of the locust

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Fast amplitude modulations are a feature common to acoustic communication signals of various vertebrate and insect species. Hence, a sufficient temporal resolution is essential for signal recognition in the auditory system. However, the encoding capacity within the auditory pathway for amplitude modulations will depend on spike rates and on the temporal precision of spikes - both of which are known to depend on sound intensity. We therefore investigated how the sound intensity influences the encoding capacities and temporal resolution of identified auditory neurones in the metathoracic ganglion of *Locusta migratoria*.

Responses of auditory neurons to sinusoidally amplitude-modulated (SAM) stimuli were recorded intracellularly and modulation transfer functions based on rate (rMTF) and vector strength (vMTF) were determined. rMTFs of receptors and local (first order) interneurons exhibited allpass characteristics, independent of intensity. rMTFs of ascending (second order) interneurons showed changes in rate with modulation frequency, corresponding to lowpass, bandpass or bandstop filters. However, at intensities of 50 dB and below these filters vanished and rMTFs became allpass.

With increasing intensity the vMTFs of receptors and local interneurons showed a systematic change from lowpass to bandpass filters. vMTF of ascending neurons were found to be bandpass even at low intensities. In comparison with receptors and local interneurons corner frequencies of ascending neurons were generally lower indicating a reduced temporal resolution at this advanced processing level. This reduction in temporal resolution became more pronounced at low intensities, but the corner frequencies of vMTFs did not correlate with spike rates.

These results show similarities to findings in the auditory pathway of vertebrates. Here, in peripheral structures, envelope information is encoded by a synchronisation of neural activity to the stimulus` amplitude modulation. In the inferior colliculus envelope information is encoded partially in average firing rate but spike locking to the envelope persists to some degree. In general, a similar trend is also seen in the metathoracic auditory network of the locust. However, in ascending neurons spike rate filter properties for different modulation frequencies were only observed for intensities above 50 dB, while for lower intensities, spike rates did not depend on modulation frequencies. Thus, a spike rate based discrimination of amplitude modulations appears to be restricted to higher intensities (above 50 dB), whereas at lower intensities information about the signal envelope is predominantly encode in spike timing.

# Comparing the neuronal encoding in two not closely related Grasshopper species: What differs is different?

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Hearing in Orthopterans has evolved several times in different behavioral contexts, e.g. as adaption for predator detection, or in a coevolutionary process together with signal production, to support mate finding and mate recognition.
To investigate the influence of different selection pressures on the auditory pathway, we compare two acridid grasshopper species, which live in disparate habitats and for which auditory signals are of different significance. Locusta migratoria belongs to the subfamily Oedipodinae. To present knowledge, this species does not communicate acoustically; rarely it produces short sound pulses but there is no observable reaction of a locust hearing these. Hence, one may assume that hearing in locusts is basically used for predator detection. Chorthippus biguttulus (subfamily Gomphocerinae) has an elaborated bidirectional communication system. The recognition of species and sex is primarily based on the temporal pattern of the song's amplitude modulations, the signal envelope.

To test wether there are differences in neuronal encoding of natural acoustic stimuli between these two species we performed intracellular recordings from identified 1st and 2nd order auditory interneurons. Earlier studies have shown an extensive congruence of the morphological characteristics of auditory neurons in these two species. Since the responses of the neurons to simple stimuli seem to be very similar between both species, the neurons are considered to be homologous (Stumpner and Ronacher 1991, J. Exp. Biol. 158: 391).
In our study we used complex test stimuli, which differ in their relevance for the two species: a natural song of a female and a male C. biguttulus. To assess differences between the spiketrains of homologous cell types of our test species, we used a spike metric introduced by van Rossum (2001, Neural Comp. 13: 751 - 763). This method measures the similarities between spike trains, and allowed to determine the distributions of intra- and interspecific spiketrain distances. The separation of these distributions can be tested for different temporal resolutions, which allows to quantify the influence of spike timing and spike count differences for discrimination.
Our preliminary results indicate that at the level of local (1st order) neurons the spike trains of the same cell type are strikingly similar between the two species. With a few exceptions this is also true for the next processing level. For some of the 2nd order ascending neurons there seem to exist higher interspecific differences between the spiketrains even though there is a remarkable intraspecific variability. Altogether our results indicate that differences in the habitats of these species had only small influence on the neuronal responses to songs, which are relevant for only one species. Note, however, that the initial selection pressures acting on the auditory system may have differed from the selection pressures acting today. These results are discussed in the context of Barlow´s "efficient coding hypothesis" (Barlow, 1961), that sensory neurons are adapted to the statistical propertiers of sensory signals to which neurons are exposed.

# How do onset cues affect song pattern recognition in grasshoppers in a noisy environment?

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For the acridid grasshopper *Chortippus biguttulus*, acoustic communication provides a central mechanism for mate recognition and species isolation. Males produce species- and sex-specific calling songs that will trigger response songs in a receptive female, but only if she considers the detected signal to be attractive. Both sexes take turns in singing, enabling the male to approach the female. Songs are recognized on the basis of the characteristic temporal pattern of amplitude modulations, the signal's envelope. Behavioural tests with model songs have shown that the ratio of syllable to pause is crucial in *C. biguttulus* (von Helversen, von Helversen 1997 J Comp Physiol A 180:373). However, in the biotope the temporal structure of signals is frequently distorted and masked by noise. Here we investigated how much signal degradation female grasshoppers will tolerate, and whether certain signal traits will increase noise tolerance.
We compared song models composed of 80 ms syllables with and without a 6 dB onset accentuation. In addition, we also varied the pause duration between syllables. The envelopes of all stimuli were degraded by broad band noise (3 dB steps; 0-1000 Hz or 100-500 Hz). In a sound attenuated chamber (kept at 30°C) the stimuli were presented repeatedly to virgin females, in a randomized order. By responding with a song, a female reliably indicates whether it has recognized a song and found it attractive.

The presence of an onset accentuation in the syllables improved the noise tolerance by ~4 dB as compared to syllables without accentuation. Contrary to the naïve expectation that a longer pause between syllables would be easier to detect in the presence of noise, lengthening the pause from 15 to 22 ms worsened the noise tolerance by 2.5 dB. Hence, an accentuation peak at the beginning of a sound pulse seems to be a feature particularly suited to improve signal recognition in noise. In contrast, the simple prolongation of a period of quietness before sound onset appears to be an inferior strategy. The necessity of reliable signal recognition in the presence of noise may have been a driving force that led to the strongly amplitude modulated structure of acoustic signals observed in many grasshopper species.
In order to estimate how a decline in song pattern recognition may correlate with an increase in the variability of neuronal responses, data of behavioural and electrophysiological experiments (on *Locusta migratoria*) will be compared. For this purpose, a spike train metric was employed to quantify the distances between neuronal responses to noise contaminated stimuli (van Rossum 2001, Neural Comput 13: 751).

# Neurite Specific Ca2+ Dynamics Underlying Sound Processing in an Auditory Interneurone

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Concepts on neuronal signal processing and integration at a cellular and subcellular level are driven by recording techniques and model systems available. The cricket CNS with the Omega-1-Neurone (ON1) provides a model system for auditory pattern recognition and directional processing. Exploiting ON1's planar structure we simultaneously imaged free intracellular Ca2+ at both input and output neurites and recorded the membrane potential in vivo during acoustic stimulation. In response to a single sound pulse the rate of Ca2+ rise followed the onset spike rate of ON1, while the final Ca2+ level depended on the mean spike rate. Ca2+ rapidly increased in both dendritic and axonal arborisations and only gradually in the axon and the cell body. Ca2+ levels were particularly high at the spike-generating zone. Through the activation of a Ca2+ sensitive K+ current this may exhibit a specific control over the cell's electrical response properties. In all cellular compartments presentation of species-specific calling song caused distinct oscillations of the Ca2+ level in the chirp rhythm, but not the faster syllable rhythm. The Ca2+ mediated hyperpolarisation of ON1 suppressed background spike activity between chirps, acting as a noise filter. During directional auditory processing the functional interaction of Ca2+ mediated inhibition and contralateral synaptic inhibition was demonstrated. Upon stimulation with different sound frequencies the dendrites, but not the axonal arborisations, demonstrated a tonotopic response profile. This mirrored the dominance of the species-specific carrier frequency and resulted in spatial filtering of high frequency auditory inputs.

# Towards a better understanding of the complex auditory behaviour in crickets: Combining a sensitive trackball system with single cell recordings from brain neurones

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Recognition and localization of sound signals is fundamental to acoustic communication. In female crickets, which orient towards the male's calling song, current models propose pattern recognition mechanisms based on the temporal structure of the song. Previously it was assumed that localization of the sound source is achieved by comparing the outputs of two separate recognition networks by integrating information over several sound pulses enabling the female to steer towards the better pattern. However, recent studies by Hedwig and Poulet (Nature 430, 2004; JEB 208, 2005) have revealed that the females started steering already to the first pulse. These rapid steering responses occurred with the latency of 55-60 ms, well before any pattern recognition could have taken place in the brain recognizers. These new findings have been made possible with development of the highly sensitive trackball system, which allows to measure walking behaviour at the level of the animal's stepping cycle. A number of behavioural experiments employing this new system have revealed previously undetectable features of complex auditory behaviour which call for carefully designed neurophysiological experiments to be carried out in order to reveal the neural basis of cricket phonotaxis.

A set-up has been developed to enable recording from brain neurones while the animal is walking on the trackball. The animal's head is fixed into a plastic dish using bees-wax and resin mixture while the rest of the body is left to move freely. The front of the head capsule is opened to reveal the ventral side of the brain. A spoon with a light guide is inserted under the brain and the metal ring is used to stabilise it. Preliminary results show that the set-up provides for stable intracellular recordings and successful staining of brain neurons.

We'll use this system to tackle questions that have arisen from behavioural experiments using the new trackball. In order to clarify how steering signals are generated as information passes from ascending to descending neurones, we will first establish how well the sound pattern of the cricket song is preserved in different types of auditory neurones in the brain and also how walking affects pattern preservation in those neurones. Recording from descending brain neurones and changing their spike activity by current injection will provide us with insight into effect of certain descending neurones on cricket's motor activity and involvement in steering. Behavioural experiments have also revealed that the pattern recognition gates unselective steering in crickets (Poulet and Hedwig, PNAS 102, 2005) and we aim to identify the modulatory pathways involved in this phenomenon.

# STRUCTURE AND FUNCTION OF *DROSOPHILA* AUDITORY NEURONS

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*Drosophila melanogaster* uses antennal hearing to detect the songs of courting conspecifics. Understanding how these songs are processed by the fly's nervous system requires detailed knowledge about the structure and function of the auditory pathway, starting from the primary neurons comprised by the fly's auditory organ, Johnston's organ (JO), up to higher auditory centeres in the brain. Using the UAS-GAL4 system to selectively label auditory sensory neurons, we systematically explored the structure of the fly's JO and its target areas in the brain. Axonal projections of JO neurons target five spatially segregated zones in the brain. These zones include the antennal mechanosensory and motor centre (AMMC), the ventrolateral protocerebrum (vlpr), and the suboesophagial ganglion (SOG). FLP-out analysis revealed that most JO neurons project to only one of these zones. Cell bodies of neurons projecting to the same zone cluster together in JO, suggesting distinct roles in sensory processing. Efferent control mechanisms, as known from vertebrate auditory systems, seem not to exist: blocking synaptic transmission by the pan-neuronal expression of tetanus toxin neither affected the mechanical amplification provided by JO neurons nor the compound action potentials recorded from their axonal projections in the antennal nerve. Functional imaging using genetically encoded calcium sensors provides first insights into the functional organization of the fly's JO.

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# Tympanal sensilla generate DPOAEs in the locust ear

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Tympanal organs of insects emit distortion-product otoacoustic emissions (DPOAEs) that are indicative of nonlinear ear mechanics. Our study seeks to define constraints of such DPOAE generation in the ear of Locusta migratoria. The peripheral auditory organ (Müller's organ) comprises ca. 80 scolopidia, each consisting of a bipolar sensory neuron that is coupled to the tympanal membrane via its accessory cells. Within the organ, 4 groups of neurons (a-d) are distinguished based on the exact attachment of their sensory dendrites to the tympanum and on their frequency tuning. The d-cells are attached to a relatively thin membrane region of the tympanum (at the "pyriform vesicle") and are most sensitive for sound frequencies above 12 kHz. The a-, b- and c-cells are coupled to a relatively thick membrane region and are most sensitive to lower frequencies.

We tested if DPOAEs in insects depend on the integrity of specific sensory neurons or their attachment to the tympanum. Mechanical lesions were set into the interconnection between the ganglion and the pyriform vesicle in order to destroy the dendrites of the d-cells. Their exclusion leads to a decrease of DPOAEs that are evoked by f2 frequencies above 15 kHz (decrease of 15 to 40 dB; mean 28 dB; n = 12 organs). DPOAEs that are evoked by lower frequencies remain unchanged. After mechanical lesions directly in the ganglion of Müller's organ in order to affect the somata of all cell groups, DPOAEs are no longer measurable across the entire frequency range examined (5 to 30 kHz). These frequency-specific changes after the exclusion of one of the cell groups suggest that auditory receptor structures are specifically required for DPOAE generation in insects.

Electrical stimulation of the auditory nerve and hence the axonal part of the sensory neurons (short current pulses of 4 to 10 µA or DC-currents of 0.5 µA) reduces the DPOAEs by as much as 30 dB. The strength of the effects depended on current intensity. The decrease is reversible, and DPOAE amplitudes recover within 1 min after the end of electrical stimulation. The electrical modulations of the DPOAE amplitude could be due to a change in the membrane potential of the sensory neurons. Severing the auditory nerve from the CNS does not change DPOAE amplitudes or their modulation by electrical nerve stimulation. Hence putative efferent fibers that could travel within the auditory nerve are unlikely to be required for a normal functioning of locust ear mechanics.

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# Directional processing of crickets differs from that in bush crickets

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Insects use directional hearing for localisation of conspecifics or prey. The directionality of the hearing system depends on peripheral factors and central mechanisms. Crickets and bush crickets both have their ears in the front legs. In both groups males are singing and females perform phonotaxis. In some bush crickets, females instead may reply to a song and males perform phonotaxis. Even though the position of the ears, the auditory function of a trachea running from the tympana to a mesothoracic spiracle and a number of central neurons indicate a common origin of hearing in the two groups, there are also speculations about independent origin (see discussion in Jost MC, Shaw KL (2006) Mol Phyl Evol 38:510).

Recent studies have demonstrated for a bush cricket (*Ancistrura nigrovittata*) that in addition to peripheral directionality (mainly sound shadow, since the ears most likely function as pressure receivers in the behaviourally relevant range) and central sharpening through contralateral inhibition by the well known omega neuron there must be one or more additional neurons involved in sharpening of directional processing (Molina J, Stumpner A (2005) J Exp Zool 303A: 1085). This has been demonstrated with photoinactivating one omega and later eliminating a whole ear while recording from the mirror image omega. For crickets data available made it more likely that omega 1 (ON1) is the only local neuron for contralateral inhibition, but this has not been tested directly.

Therefore we performed the same sequence of experiments in the cricket *Gryllus bimaculatus* as in the bush cricket *A. nigrovittata*. The only difference is elimination of the contralateral ear: in bush crickets this had been achieved by cutting the leg housing the ear, since the auditory tracheae of the two legs are not functionally connected. In crickets, however, the tracheae and tympana of both sides are functionally coupled. Therefore cutting a leg would have changed the impedance of the whole system and by this might have changed properties of the contralateral ear. As a consequence, we waxed the tympanic membranes of one leg with hot wax, which at the same time most likely damaged the sensory neurons by the heat. Control experiments demonstrated efficacy of the procedure. As a result, we were able to demonstrate that killing one omega reduced directional responses of its mirror image partner and that additionally eliminating the contralateral ear lead to no additional change in directionality. This was clear with 16 kHz, but less clear with 5 kHz (where variability of responses was very high and directionality often was low in the beginning). We interpret the results in the following way: While in bush crickets the omega neuron and other interneurons together perform directional inhibition of auditory interneurons, in crickets this task is exclusively performed by the omega neuron 1 (ON1). Therefore, the auditory network of crickets and bush crickets differs in basic properties.

# Adaptation of auditory neurons in a bushcricket

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The adaptation of auditory neurons was examined in the ensiferan bushcricket *Ancistrura nigrovittata*. This species processes frequencies from about 2 to above 50 kHz and its receptors show tonotopic organisation (Stumpner 1996). Each receptor represents in its responses a specific, more or less broad frequency range. The tonotopy is also present in the first thoracic ganglion. Excitation of interneurons depends on their arborisations in the auditory neuropil and thus on the frequency range they receive by receptors. The omega-neuron for example processes a broad range of frequencies and is important for directional hearing (Molina und Stumpner 2005).

Coding of stimulus strength at high intensities is not possible, especially when the operating capacity of the neuron is restricted by the absolute refractory period. To increase this operating capacity at long lasting stimuli neurons start to reduce their firing rate while adapting to the stimulus.

The adaptation of receptors and a local interneuron, the omega-neuron, was measured in this study. The effect of adaptation of auditory neurons in the first thoracic ganglion is shown via intracellular recordings while stimulating the animal with sound of different intensities at 16 or 28 kHz. A standardised method for analysis was applied for all tested neurons, in which the onset of firing to a 300 ms stimulus was compared to the adapted state near the end of the stimulus. The mean firing frequency at the onset is about 380 Hz for omega-neurons, but only 310 Hz for receptors. The adapted state shows a frequency of 80 Hz for omega-neurons and 180 Hz for receptors. A typical adaptation of an omega-neuron therefore is about 75 to 80 %. The receptors only show a response reduction of nearly 50 %. The omega-neuron is in series to the receptors. Therefore the receptor adaptation must be taken over and additional adaptation seems to take place in the omega-neuron.

In this state of research it is unclear whether the omega-neuron receives excitatory input from other auditory interneurons. Intracellular depolarisations have revealed an intrinsic adaptation of the omega-neuron of about 20 %. Inhibitory input from the contralateral ear might add to the adaptation.

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# Localization of a sound source by the parasitoid fly *Emblemasoma auditrix*

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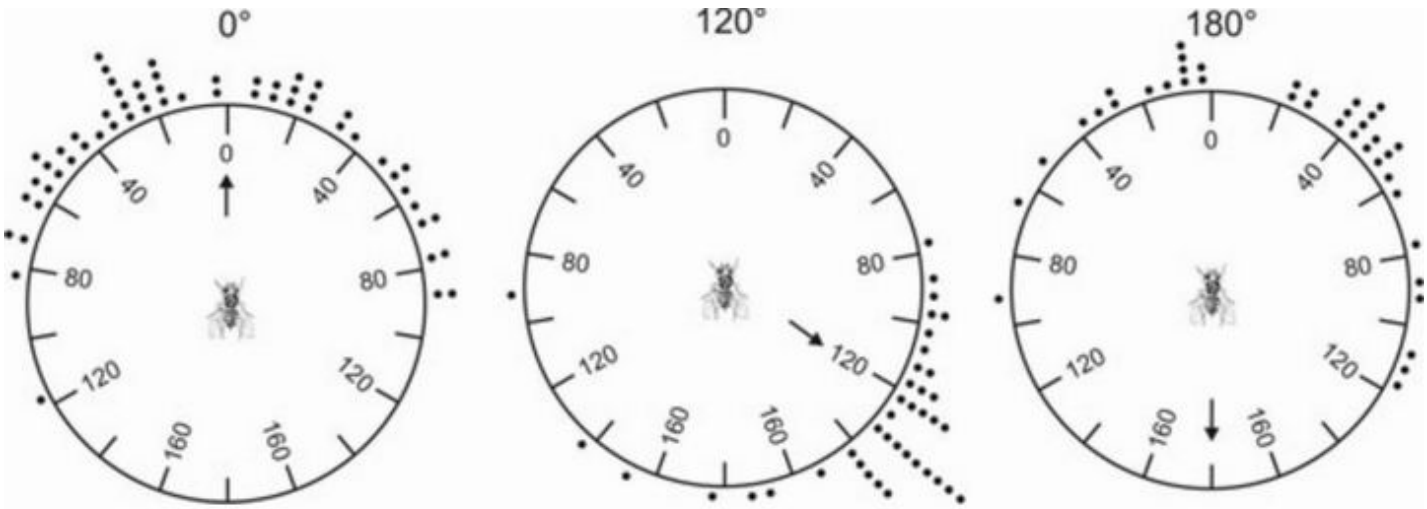
The parasitoid fly *Emblemasoma* auditrix acoustically locates its cicada host. Major cues for this phonotaxis are intensity differences either between left and right ear. We analysed this positive phonotaxis in behavioural experiments. The localization ability was analyzed in the horizontal plane. After perception of the hosts calling song, the fly locates the direction of the sound source often by turning movements. These movements correlate to different azimuth loudspeaker directions:

1. A frontal sound incidence usually elicits a turn either to the left or right side (Fig.).
2. A sound from behind (180°) results in a turn similar to that from frontal (Fig.). Thus, the fly cannot discriminate sounds from frontal or behind.
3. The laterality of a sound source is located correctly between 20° and 100°. Azimuth locations between 100° and 180° can result in incorrect turns towards the opposite direction.
4. In most cases the fly does not turn precisely towards the direction of the loudspeaker (Fig.). The mean error is approximately 40°. This error is due to a stepwise turning resulting in an "overshoot". In a number of cases the overshoot is corrected by a second turn in the opposite direction, although in summary this does not improve the precision.

Thus, horizontal localization is possible, despite minute intensity differences between both ears.




Turning movements of a fly in response to three azimuth loudspeaker directions. Each dot represent the direction before forward movement started. Loudspeaker direction is indicated b an arrow.

# Are developmental constrains important for the formation of an insect auditory system?

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Insect audition evolved mainly for three functions: intraspecific communication, predator detection and host location. The morphology and physiology of the very divers insect ears have been viewed in the light of these functions and their ecological constrains. However, the formation might also be constrained by development.

An example might be the auditory system of cicadas. Adult cicadas have an elaborated auditory system, with up to 2000 sensory cells. No plausible explanation for this comparatively very large number has been given so far. Many other species perform intraspecific communication with less than 100 sensory cells. We propose that the number in cicadas is a result of the development of the sense organ. By contrast to the adults, larval cicadas live underground and do not possess an auditory organ. However, they might need the very same sensory cells for other purposes like vibration reception.

We investigated whether the nymphs already possess the "auditory" sensory cells of the adults and studied the transformation of the sense organ. Scanning electron microscopy was used to analyse the external structures of the second abdominal segment of larvae. The ear in the adults is located in the second segment with a thin tympanic membrane backed by a tracheal airsac.
Neuroanatomically we investigated the sense organ by serial sectioning and backfilling of the axons. Backfills show that the central projection of the larval sensory cells into the thoracic ganglia is similar to that of the adult auditory organ. Behavioral and physiological experiments were designed to reveal the function of the larval sense organ. We propose that the sensory organ is a vibration receiver. Such a receiver seems important for the underground life style in respect to predators and competition. The larvae suck on tree roots where they form small holes occupied by a single larva in the ground. In behaviour experiments the larvae can hurt each other resulting in deadly injuries. Thus, it might be important to avoid other individuals.

For this proposed function a complex vibration sensor might be needed. The comparative data indicate that vibration receptors often have large numbers of sensory cells. Thus the large number found in adults might be a result of the necessities of the larvae. This cenogenesis might also apply for the formation of auditory organs in other insect orders, like the Coleoptera.

# Signal transmission and detection for a public and private mode of communication in a neotropical katydid

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Due to the conspicuousness of long distance sound signals, they do not remain private to intended receivers, but are subject to eavesdropping by unintended receivers. If the eavesdropper is a parasitoid or predator, this may have dramatic consequences for the signaller´s fitness (1). This strong selection pressure resulted in evolutionary adaptations that reduce the conspicuousness to the predators. Some species of neotropical katydids (Pseudophyllinae) are known to replace their airborne sound signals by tremulations (substrate-borne vibrations) in response to predation by foliage-gleaning bats which are attracted by calling songs of their prey (2). Here we report a facultative shift in signalling behaviour of a katydid, from air-borne sound to tremulation signalling, depending on the ambient light conditions. To characterize the costs and benefits of the two communication channels we investigated the active range of both signal types and their detection under natural conditions.

The relationship between air-borne sound and tremulation signals correlated significantly with the lunar cycle, and thus the ambient light conditions at night. Under new moon conditions, males signalled on average 7.5 more often by air-borne sound compared to tremulation, in contrast to a ratio of only 2.2 under full moon conditions. Thus, under higher illumination at night males spent significantly more time signaling in the more private mode of communication.

The katydid *Docidocercus gigliotosi* produces a tremulation signal with a duration of about 1s and an unusual low carrier frequency of 13Hz. The preferred roost site is a plant of the pineapple family, *Achmea magdalena* (3). The long, fleshy leaves of the plant have resonator-like properties at frequencies between 10 – 20 Hz, and thus transmit the tremulation signal with almost no attenuation at all. The signal is therefore suprathreshold for potential receivers at any position on the plant; the active range of the signal is about 4 meters. By contrast, the airborne sound signal is a short call of 20 ms at a carrier frequency of 20 – 25 kHz, repeated every 8 – 10 seconds. The active range of the airborne-sound signal in the understory of the rainforest was determined with the “biological microphone”-approach (4) and varies between 22 and 35 meters, depending on vegetation density.

The detection of both types of signal was studied by recording afferent activity of airborne-sound and vibration receptors under conditions including the natural background noise in both communication channels. Background noise in the airborne-sound channel is high at night and produces significantly more false alarms compared to the vibration channel, where background noise levels are low.

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# Sensory bias in the peripheral auditory system of field crickets: Directional hearing and preference for certain carrier frequencies

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Female crickets are attracted to the calling songs of males. These songs are composed of chirps with a species-specific temporal pattern of syllables and inter-syllable intervals. The calling song in Gryllus bimaculatus is broadcast at a carrier frequency relatively narrow tuned to 4.7 kHz. We investigated two possible cases of a sensory bias in this communication systems related to the carrier frequency:

A) Because carrier frequency was found to be negatively correlated with body size of males it has been suggested that female preference for larger males might be based on this acoustic parameter (1). We performed behavioural two-choice experiments with females on a trackball system where females could choose to orient to otherwise identical calling songs, which differed in the carrier frequency within the natural range of variation of a population (4.2 - 5.2 kHz). Contrary to previous results females always preferred the frequencies corresponding to the best frequency in the tuning of the interneuron AN1, which has been shown to represent the afferent interneuron carrying information for phonotaxis (2). Thus, as suggested from the sensory bias hypothesis, females prefer the signal which generates the greatest amount of afferent excitation, rather than lower frequencies with a higher threshold in the AN1-neuron and less afferent action potentials. Female receivers differ in the best frequency of the AN1-tuning (range 4.5 to 5.2; most females at 4.8-5.0 kHz). Individual females prefer in choice experiments the frequency which corresponds to the best frequency of the AN1-neuron, which further corroborates the sensory bias hypothesis.

B) The peripheral auditory system of field crickets represents a complicated pressure gradient receiver with three relevant sound inputs, and a phase shifter providing a phase delay for sound from the contralateral side (3). The system provides highest directionality only at one frequency; thus the ear also shows a directional tuning. Behavioural experiments on the trackball demonstrate that the phonotactic path of females with only little deviation from a straight line is elicited at one particular frequency, and calling songs deviating by 200-300Hz from this frequency cause the female to perform larger turns. The frequency of highest directionality in behaviour of individual females correlates with the frequency which produces best directionality in the ears of these females. The frequency optimum of directional tuning varies between individuals, with a mean value at about 4.4-4.6 kHz.

C) The frequency optimum of the directional tuning in individuals may differ from the frequency optimum of the neuronal tuning. Thus, there is a trade-off between attraction to a signal providing strongest afferent excitation, but smaller directional cues for orientation.

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# Poster Topic

## T19: Auditory system II: Vertebrates

**T19-1A** Binaural Masking-Level Difference in the Barn Owl: Electrophysiological Correlates
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**T19-2A** Localization Behavior of Free-flying Barn Owls (Tyto alba): Effects of Frequency and Level on Latency
*L. Hausmann, M. Singheiser, DTT. Plachta and H. Wagner, Aachen*

**T19-3A** Electrophysiology of identified chicken auditory brainstem neurons in primary culture
*T. Künzel, MJ. Wirth, H. Wagner and H. Luksch, Aachen*

**T19-4A** Localization Behavior of Free-flying Barn Owls (*Tyto alba*): Effects of Frequency and Level on Striking Accuracy
*M. Singheiser, DTT. Plachta and H. Wagner, Aachen*

**T19-5A** Binaural Masking-Level Difference in the Barn Owl: Behavior
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**T19-6A** Expression of Chloride-Transporters in the Avian Auditory Brainstem
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**T19-14A** Converting an ion transporter into a molecular motor for sensory amplification
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**T19-15A** Multiple Pathways for Novelty Detection in the Auditory System of the Barn Owl.
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**T19-2B** Identifying Effective Stimulation Parameters for an Auditory Midbrain Implant
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**T19-3B** Electrical Stimulation above the Hearing Threshold Enhances the Effect of Neurotrophic Factor Treatment in Deafened Guinea Pigs
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**T19-11B** Perception of environmental sounds - theoretical considerations
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**T19-12B** Is the primary auditory cortex unimodal?
Anatomical connections of field AI with other sensory systems
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**T19-13B** States in the ongoing cortical activity carrying information in discrimination learning of differential electrical stimulation
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*M. Deliano, H. Scheich and FW. Ohl, Magdeburg*

**T19-14B** Backward masking effects produced by intracortical microstimulation in the auditory cortex
*A. Engelhorn, B. Dann, M. Deliano and FW. Ohl, Magdeburg*

**T19-1C** Structural left-right asymmetries in rodent auditory cortex
*A. Laszcz, J. Goldschmidt, FW. Ohl, H. Scheich, M. Schildt, H. Schulze, W. Wetzel, W. Zuschratter and E. Budinger, Magdeburg*

**T19-2C** Auditory-nerve first-spike latency: a physiologically motivated model
*H. Neubauer and P. Heil, Magdeburg*

**T19-3C** Differential effects of iontophoretic application of the $GABA_A$-antagonists Bicuculline and Gabazine on tone-evoked local field potentials in primary auditory cortex.
*H. Schulze, C. Möller and S. Kurt, Magdeburg and Ulm*

**T19-4C** Two types of neuronal firing in the auditory cortex of monkeys during the categorical task performance
*E. Selezneva, H. Scheich and M. Brosch, Magdeburg*

**T19-5C** Activity-dependent changes of the inhibitory MSO input after hearing onset
*B. Hassfurth, F. Werthat, O. Alexandrova, A. Magnusson, B. Grothe and U. Koch, Martinsried*

**T19-6C** ITD discrimination ability of the Mongolian Gerbil (*Meriones unguiculatus*) in a six alternative choice setup
*T. Kindermann, L. Wiegrebe and B. Grothe, Martinsried*

**T19-7C** Auditory size normalization in the auditory pathway of the Mongolian Gerbil *(Meriones unguiculatus)*
*G. Schebesch, U. Firzlaff, L. Wiegrebe and B. Grothe, Martinsried, Planegg*

**T19-8C** Comodulation masking release determined in the mouse (*Mus musculus*) using a flanking-band paradigm
*KB. Klink, VN. Weik and GM. Klump, Oldenburg*

**T19-9C** Localisation dominance in the Mongolian Gerbil, Meriones Unguiculatus and the effect of stimulus frequency
*M. Wolf, M. Schuchmann and L. Wiegrebe, Planegg - Martinsried*

**T19-10C** Mechanisms for Echo Roughness Encoding in the Inferior Colliculus of the Spearnosed Bat *Phyllostomus Discolor*
*F. Borina, G. Schuller, U. Firzlaff and L. Wiegrebe, Planegg-Martinsried*

**T19-11C** Sonar hyperacuity revisited: echo-acoustic evaluation of a jittering surface in the bat *Glossophaga soricina*
*HR. Goerlitz and L. Wiegrebe, Planegg-Martinsried*

**T19-12C** Processing of Complex Activity Patterns at the Calyx of Held Synapse: A Computational Analysis
*J. Hermann, B. Grothe and A. Klug, Planegg-Martinsried*

**T19-13C** Reversible Plasticity of Binaural Neurons in the Mammalian Auditory Brainstem
*E. Schiller, I. Siveke and B. Grothe, Planegg-Martinsried*

**T19-14C** Cross-modal integration of sensory information in auditory cortex
*C. Kayser, C. Petkov, M. Augath and N. Logothetis, Tuebingen*

# Binaural Masking-Level Difference in the Barn Owl: Electrophysiological Correlates

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Humans and animals are able to sense signals in noisy environments. This task is easier when the noise and the signal have different spatial locations. In the auditory system, binaural masking level difference (BMLD) is the release from masking caused when masker and target have different binaural configurations. We investigated neural mechanisms underlying release from masking in the inferior colliculus in 55 neurons of 4 of barn owls. The level of the masking noise remained constant, while the level of the target signal, a pure tone, varied. The detection threshold of IC neurons to best delay tone in presence of best delays noise ($N_{BD}S_{BD}$) was compared to the detection threshold of tone at its best delay in a best delay noise with 180° phase shift ($N_{WD}S_{BD}$). Detection threshold in $N_{WD}S_{BD}$ was about 4 dB lower than $N_{BD}S_{BD}$ configuration. Measuring BMLDs at higher noise levels led to BMLDs of about 10 dB, which is comparable to psychophysical observations.

# Localization Behavior of Free-flying Barn Owls (Tyto alba): Effects of Frequency and Level on Latency

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Barn owls (Tyto alba) are able to catch prey using auditory information alone. Target localization suffers when frequencies >5 kHz are removed, but in contrast to Payne`s findings (Payne, 1971: Acoustic Location Of Prey By Barn Owls (Tyto alba). J.Exp.Biol. 54; 535-573), owls still achieve a hit rate of about 50% under closed-loop conditions, even at a given stimulation bandwidth of 1-2.5 kHz. Even under open-loop conditions frequency bands below 5 kHz don't prevent the owl from striking, but cause a strong decrease in striking precision and hit rate down to less than 10% hit.

The owl localizes its prey by turning the head towards the direction of a sound source before take-off. It is a worthwhile investigating whether a longer latency is associated with a higher striking precision; or if the hit rate is independent from the time spent in localization behavior until the owl becomes airborne. In a series of free-flight experiments with barn owls, conducted in a darkened free-flight room, the owls had to localize a target by their auditory system solely. The owls were trained to fly from a perch to one of four target loudspeakers, one of them emitting a pulsed sound signal of 500 ms signal length interrupted by a 1 s silence. The signal was stopped as soon as the owl left the perch (open-loop condition). The positions of the speaker ramps were altered between three distances (2.35 - 3.35 m) to the perch, to prevent the owls from memorizing the spatial position of the target speakers. Correct flights were rewarded with a piece of meat after every trial. A constant masker noise at 33 dB SPL reduced the background noise and allowed discrete manipulation of the signal-to-noise (S/N) ratio between masker noise and tested band-limited noise stimulus from -10 dB to +10 dB. Frequency bands of the test stimuli were varied (1-2.5 kHz, 1-10 kHz, 5-10 kHz) to analyze their influence on latency. The latter was measured using a laser barrier: time count started with stimulus onset and stopped with the owl`s take-off.

A reaction time task can be parsed into perception, decision making and action. The decision component is a "bottleneck" of serial and hence limited processing. Thus, latency should increase with increasing task difficulty (decreasing S/N ratio). In our experiments this general assumption could not be confirmed: no significant difference in latencies for both owls at any frequency band was found. This lack of correlation between latency and S/N ratio or signal bandwidth suggests that latency could be more related to individual strategies than to physical stimulus parameters like frequency band and S/N ratio. This hypothesis is further supported as owl Huibu constantly showed shorter latencies than owl Unkas within the 1-10 kHz- and 5-10 kHz-signal, even at significant levels (U-test, p-value <0.035) for the 5-10 kHz signal and the 1-10 kHz signal, for a S/N ratio of 5, 0 and -5 dB. Interestingly, for the low-frequency band (1-2.5 kHz), Huibu`s latencies were no longer significantly shorter than for Unkas. Huibu was more experienced in performing free-flight experiments; differences in latencies may be due to her familiarity to the task.

# Electrophysiology of identified chicken auditory brainstem neurons in primary culture

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In birds, the brainstem nuclei N. magnocellularis (NM) and N. laminaris (NL) contain the second and third order neurons of the auditory pathway. The neurons are part of a circuit that performs a computation similar to a multiplication, thus achieving the detection and initial representation of interaural time differences. The neurons are specialised for fast, faithful and unambiguous transmission of stimuli. It is not yet known by which mechanisms the physiological specialisations of this neurons emerge in development.
We developed a serum-free culture system of NM/NL neurons that were prelabelled by injection of fluorescent retrograde tracers. The auditory region of the hindbrain was excised, dissociated and cultivated in serum-free conditions after ex-vivo tracing. Whole cell patch recordings (current and voltage clamp) were performed using a fluorescent microscope to identify the neurons prior to recording.
We compared basic electrophysiological properties, action potential firing patterns, action potential kinetics and voltage clamp behaviour of the neurons at different culture days. During the first 5 days in vitro NM and NL neurons had a resting membrane potential of ~ -47 mV and input resistances of 1298 and 1490 MΩ respectively, indicating an immature state of development. Most of the neurons (NM: 41%; NL: 47%) recorded between DIV 1 and DIV 5 fired short, rapidly damped action potential trains upon a depolarising current step, but the typical phasic firing pattern known from slice recordings in older stages of these nuclei was also found. The action potential duration (half-width NM: 5 ms; NL: 4,48 ms) was long compared to slice recordings in older stages, but shorter than recordings from a culture system of E7 brainstem neurons made in our lab at corresponding culture days.
These results show, that the prelabelled NM/NL neurons can be maintained and analysed in vitro. The damped action potential firing pattern appears to be a developmental step towards the specialised phasic firing patterns and may correspond to an incomplete composition of voltage-activated potassium channels (Kv). In the next step we want to analyse the development of Kv in further detail and will try to elucidate the influence of normotypic synaptogenesis (NM-NL synapse) in the culture. Eventually, our aim is to reconstruct the NM-NL circuitry using biohybrid culture techniques.

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# Localization Behavior of Free-flying Barn Owls (*Tyto alba*): Effects of Frequency and Level on Striking Accuracy

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As nocturnal hunters, barn owls (*Tyto alba*) are perfectly adapted to localize and strike prey in total darkness by sole use of audition[1,2,3]. As most free-flight experiments were conducted under closed-loop conditions[1,3] or without determination of the signal-to-noise ratio (S/N ratio)[2], this experimental series was an attempt to investigate the effects of different frequency bands and various S/N ratios on the striking accuracy of freely flying barn owls.

Two owls were trained to fly from a perch to one of four target loudspeakers inside a completely dark (<< 0.001 $cdm^{-2}$) sound reduced free-flight room. To prevent the owls from learning fixed spatial speaker coordinates, the distance between target speaker and perch was altered prior to each experimental session. Target signals consisted of three different noise bands: 1-2.5 kHz, 5-10 kHz and 1-10 kHz. We used an open-loop mode for stimulation where the presentation of the pulsed target signal (pulse duration: 500 ms and silent interval: 1 s) was terminated by the owl leaving the perch. For presentation of five different S/N ratios, a constant masker (Gaussian noise, bandwidth 1-12 kHz, 33 dB SPL measured at perch position) was presented, thus allowing the adjustment of S/N ratios spanning from +10 dB to -10 dB in steps of 5 dB. Flight data and landing positions were recorded using infrared cameras and an infrared tracking system.

A total of 500 flights were performed for both owls. Overall striking percentage at the low frequency signal, regardless of S/N ratios, was significantly reduced compared to the broadband signals (0.0071, $\chi^2$ test) as well as high frequency signals (0.0217, $\chi^2$ test) for owl Huibu. In general, owl Unkas displayed similar results including significantly higher striking percentages for the signals with high frequency components of 5-10 kHz ($p< 0.0001$, $\chi^2$ test). Additionally, the novice owl Unkas provided a higher striking percentage as well as accuracy than the experienced owl Huibu (0.0297, $\chi^2$ test). Only during broadband stimulation (1-10 kHz) a clear trend became evident for the variation of the S/N ratios: both owls displayed a significantly higher striking percentage at the S/N ratios of +10 and +5 dB compared to the other ones. The two other stimuli (1-2.5 kHz, 5-10 kHz) did not elicit any trend of this kind. When the high frequency signals were presented, a S/N ratio of -5 dB provoked higher striking rates than S/N ratios of ±0 dB and +5 dB, which is consistent for both owls. In summary, both owls are able to approach targets under open-loop stimulation but striking percentage is inferior to closed-loop situations or to behavioral tasks, where the owls received at last one additional in-flight stimulus[2].

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# Binaural Masking-Level Difference in the Barn Owl: Behavior

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In humans, binaural masking-level difference (BMLD) is a measure of detection. When a tone is played over headphones together with masking noise tone detection is improved when either tone or noise is inverted in one ear. BMLD is assumed to be computed by binaural correlation and is, therefore, dependent on phase locking. Until now BMLD has been tested only in animals in which phase locking is limited to low frequencies. Since the barn owl shows phase locking up to 9 kHz, we expected BMLD also at high frequencies.

We trained two barn owls in a tone in noise detection task. A tonal signal (S) was played together with a correlated broadband noise (N). Tone and noise had either no interaural phase difference (IPD) ($N_0S_0$ or diotic configuration) or the tone had an IPD of half the tone period ($N_0S_\pi$ or dichotic configuration). The signal to noise ratio (SNR) was changed randomly between trials. The SNR spanned a range of up to 60dB around signal detection threshold. Occasionally a catch trial was added where only the noise was present. During a session the owl was sitting in a sound attenuating chamber. The acoustic stimulus was played to the subjects via headphones. Two response keys were placed in front of the subjects, one to the right and one to the left. The owls have learned to peck on the key to the right when they detected a tone and to peck on the key to the left if they only hear the masking noise.

We found that the percent of tone detection was strongly correlated with SNR. When the tone became fainter tone detection decreased in a psychometric curve. In both owls the psychometric curve for dichotic stimulus configuration was shifted towards lower SNR compared with diotic stimulus configuration. We observed a BMLD up to 9 dB at 5 kHz and a BMLD of about 16 dB at 2.5 kHz. These values are close to what is found in humans at low frequencies. These are the first data for high-frequency BMLD.

# Expression of Chloride-Transporters in the Avian Auditory Brainstem

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GABA is the major inhibitory transmitter in the brain. In most brain regions of adult vertebrates the inhibitory effect is based on hyperpolarisation. Chloride ions enter the neurons via $GABA_A$ receptors following the electrochemical gradient. The potassium-chloride-cotransporter 2 (KCC2) is presumably responsible for the low intracellular chloride level. Early in development, the electrochemical gradient for chloride is reversed in cortical and sub-cortical neurons. Chloride ions leave the neurons via $GABA_A$ receptors and induce a depolarisation. Functionally, this seems to support calcium homeostasis during development. Many authors hold the sodium-potassium-chloride-cotransporter 1 (NKCC1) responsible for the high intracellular chloride level.

In the auditory brainstem of birds (chicken), the depolarising effect of GABA persists into adulthood. Nucleus laminaris and N. magnocellularis receive GABAergic input from the Nucleus olivaris superior. This input is depolarising but inhibitory due to a shunting inhibition. The intracellular chloride level seems to be increased. The molecular basis for this increase is still unknown.

We report here the expression patterns of several chloride transporters in the avian auditory brainstem on protein and mRNA level. We find expression of NKCC1 protein in the auditory nuclei of E10 chicken. We also find KCC2 expression at the same time point. We shall further analyse the developmental profiles of expression in chick and barn owl.

# Water wave analysis with the lateral line system in the clawed frog, *Xenopus*: Multiple simultaneous waves, change of wave speed

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Our previous studies had shown that *Xenopus* localizes and distinguishes water surface waves with undiminished accuracy when two waves impinge simultaneously. Evidence was presented that this analysis is based on comparison of the superposition patterns at several lateral line organs (Elepfandt et al., Göttingen Neurobiology Report 2005). In the present study we tested capabilities of wave analysis with three concurrent waves impinging from different directions. We further tested if change of wave velocity affects localization.

**1. *Xenopus* can localize and distinguish waves when three waves impinge simultaneously**

With three waves, two experiments were done. In the first, waves of identical frequency were presented simultaneously to test wave localization. The criterion was turn of *Xenopus* toward one of the wave sources. Under such conditions, *Xenopus* had no problem to locate individual wave directions.

In the second experiment with three waves, one of the waves had a different frequency and the capability was tested to localize this wave. Controls were made that waves could only be distinguished on the basis of frequency. *Xenopus* had no problem in identifying and localizing the different wave.

**2. Wave localization in *Xenopus* is accomplished by a mechanism other than simple measurements of time differences**

General opinion is that *Xenopus* identifies waves by measuring when exactly the crests and troughs of the waves pass different organs and by subsequent analysis of the time differences between organs. Such analysis of time differences should midlead when waves run with altered velocity. Under natural conditions, surface waves of a given frequency run with a constant velocity. By changing water surface tension by, e.g. adding surfactants, wave velocity can be changed. We managed to reduce the velocity of a 28 Hz wave by 30%. Nevertheless, *Xenopus* correctly localized the wave.

# Modeling the Activity of the Auditory Nerve after Sensorineural Hearing Loss

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Hearing loss can be caused by a variety of factors, for example damage to the middle ear, damage to or loss of the hair cells in the cochlea, or even by damage to the auditory nerve. The resulting changes in the auditory nerve activity after hearing loss can only be measured to a very limited extent and length. In a model ear, however, the desired stimuli can be presented as long as needed, and different types of hearing loss can be induced and studied extensively.

To quantify the impact of hearing loss on auditory nerve activity, we use the Auditory Modeling System (AMS), which has been developed in the group of Ray Meddis in Essex, UK (see e.g. Meddis et al. 2001, JASA). The model is based on a phenomenological description of the ear, from the pinna to the cochlea, cochlear hair cells, and synapses to the auditory nerve. AMS therefore is able to reproduce several key features of basilar membrane and auditory nerve response in healthy ears.

Here we implemented various types of hearing loss in AMS by changing the relevant, biophysically plausible parameters. We focus on hearing loss due to cochlear disfunctions that are caused by malfunctioning or loss of hair cells. Cochlear hair cells are often affected by acoustic trauma, aging and ototoxic drugs like cisplatin, which is used in cancer treatments.

We describe and analyze the statistics of typical changes in auditory nerve activity caused by damage to outer hair cells or the stereocilia of inner and/or outer hair cells. Our results show that the changes in the auditory nerve activity strongly depend on the type of the hearing loss, which might help in developing more specific hearing aids. Results based on this study may also give detailed input for studies of the auditory nervous system such as modeling studies on tinnitus.

# Differences in effect profiles of two brominated environmental compounds on the auditory brainstem response in rats and relation to thyroid hormones - benchmark analysis of effects

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The development of the auditory system is known to depend on sufficient supply with thyroid hormones in the early postnatal phase in rats. Consequently, substances which affect the thyroid hormone system may induce hearing impairment when applied during critical developmental phases. Here, we present effects of two abundant brominated flame retardants - tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD) - which are widely used in personal computers and other electronic equipment, on the auditory brainstem response in adult rats after pre- and postnatal exposure. Like other halogenated compounds which are persistent in the environment, TBBPA and HBCD are reported to exert influences on the thyroid hormone system in reporter gene assays and in vivo after subacute treatment. To examine effects on auditory function, rats were exposed to TBBPA at 8 dose levels, ranging from 0 to 3000 mg/kg body wt, or 8 dose levels of HBCD in the range from 0 to 100 mg/kg body wt. Exposure started in the parental generation and was continued in the offspring after birth. Adult rats from the offspring were sedated with ketamine and xylazine and brainstem auditory evoked potentials (BAEP) were recorded after stimulation with clicks and tone pips. TBBPA caused marked dose-related elevations in BAEP thresholds in the low frequency range from 0.5 to 4 kHz only in female offspring. In both sexes, latencies of waves II and IV were prolonged by exposure with more pronounced increases seen on wave IV compared to wave II and in males compared to females. This outcome suggests that the apical part of the cochlea may be the primary site of the TBBPA-induced effects in female rats, while the progressive increases in latencies of later waves in male rats indicates a predominant neural effect. In contrast, HBCD caused effects on the BAEP only in male rats. Elevated thresholds and prolonged latencies were seen in the low frequency range up to 4 kHz. No progressive latency increases were detected on later waves, suggesting a predominant cochlear effect. In littermates of TBBPA-exposed rats, effects on circulating thyroid hormones were observed which is consistent with the assumption of a thyroid hormone-mediated effect. On the other hand, circulating thyroid hormones were not affected in HBCD-treated littermates, while they could be detected in rats with subacute treatment, so that the endocrine relation of HBCD-induced effects on the BAEP remains unclear at present. A benchmark analysis was used to determine critical effect doses (CED) and the lower confidence limits (BMDL) for exposure-related effects. Benchmark analyses revealed BMDL values in the range from 1 to 10 mg/kg and <1 to 25 mg/kg for auditory effects of TBBPA and HBCD, respectively, which in the case of TBBPA were similar to effects on thyroid hormones. (Supported by EU contract QLK4-CT-2002-00596).

# Oscillating neurons in the cochlear nucleus: Experimental evidences for a new simulation topology, simulation results, and consequences for pitch perception

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Intrinsic oscillations in the millisecond range were observed in different processing centers of the auditory system, like the cochlear nucleus (Young et al., 1988 in Bahmer and Langner, 2006a) and the inferior colliculus (IC, Langner 1981, 1983, and Langner and Schreiner 1988). It was hypothesized that the oscillations in the IC originate from the cochlear nucleus (Langner, 1992).

Our analysis of chopper intervals (Bahmer and Langner, 2006a) measured by Young et al. (1988) in the cat revealed a significant preference for multiples of 0.4 ms. ($P = 0.05$).

Our hypothesis is that the intrinsic oscillations play a role as an internal time reference for pitch estimation. The pitch of an amplitude modulated signal corresponds approximately to its modulation frequency. However, when the carrier frequency is varied the elicited pitch corresponds more closely to a subharmonic of the carrier. Moreover, with high resolution of the carrier steps in the resulting pitch curves could be observed (Langner, 1981). Because the corresponding pitch periods were multiples of 0.4 ms, the pitch steps were postulated to result from a correlation process that includes intervals of intrinsic oscillations as a time reference. On the basis of these and additional (e.g. Ferragamo et al., 1998) evidences we suggest a model of chopper neurons which are arranged in a circular network with a synaptic delay of 0.4 ms (Bahmer and Langner, 2006b).

The model (executed in NEURON and Matlab) consists of Hodgkin-Huxley-like and leaky integrate-and-fire models of chopper and onset neurons. The more or less spectral integration of the onset neuron is transferred to the chopper neurons and enhances the dynamic range of periodicity encoding. The broad band input from the onset and the narrow band input from the auditory nerve enables chopper neurons to combine different ways of auditory signal analysis.

Our simulation shows that the width of the spectral integration is important for example for the resolution of single components of sinusoidal amplitude modulated sine waves (SAM) and the ability of chopper neurons to encode periodicity information over a wide dynamic range but it is unsuitable for encoding of single frequency components. While a small integration width enables chopper neurons to code resolved frequency components, it is of disadvantage for encoding periodicity at high levels, whereas a wide integration width allows encoding of encoding periodicity at low levels. A preference for different integration widths in different subjects may account for a dichotomy found in pitch perception (Schneider et al., 2005).

In an improved version of this model longer intervals are created by neurons with appropriate time constants which sample down fast oscillation frequencies. Both models can reproduce the mean, standard deviation, and coefficient of variation of the interspike intervals of real chopper neurons as well as the dynamic features of periodicity coding of chopper neurons.

Small networks of chopper neurons may be a prototype for oscillating networks in the whole brain.

# Effect of amplitude and modulation depth on periodicity coding in the auditory midbrain

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In communication signals the temporal envelope, i.e. its periodicity, is a characteristic and important signal feature. Processing of this feature depends on a highly specialised interaction between different centres of the auditory brainstem and midbrain. Our research therefore focuses on central inferior colliculus (ICC) of the auditory midbrain, because it integrates periodicity information from preceding processing centres. The important role of the ICC in processing signal periodicity has been shown in many earlier studies (for review see Langner, 1992, Hear.Res. 60:115). In this study we address the question whether and how amplitude and modulation depth influence the periodicity processing in ICC-neurons of the Mongolian gerbil (meriones unguiculatus).

We use sinusoidal amplitude modulated signals (SAMs) as model of complex tones with a distinct periodicity. Their relatively simple structure - compared to more complex harmonic sounds - facilitates the tracking and interpretation of their processing. We varied signal amplitude over a range of 60 to 90 dB in 5dB steps depending on the neurons threshold and modulation depth from 10% to 100% in steps of 10% of such stimuli during the recording of single cells in the ICC. The influence of these stimulus parameters on overall rate and timing of neural activity was evaluated.

Our findings suggest, that neurons in the ICC show an average dynamic range of 50 dB for SAM signals, which is similar to that of pure tones. Neuronal synchronization is strongly influenced by signal amplitude. On average best synchronisation can be found at about 20 to 30 dB above threshold and decreases with increasing sound intensity. The impact of modulation depth seems to be smaller in comparison. An increase of modulation depth of 90% equals an sound level increase of just about 30dB in means of spike rate. Increasing the modulation depth by 10% improves synchronisation by about 3%, quantified by vector strength.
In summary, the results show that some neurons in the ICC (20%) have optimal ranges for intensity of SAM signals. 80% of the ICC neurons show either an monotonic increase of the spike rate or saturate for high sound intensities. Modulation depth is coded best, in terms of spike rate, for higher intensities and leads to the best synchronisation for low or medium intensities. The present findings allow an optimisation of stimuli requirements for ICC Neurons in Gerbils. This will be useful in further studies of periodicity coding.

# Short term light deprivation increases accuracy of sound localization in humans

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Crossmodal reorganization processes in the brain are mainly associated with early blindness, on the assumption that recruitment of genuine visual areas, such as primary visual cortex, for non-visual functions results in superior auditory and tactile performance of blind, compared to sighted, humans.
This study shows that in sighted subjects the accuracy of sound localization, measured by a task of head-pointing to acoustic targets, is reversibly increased after short-term light deprivation of 90 min. However, only the systematic deviations from target positions (constant error) were reduced after light deprivation, while the general precision of head pointing remained unchanged. Return to pre-deprivation values was observed after 180 min of re-exposure to light. The post-deprivation change was similar, though less in magnitude, to the effect of blindness that was demonstrated previously [1].
Generally, these findings indicate that auditory-visual crossmodal plasticity can be quite rapidly initiated by deprivation of the visual cortex from visual input. It seems possible that visual deprivation has an influence on neuronal circuits, that are involved in processing of auditory information in visual brain areas of normal sighted humans. Since exclusively the constant error in sound localization, not general performance, was changed, the present effect of visual deprivation may, however, not be attributable to reorganization processes in the sense of a compensation for the absence of vision. It is more likely that the observed change in accuracy was specifically induced by the absence of visual calibration of the neural representation of auditory space [2] during light deprivation.

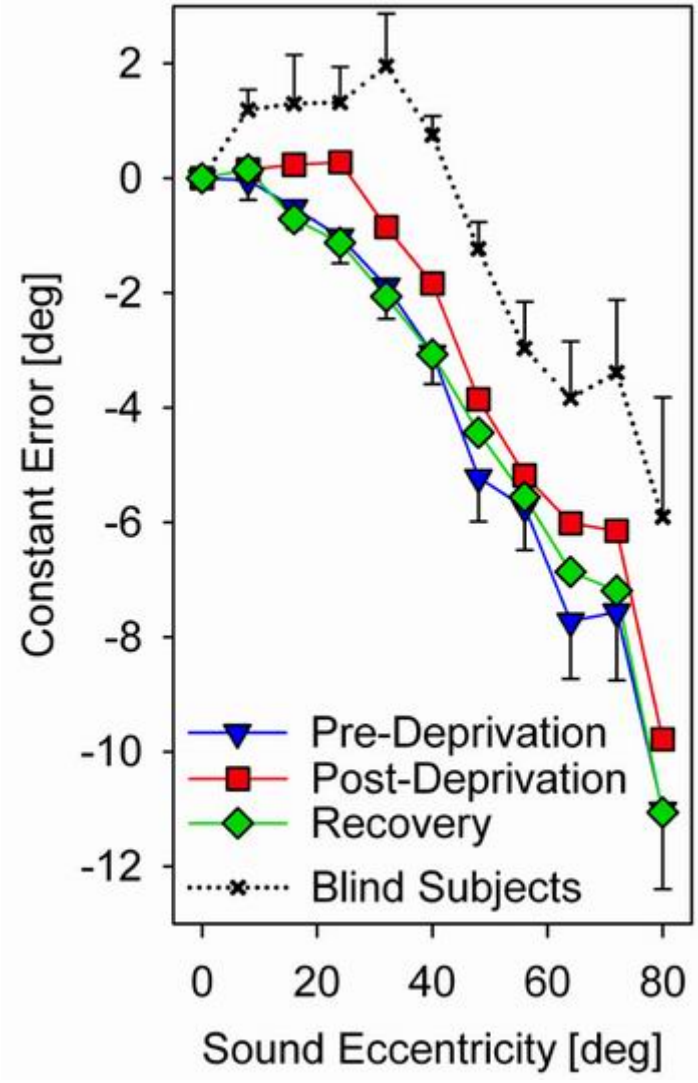


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Figure. Mean constant error of head-pointing responses, plotted as a function of sound eccentricity. Data are shown in comparison with results from a previous study [1], in which blind subjects were tested using the same task of head pointing as in the present study. Negative values indicate underestimation of eccentricity, positive values overestimation.

# Neuronal Adaptation in the Awake Rat Auditory Cortex

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Clear representation of behaviorally relevant stimuli in a noisy environment is one of the major challenges for the auditory system. One possible solution to this problem are differentiated responses according to stimulus statistics. In this respect, rare sounds (deviants) are more effective in directing attention and processing resources than more frequent ones (standards). This effect is paralleled by the well established 'mismatch negativity' in event-related potentials (Näätänen et al. 2001) when stimuli are presented in an 'oddball' stimulation paradigm. Only few studies have investigated adaptation under such conditions at the single neuron level (e.g. Ulanovsky et al. 2003) and most of them were done under anaesthesia.

We examined how low probability sounds are represented by single neurons in awake rats. Chronically implanted animals with up to 4 movable electrodes (SE and tetrodes) in both hemispheres of the auditory cortex were tested with sequences of pure-tones (duration 100-200 ms, 50 dB SPL) under free field conditions. Sequences of repetitive standard stimuli were randomly interspersed with low probability deviants (10% to 30%) of different sound frequency (up to 1 octave).

A high percentage of neurons showed clear effects of adaptation. A stimulus specific adaptation index (SI) (after Ulanovsky et al. 2003) was calculated for analyzing the neuronal response to a tone as standard compared to as deviant. The magnitude of the effect depended on stimulus statistics. Adaptation increased with lower deviant probability (and higher standard probability) significantly. Additionally, the adaptation index was computed for different time windows after stimulus onset and, as a control, 100 ms before stimulus onset. Most significant adaptation effects were present in the phasic On-response of neurons. Integrated over the whole stimulus duration, the adaptation effect decreased rapidly.

# Converting an ion transporter into a molecular motor for sensory amplification

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Prestin (SLC26A5), a member of the SLC26 anion transporter family, is the motor molecule of mammalian outer hair cells (OHCs). It contributes to cochlear amplification in mammals. As a result of prestin's motor activity, OHCs respond to oscillating voltage changes elicited by incoming sound waves with macroscopic length changes, thereby greatly enhancing hearing sensitivity and frequency selectivity. This phenomenon is termed electromotility. Prestin´s electromotile properties are thought to result from voltage-dependent movements of an electrically charged voltage sensor, which subsequently trigger a force-producing conformational reorientation of the protein. Movement of the voltage sensor through the membrane's electrical field gives rise to an electrical current upon changes in membrane potential. This can also be measured as a voltage-dependent membrane capacitance, usually termed 'non-linear capacitance' (NLC). Due to the tight coupling between voltage sensor movement and electromechanical output, NLC can be used as an electrical indicator of electromotility. We have previously suggested that electromotility may be related to an incomplete anion transport cycle of prestin.

To investigate this hypothesis, we cloned two prestin-orthologs from chicken and zebrafish. Here we show that these non-mammalian SLC26A5 proteins are electrogenic divalent/chloride exchangers with a 1:1 stoichiometry. However, they do not generate electromotility. By contrast, rat Prestin (rPres) is not a functional anion transporter, but generates electromotility. Mutational exchange of small amino acid sequence stretches conferred motility-related NLC to non-mammalian SLC26A5 anion transporters. Equivalently, transferring circumscribed parts of zebrafish SLC26A5 onto the mammalian ortholog enabled functional divalent anion transport by rPres.

Thus, minor changes in amino acid sequence can convert an SLC26A5 anion exchanger into a voltage-driven membrane motor and vice versa.

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# Multiple Pathways for Novelty Detection in the Auditory System of the Barn Owl.

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The behavioral response to a stimulus is largely dependent on its context. Novelty detection is an example of a vital context-dependent task performed by the brain. Here we attempt to characterize a neuro-correlate of novelty detection, stimulus-specific-adaptation (SSA), in the midbrain and forebrain sound localization pathways of the barn owl.

Two paradigms were used to characterize SSA. One is the oddball paradigm, based on presenting probabilistic stimuli in which two differing stimuli are presented with different probabilities - rare and frequent. The second is a novel paradigm of repetitive stimulations in a constant order, creating the equivalent effect of rare and frequent stimuli. We find that responses to rare stimuli are indeed significantly stronger (SSA effect) both in the midbrain and forebrain. However, in the midbrain we report a distinction between the inferior colliculus (IC) and the optic tectum (OT). Similar to the forebrain, the OT showed an SSA effect both to changes in frequency and in interaural time difference (ITD), whereas in the IC SSA effect emerged only in frequency and not in ITD. Interestingly, we find that there is a corresponding SSA effect in response latency in the forebrain and OT, but not in the IC. In addition we report SSA effects for sound intensities which can not be explained by synaptic depression. SSA effects in the intensity domain were absent in IC but clear in the OT.

Our results demonstrate that novelty detection is an invariant property of the localization pathways of the barn owl. Different sound properties with diverse neural representations show similar SSA effects, highlighting the importance of novelty in the internal representation of sensory information. The absence of SSA effects in the IC leads us to suggest that the forebrain circuitry is engaged in novelty detection and that novelty signals are conveyed from the forebrain to the midbrain to control behavioral responses in a context dependent manner.

# Binaural interactions in cortical field A1 of congenitally deaf cats

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Congenitally deaf white cats are a model of human congenital deafness. In their auditory system effects of auditory deprivation on auditory functional properties can be studied. The present investigation was aimed at representation of binaural cues at the level of the auditory cortex. In hearing controls the hair cells were pharmacologically destroyed at the beginning of the experiment to prevent electrophony. To stimulate the auditory system, electrodes of cochlear implants in monopolar or wide bipolar configurations were used. Mapping of the field A1 using microelectrode-recorded local field potentials with stimulation at the ipsi- and contralateral ear was performed in anaesthetized animals using a train of three biphasic pulses (200µs/phase) at a frequency of 500 Hz. In a cortical region of interest (1 $mm^2$, positions of LFPs with largest amplitudes) LFPs obtained with ipsilateral and contralateral stimulation were compared. The results revealed differences in the ipsilateral representations in deaf animals when compared to controls. Additionally, single- and multi-units were recorded in the ROI during binaural stimulation. For each unit, thresholds to stimulation at ipsi- and contralateral ears were determined first with pulsatile stimulation. Sensitivity to interaural time difference (ITDs) in the range of 0-1000 µs was tested with single pulses and pulse trains (500 Hz, 3 pulses) at intensities of 0 - 10 dB above unit's threshold. Sensitivity to interaural level differences (ILDs) were investigated under constant average binaural intensity at 0-10 dB above threshold. In congenitally deaf animals sensitivity to both ITD and ILDs were found with electrical stimulation. These results demonstrate a sensitivity of the auditory cortex to binaural cues also in congenitally deaf. However, responses in deaf animals differed from hearing controls in their intensity dependence. Also, temporal structure of the responses differed significantly, demonstrating the effect of auditory experience on binaural interactions at the cortical level.

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# Identifying Effective Stimulation Parameters for an Auditory Midbrain Implant

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The auditory midbrain implant (AMI) is now in clinical trials. Although the first implanted patients achieve different loudness, pitch, and temporal percepts using the AMI, it is still not clear how to stimulate the inferior colliculus central nucleus (ICC) to achieve intelligible speech perception. As an initial step towards identifying effective stimulation strategies, we investigated the effects of different ICC stimulus parameters (i.e. phase duration, pulse rate, amplitude modulation depth and frequency) on auditory cortical activity in a guinea pig model. Multi-site silicon probes were used to record from different layers and isofrequency regions within the primary auditory cortex (A1) in response to stimulation (monopolar, cathodic-leading pulses) of different isofrequency locations within the ICC using our human AMI penetrating array. As expected, A1 neural thresholds decreased as the phase duration (41-984 µs) increased where the lowest charge per phase was observed at 41 µs. However, the threshold versus phase duration curves varied across different stimulation locations along the ICC laminae suggesting that different population of neurons within the ICC and higher auditory regions may be activated. Similarly, depending on stimulation location, cortical neurons exhibited different synchronization properties that could span a wide range of pulse rates (~10 to 70 pps) and modulation frequencies (~20 to 60 Hz). For higher pulse rates and modulation frequencies, cortical neurons usually ceased firing after the onset response and exhibited larger rebound activity. Furthermore, different cortical neurons responded optimally to different combinations of pulse rate, modulation depth, and modulation frequency. Overall, these findings demonstrate that location of implantation within the ICC and selection of certain stimulus parameters can affect the extent and effectiveness of auditory cortical activation. Different combinations of stimulus parameters identified in this study will be implemented in our AMI patients and hearing performance will be assessed.

# Electrical Stimulation above the Hearing Threshold Enhances the Effect of Neurotrophic Factor Treatment in Deafened Guinea Pigs

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Sensory-neural hearing loss leads to degeneration of spiral ganglion cells (SGCs). Several studies indicate that the SGC degeneration can be reduced by electrical stimulation (ES). Furthermore, treatment with neurotrophic factors (NF) such as GDNF and BDNF provides a protective effect to SGCs. To investigate if the combination of both interventions has a synergistic effect, we investigated the SGC-density in deafened guinea pigs after delayed treatment with GDNF or BDNF and ES above the hearing threshold. Additionally, we investigated the potential of ES to maintain the effect of a treatment with BDNF on spiral ganglion cells.

Pigmented guinea pigs were systemically deafened by a co-administration of kanamycin and ethacrynic acid. Three weeks after deafening the left cochleae were implanted with an electrode/cannula device. The cannula was attached to a mini-osmotic pump (flow rate: 0.5 µl/h) filled with GDNF (100 ng/ml), BDNF (100 ng/ml) or artificial perilymph (AP). The drugs were administered into the inner ear for four weeks. In additional groups of animals drug treatment was combined with electrical stimulation via a portable stimulator for 24 days, 24 hrs a day. Another group of animals was treated with BDNF for two weeks followed by a two weeks period of electrical stimulation. 48 days after deafening the cochleae were extracted and prepared for histology. The outline of each Rosenthal´s canal profile was traced and all SGCs in this area were counted to generate a SGC-density, expressed as the number of SGCs in an area of 10.000 $\mu m^2$.

Our results demonstrate that both, ES and NF treatment caused significant spiral ganglion cell survival when compared to the control group with the effect being more pronounced for the NF. The combination of ES and GDNF application resulted in a significant enhancement in SGC survival compared to the application of GDNF or ES alone.

We conclude that the combination of local intracochlear delivery of NF and simultaneous electrical stimulation of the inner ear above the hearing threshold has more potential for SGC-protection than one of the two interventions alone.

# High resolution representation of left and right hemispace in auditory cortex: a comprehensive electrophysiological and behavioral study

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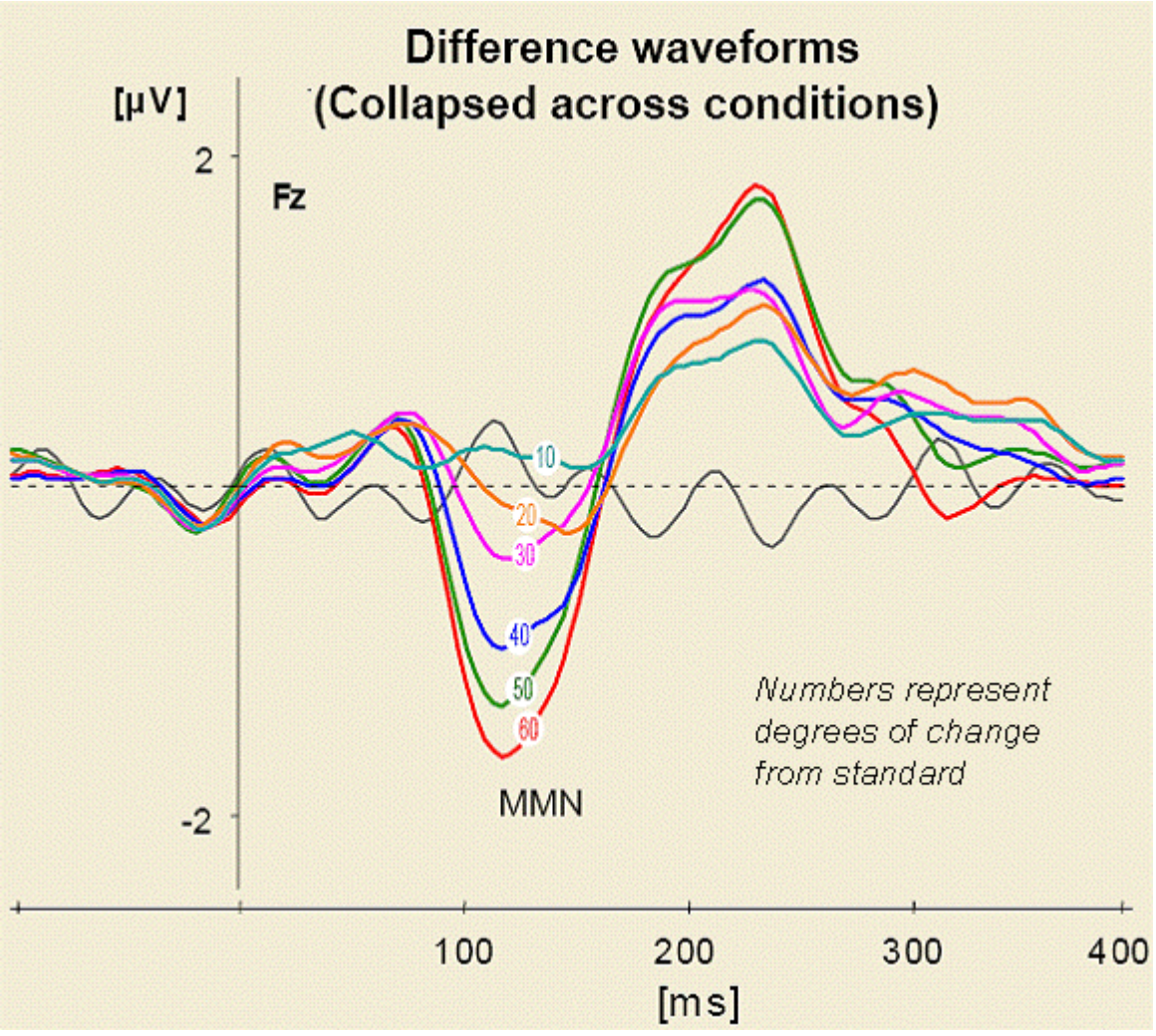
The representation of auditory space in the human cortex is unclear. While behavioral evidence suggests that the human auditory system is sensitive to azimuthal differences of less than 10°, neurons in the auditory cortex of cats and monkeys show broad receptive fields. We measured the neural correlate of human spatial change detection in 17 subjects, using the mismatch negativity (MMN) event related potential, a marker of preattentive auditory processing with major sources at the auditory cortex. We assessed both the electrophysiological and the behavioral change detection measures for 10, 20, 30, 40, 50 and 60° of deviation of sound location from the standard location, comparing the left and right hemispaces, as well as the direction of deviation (towards or away from the midline). The MMN was measured in an unattended session, while during the attended session we asked the subjects to detect auditory spatial changes. The MMN response displayed a double peak, more evident in the case where the standard was closer to the midline. Both peaks showed a significant effect of deviance, with a near linear relationship between the magnitude of spatial change and the MMN amplitude throughout the range (see figure). In contrast, speed and accuracy of performance improved up to 30° and then reached a plateau. There was a significant interaction between the direction of deviation and deviation magnitude for the early peak, with a steeper slope for the case in which the standard was lateral and the deviation was towards the midline. No difference was found between deviations on the left and on the right for either MMN peak. We conclude that the positions of auditory events are accurately tracked at the level of the auditory cortex, with no indication of hemispace differences.



# CI stimulus artifacts in CAEP recordings from cochlear implant patients

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Cortical auditory evoked potentials (CAEP) are important tools for evaluating the benefit of a cochlear implant (CI) and the maturation of the auditory system in children (Beynon et al. 2002, Sharma et al. 2004). A major problem in recording CAEPs in CI patients are electrical artifacts resulting from the activity of the implant device. In contrast to other artifacts, the CI-generated artifacts are time-locked to the stimulus and, thus, are preserved in the averaged signal. To avoid masking of the biological response, offline minimization of the CI artifact is attemped with various techniques, e.g. independent component analysis or optimized differential reference (Gilley et al. 2006). The effect of remote current sources on the recorded signals depends on the properties of the recording system (electrodes and amplifiers). Therefore, CI-generated artifacts might mask the biological signal to a different extent in different recording systems. Consequently, different recording systems might be differently suited for recordings of CAEPs in CI patients.

In the present study, we compared the amplitude and spatial distribution of the CI artifacts in EEGs recorded with two EEG systems with low and high input resistance, repectively: 1) a 32 channel Neuroscan 32 (Neuroscan) working with electrode impedances below 5 kOhms and 2) a 128 channel GES 250 (Electrical Geodesics) working with electrode impedances in the range of 50 kOhms.

Experiments were performed with 4 adult CI patients, each measured on both systems. CAEPs were recorded during auditory discrimination tasks (oddball paradigm) with tonal stimuli (1 kHz , 2 kHz) and with speech sounds (/da/,/ga/). Stimuli were presented with a free field loud speaker at 30 dB hearing level. Data acquisition and analysis were performed with frequency filters open.

Results: From all patients, CI artifacts appeared in the responses from electrodes in the vicinity of the CI. According to the position of the CI device, the spatial distribution of contaminated electrodes varied between subjects. For two patients, the distribution was comparable between the recording systems. For the other two, however, the GES 250 recordings showed the artifact also on ipsilateral frontal electrodes including FP1 and FP2, respectively, which were not affected in the Neuroscan recordings. In spite of the short stimulus duration (80 ms for the tonal stimuli), the artifacts lasted for more than 1 s. With the GES 250 system, the amplitudes of the artifacts were severalfold higher as compared to the Neuroscan system, reaching values of 300 µV. The results are discussed.

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# Comparison of protein profiles in the rat inferior colliculus and cerebellum by two-dimensional gel electrophoresis and mass spectrometry

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Proteins, e.g. enzymes, transcription factors, transporters, and receptors, form the basis of most cellular processes. Functional differences between tissues are therefore reflected by differences in the composition, abundance, and subcellular localization of proteins. The mammalian brain consists of numerous anatomically and functionally distinct regions and modern proteomic technology allows us to pinpoint differences in the protein profiles of two such regions at large scale. As a first step in a series of comparative protein profiling studies, we have analyzed the proteome of the inferior colliculus and the cerebellum of adult rats. Proteins were isolated from the tissues and separated into two fractions by differential centrifugation, enriched for cytoplasmic and nuclear proteins, respectively (Guillemin et al., 2005; Proteomics). These subproteomes were separated on two-dimensional polyacrylamide gels and stained with fluorescent dye, yielding protein maps for both regions on which a total of 322 protein spots were identified using MALDI-ToF mass spectrometry. Because more than 95% of the identified proteins were present in both brain regions analyzed, we quantified differences in protein abundance by comparing intensities of matching spots with specialized image analysis software, resulting in a relative abundance ratio (regulation factor) for each spot pair. Fifty proteins displayed differences in abundance between the two regions. More than half of the proteins up-regulated in the inferior colliculus could be categorized into a group pointing to increased metabolic activity. In the cerebellum, most up-regulated proteins play key roles in processes such as plasticity and learning. This demonstrates that a global proteomic approach can resolve functional differences between two brain regions: The inferior colliculus is a hub of the auditory pathway and receives a large number of inputs that fire action potentials at high frequency. It was identified previously as the brain region with the highest metabolic rate, whereas the cerebellum is a well-known center for motor learning.

Focusing on specific brain regions, instead of analyzing the brain in its entirety, provides the advantage of a reduced sample complexity and facilitates the detection of individual differences. To underline this fact, we prepared an additional set of gels loaded with whole brain samples. Using this reference, characteristic differences were less pronounced, and partially fell below the detection limit. We suggest that for the study of protein patterns in the mammalian brain, focus on anatomically and functionally defined substructures is a superior approach than analyzing the brain as a whole.

# Impaired structural development of the superior olivary complex of mice lacking the CaV1.3 subunit of L-type calcium channels

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CaV1.3-/- mice lack functional L-type calcium channels which are essential for normal transmitter release from cochlear inner hair cells. As a consequence, their central auditory system is virtually deprived of incoming activity, both during early development and after the onset of hearing (at around P12 in normal mice). Spontaneous activity triggered by Ca2+ spikes in the inner hair cells is thought to sculpture normal auditory brainstem circuitry. Here we analyzed the effect of missing CaV1.3-/- channels on the structural development of the superior olivary complex during early postnatal life. Interestingly, the lateral superior olive (LSO) of 12-day-old CaV1.3-/- mice displayed a much smaller volume (see poster by B. Müller et al., this meeting) and showed a striking deformation in that the typical U-shaped appearance normally apparent in coronal sections was absent (present study). The misshapen LSO implies a crucial role for CaV1.3 channels in sculpturing the cytoarchitecture of this nucleus. Remarkably, the other nuclei analyzed, the superior paraolivary nucleus (SPN) and the medial nucleus of the trapezoid body (MNTB), did not display such gross abnormalities. As the LSO deformation suggested to us an abnormal input circuitry, we assessed whether synapse formation was also impaired. To do so, we performed immunohistochemistry employing antibodies against three synaptic marker proteins, the vesicular glutamate transporter 1 (VGLUT1), the glycine transporter 2 (GLYT2), the vesicular inhibitory amino acid transporter (VIAAT), as well as the 28 kDa calcium binding protein (Calbindin). Immunofluorescent sections were analyzed by confocal microscopy and immunoreactivity (ir) was quantified. Some significant changes occurred. For example, VGLUT1-ir was weaker in the LSO, the MNTB, and the SPN of P12 CaV1.3-/- mice. By contrast, both GLYT2-ir and calbindin-ir were increased in the MNTB of mutants. Nonetheless, the differences amounted to less than 20%, indicating that the changes in the amount of synaptic proteins were only subtle. Concerning the labeling pattern, none of the marker proteins displayed any changes; immunoreactivity occurred throughout the nuclei and did not show any temporo-spatial redistribution. Together, our results demonstrate that CaV1.3 Ca2+ channels are essential for normal development of the LSO cytoarchitecture. However, the deformation of the LSO does not appear to be paralleled by abnormalities in the glutamatergic and glycinergic inputs. Likewise, Ca2+ buffering capacity is likely to be unaffected. Our results of differential effects are consistent with those of a recent study performed in the peripheral auditory system of CaV1.3-/- mice, which demonstrated an impaired regulation of Ca2+-activated K+ channels, yet normal ribbon synapse formation in inner hair cells (Nemzou et al., 2006 Neuroscience).

# Optical imaging with voltage-sensitive dyes reveals delay lines in the chick nucleus laminaris

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**Optical imaging with voltage-sensitive dyes reveals delay lines in the chick nucleus laminaris**

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The localization of a sound source in space is realized by processing the inputs from the two ears. Two parameters are involved in this process, interaural level differences and interaural time differences. The 'place theory'-model formulated by Jeffress (1948, J Comp Psychol) claims that two physiological elements are essential for the computation of interaural time differences, namely delay lines and coincidence detectors. The Jeffress-model is almost ideally implemented in the auditory pathway of bird: Neurons of the nucleus magnocellularis (NM) have axons that form delay lines; the somata of neurons in the nucleus laminaris (NL) receive NM-input and operate as coincidence detectors. In the chick, electrophysiological recordings at different positions along the NM axon trajectories have indirectly shown the presence of delay lines (Overholt et al., 1992, J Neurosci). So far, the continuous recording of a signal that travels along in a single axon or a bundle of axons has not been done. Here we investigated the spread of excitation over the entire NM-NL projection with an optical imaging approach. Brainstem slices from embryonic day (E) 14-18 chicks were incubated with the voltage-sensitive dye RH795. The ipsilateral NM-projection to the NL was activated by placing an electrical stimulus electrode into the NM of the same side. The contralateral NM-projection to the NL was activated by stimulating the axon trajectories from the contralateral side at midline. Recordings with a high spatio-temporal resolution were obtained at 34ºC with a photodiode-array containing 464 single diodes (Neuroplex, RedShirtImaging). Optical signals were simultaneously acquired with a sample frequency of up to 100 kHz. At ipsilateral stimulation, the average interval between the peak amplitudes obtained at different positions along the NL amounted to 144 ± 147 µs per 100 µm (n = 11 slices). When stimulating the contralateral inputs to the NL, the mean peak amplitude interval per 100 µm amounted to 427 ± 156 µs (n = 9 slices), which was significantly longer than the situation at ipsilateral stimulations (p = 0.0006). Our data indicate that in the chick the contralateral NM-NL projection reflects a delay line, whereas the ipsilateral NM-NL projection does not.

# CaV1.3-/- mice show a disturbed development in the lateral superior olive of the auditory brainstem

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CaV1.3 channels belong to the group of L-type calcium channels involved in the regulation of transmitter release. Mice lacking CaV1.3 (CaV1.3-/-) have a strongly reduced transmitter release in cochlear inner hair cells, demonstrating the major role of this channel in depolarization-induced calcium entry (Glueckert et al., 2003, Hear. Res. 178, 95-105). CaV1.3-/- mice show a degeneration of the inner and outer hair cells in the cochlea and are deaf (Platzer et al., 2000, Cell 102, 89-97). Due to the altered synaptic transmission, CaV1.3-/- mice generate no spontaneous activity in the auditory nerve. This experience-independent activity (before hearing onset) is thought to be important for the development of the auditory pathway. In order to test this hypothesis, we compared anatomical and electrophysiological properties of the lateral superior olive (LSO), a prominent nucleus of the central auditory system, of wild type (WT) and CaV1.3-/- mice. Nissl stained slices of the auditory brainstem from postnatal day (P) 12 old animals were used to investigate the anatomical properties of the entire LSO. In addition, passive and active membrane properties of single LSO neurons around P3 and P12 were analyzed with whole-cell patch-clamp recordings. The volume of the LSO in CaV1.3-/- mice amounted to 0.016 ± 0.002 mm3 which was smaller than that in WT mice (0.037 ± 0.002 mm3; $p = 0.0001$). Counting the number of LSO neurons, it turned out that the cell density was higher in CaV1.3-/- (79,511 ± 10,386 cells/mm3) than in WT mice (44,476 ± 2,963 cells/mm3; $p = 0.001$). These results indicate soma-dendritic morphological differences between LSO neurons from WT and CaV1.3-/- mice. Regarding the resting membrane potential, no developmental difference (P3 vs. P12) and no difference between WT and CaV1.3-/- neurons was observed (WT: -55 ± 5 mV; CaV1.3-/-: -56 ± 4 mV). In WT cells, the capacitance increased with age (P3: 22 ± 6 pF; P12: 27 ± 7 pF; $p = 0.0086$), whereas in CaV1.3-/- cells it remained unchanged (P3: 19 ± 5 pF; P12: 21 ± 8 pF; $p = 0.2758$). The membrane resistance decreased in WT cells (P3: 163 ± 104 MOhm; P12: 64 ± 35 MOhm; $p = 0.001$), but was similar in P3 and P12 CaV1.3-/- cells (P3: 190 ± 121 MOhm; P12: 146 ± 99 MOhm; $p = 0.2837$). The transient and delayed potassium currents revealed no developmental differences in current density and channel conductance, in LSO neurons from both WT and CaV1.3-/- mice. In contrast, the activation thresholds of these currents were significantly lower at P12 in WT, but stayed the same in CaV1.3-/- cells. Concerning the transient sodium current, current density significantly increased in both groups, whereas the channel conductance showed an increase only in WT cells. Contrary to the situation with the potassium currents, the activation threshold of the sodium current declined in CaV1.3-/- cells but not in WT cells. Together, we found a retarded maturation of some passive membrane properties and differences in the development of voltage-activated channels in neurons from CaV1.3-/- mice. We conclude that the lack of CaV1.3 influences the development of peripheral as well as central auditory structures.

# Maturation of inhibitory synaptic transmission in the auditory brainstem depends on thyroid hormone

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Thyroid hormone (TH) plays an essential role in the development of the auditory system. Its deficiency causes irreversible damage both to the cochlea and the central auditory system (Knipper et al., 2000; J Neurophysiol). To gain further insight into the function of TH during development of the central auditory system, we investigated the influence of TH on the maturation of inhibitory synapses in the lateral superior olive (LSO). This auditory nucleus is an important processing center in the brainstem and involved in sound localization. For comparison, we also analyzed the role of TH in the maturation of inhibitory synapses in hippocampal neurons.

Inhibitory synapses undergo an age-related shift from depolarization to hyperpolarization, which is attributable to a decrease in intracellular chloride concentration $[Cl^-]_i$. To investigate whether this shift is under the control of TH, hypothyroidism was induced in pregnant and lactating rats by applying the antithyroid drug 1-methyl-2-mercaptoimidazole (MMI). In the LSO of untreated control animals, $[Cl^-]_i$ decreased from 44±22 mM at P3 to 8±5 mM at P12, resulting in a hyperpolarizing action of glycine at this stage (Balakrishnan et al., 2003, J Neurosci). In contrast, in MMI-treated rats, $[Cl^-]_i$ decreased only from 34±20 mM at P5-6 to 26±16 mM at P10-12. In line with this, 7 out of 9 P12 LSO neurons were depolarized upon glycine application. Thus, maturation of inhibitory synapses is delayed, if not prevented, in the absence of TH. The lowering of $[Cl^-]_i$ is caused by activation of the K/Cl-cotransporter KCC2. We therefore analyzed KCC2 in the LSO at P12 by immunohistochemistry. Both in untreated and MMI-treated animals, KCC2 was abundant and localized at the plasma membrane. This argues against an influence of TH on transcription or trafficking of the cotransporter in the LSO and suggests posttranslational modulation, such as age-related KCC2 oligomerization (Blaesse et al., 2006; J Neurosci).

To assess whether the TH effect on the maturation of inhibitory transmission is specific to the LSO, we determined the developmental regulation of $[Cl^-]_i$ in the hippocampus. Perforated patch recordings revealed no statistical difference in $[Cl^-]_i$ between untreated and hypothyroid rats both at P12 (control: 13±14 mM; MMI-treated: 11±7 mM) and P16 (control: 5±2 mM; MMI-treated: 7±3 mM).

In summary, our results show that TH deficiency influences age-related $[Cl^-]_i$ regulation in LSO neurons, yet not in the hippocampus. These data point to a novel target of TH in the auditory system and suggest a novel signaling pathway for KCC2 regulation. Furthermore, the data corroborate our previous finding that distinct mechanisms of age-dependent KCC2 activation occur in different brain areas (Blaesse et al., 2006; J Neurosci).

# Perception of environmental sounds - theoretical considerations

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Behaviour is always embedded in an environment whose properties provide the context for action. The extent to which these environmental properties can be determined by an animal depends largely on the neural structures involved in the analysis of sensory information and memory. Conversely, observing the processing of well-characterized stimuli can help to test hypotheses about the neuroarchitecture behind specific perceptual phenomena. In this paper, the neural pathways involved in the evaluation of the emotional contents of mammal vocalizations will be discussed from a theoretical perspective on the basis of behavioural, bioacoustic and neuroimaging data.

# Is the primary auditory cortex unimodal? Anatomical connections of field AI with other sensory systems

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It is still a popular view that primary sensory cortices are unimodal, but recent physiological studies have shown that they can also be activated by multiple other modalities. Here, we investigate the anatomical connections, which may underlie multisensory processes at the level of the primary auditory cortex (field AI), and which may, in turn, enable AI to influence other sensory systems. We approached this issue by means of the bidirectional axonal transport of fluorescein-labeled dextran which was injected into AI of Mongolian gerbils (*Meriones unguiculatus*).

Of the total number of retrogradely labeled cell bodies found in non-auditory sensory and multisensory brain areas, approximately 40% were in cortical and 60% in subcortical areas. Of the cell bodies in the cortical areas about 82% were located in multisensory cortex, *viz.* posterior auditory association (fields DP, VP) and posterior parietal cortex (PPC), the claustrum (Cl), and the endopiriform nucleus (En), 10% were located in the primary somatosensory (S1) and 8% in secondary visual cortex (Oc2). DP/VP, PPC, Cl/En, S1, Oc2 as well as the primary olfactory cortex also contained anterogradely labeled axons and their terminations. The laminar pattern of corticocortical connections suggests that AI receives primarily cortical feedback-type inputs and projects in a feedforward manner to its target areas.

Of the labeled cell bodies in the subcortical structures, approximately 90% were located in multisensory thalamic, 4% in visual thalamic, and 6% in multisensory lower brainstem structures. At subcortical levels, we observed a similar correspondence of retrogradely labeled cells and anterogradely labeled axons in visual (posterior limitans thalamic nucleus PLi) and multisensory thalamic nuclei (dorsal and medial division of the medial geniculate body, suprageniculate nucleus, posterior thalamic cell group, zona incerta), and in the multisensory nucleus of the brachium of the inferior colliculus. Retrograde, but not anterograde, labeling was found in the multisensory pontine reticular formation. Conversely, anterograde, but no retrograde, labeling was found in the visual laterodorsal and lateroposterior thalamic nuclei, in the multisensory peripeduncular, posterior intralaminar, and reticular thalamic nuclei, as well as in the multisensory superior and pericentral inferior colliculi, pontine nuclei, and periaqueductal grey.

Our study supports the notion that AI is not merely involved in the analysis of auditory stimulus properties but also in processing of other sensory and multisensory information.

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# States in the ongoing cortical activity carrying information in discrimination learning of differential electrical stimulation applied through a sensory cortex interface

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Electrical stimulation of sensory cortex has regained interest among researches as a tool for directly studying the relationship between cortical physiology and perception. Also, electrical stimulation is a promising approach for directly interfacing sensory cortex with a neuroprosthesis, in order to replace lost sensory function of its afferent pathways. Thereby, it is often assumed that behaviorally relevant information transmitted to the cortex is carried by populations of neurons directly excited through the electrical stimulus. However, recent studies have shown that even with current amplitudes close to threshold, activity in the cortex evoked by intracortical microstimulation (ICMS) techniques available today is highly synchronous, spatially widespread and cellular unspecific (Butovas & Schwarz, 2003). Due to the "unphysiological" properties of electrically evoked activity, ICMS is likely to interfere with the ongoing cortical dynamics, and thus might limit the capacity of cortex for neural integration and plastic reorganization. Therefore, behaviorally relevant effects of ICMS might rather emerge from transsynaptically activated neuronal populations distal to the stimulation site. In order to make inferences about the neuronal populations carrying the behaviorally relevant information in response to ICMS, we analyzed the relationship between behavioral measures of discrimination learning with ICMS and the ongoning cortical dynamics derived from electrocorticograms (ECoGs) recorded at high spatial resolution concurrently in freely behaving Mongolian gerbils (*Meriones unguiculatus*). Animals learnt to discriminate different ICSM-sites along the tonotopic gradient of the primary auditory cortex. Task related spatial cortical activity patterns were identified in the β- and γ-band of the ongoing ECoG by a multivariate pattern classification procedure. "Early" patterns occurred about 100 ms after stimulus onset, were time-locked to the stimulus, and already present in naïve animals. "Late" patterns emerged in the course of training about 1 to 2 s after stimulus onset not time-locked to the stimulus. A detailed spatial analysis of the patterns revealed remarkable differences between "early" and "late" patterns: "early" and "late" patterns carried different spatial information and were non-overlapping. In the "early" patterns spatial information was mainly carried by cortical sites strongly activated by the electrical stimuli. In the "late" patterns, spatial information was carried by cortical sites, which had been rather unaffected by the preceding electrical stimulation. Whereas the "early" patterns were evoked by the electrical stimuli reflecting the stimulation sites, "late" patterns emerged from a plastic reorganization of the ongoing dynamics, and were related to the behavioral interpretation of the electrical stimuli learnt by the animal. This reorganization might only occur in transsynaptically activated neuronal populations not driven by the electrical stimulus in an "unphysiological" way. (Supported by the BioFuture grant 0311891 of the BMBF)

# Backward masking effects produced by intracortical microstimulation in the auditory cortex

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Intracortical electrical microstimulation was used to investigate the relevance of the time structure of an acoustically evoked potential for auditory perception. Mongolian gerbils (*Meriones unguiculatus*) were trained in a go-nogo paradigm (shuttle box) to discriminate between high pitch (4 kHz) and low pitch (1 kHz) tones (250 ms, 5 ms onset and offset ramps, 65 dB SPL). After performance reached a predefined criterion, a stimulation electrode was implanted in the center of primary auditory cortex, field AI, and further trainings were performed with interspersed test trials, in which acoustical and electrical stimulation (300 ms pulsetrains of biphasic pulses (50-100µA) ) were presented concurrently. The temporal overlap was varied systematically by varying the delay between the electrical and the acustical stimuli from 0 ms to 150 ms. Results indicate different relevance of early and late evoked potential components for stimulus discrimination, i.e. early components (up to 100 ms) seemed to be more vulnerable to electrical cortical stimulation.

# Structural left-right asymmetries in rodent auditory cortex

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There are several indications of functional lateralization in auditory cortex with respect to the processing of certain temporal and spectral aspects of auditory stimuli. This is most evident in humans (e.g., left hemispheric dominance of speech processing) but also in rodents where a left auditory cortex specialization for processing pure tone sequences and right auditory cortex specialization for processing frequency modulated tones was found.

Such functional asymmetries encouraged investigations of potential corresponding anatomical asymmetries in auditory cortex. Studies on human post mortem material have described several interhemispheric differences in gross anatomical features and cortical microcircuitry of auditory cortex, however comparable studies in animals are rare and have not clearly demonstrated such asymmetries.

Recently we approached this issue by investigating the anatomy of the left and right auditory cortex in Mongolian gerbils, a frequently used animal model in auditory research. A study of the general cortical architecture, e.g. of the size of koniocortical areas, of the degree of fiber myelinization, or of the distribution of cortical marker proteins did not reveal any conspicuous differences. Consequently, we investigated here the auditory cortex anatomy at the two subsequent levels: (a) at the level of microcolumnar organization using TIMM staining; (b) at the level of single auditory neurons using juxtacellular in vivo staining with biocytin.

The comparison of size and spacing of cortical microcolumns, visualized by the specific labeling of their zinc-enriched axonal terminals (TIMM) in superficial layers showed consistent inter-hemispheric differences. The average diameter, surface area and perimeter of microcolumns as well as mean distances between them were smaller on the left side than on the right.

On the cellular morphology level, staining of electrophysiologically characterized pyramidal neurons of layer II and III revealed a inter-hemispheric asymmetry in the spatial distribution of synaptic contacts in relation to neuronal soma. For example, for neurons with the same best frequency the largest maximum of the concentration of axonal synapses fell in a closer proximity to the soma in the left hemisphere than in the right.

These data seam to be consistent with the inter-hemispheric asymmetry between cortical microcolumns.

# Auditory-nerve first-spike latency: a physiologically motivated model

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Auditory-nerve (AN) fibres are potentially able to code sounds by timing and rate of single action potentials (spikes). We have shown previously (Heil and Neubauer, J.Neurosci. 21:7404-7415, 2001) that the timing of the first spike after the onset of a stimulus can be used to specify the nature of absolute thresholds of AN fibres and the relevant physical quantity. We demonstrated that first spike thresholds of AN fibres can be specified by the integral of the temporal amplitude envelope over time rather than by the pressure amplitude or Sound Pressure Level itself. However, until now it is not clear how this finding relates to physiological processes at levels of the auditory system peripheral to the first spike. It has been speculated that the observed long latencies might arise from stochastic summation of events or of the probability of events over time (Krishna, J.Comp.Neurosci, 13:71-91, 2002; Heil and Neubauer, Proc.Natl.Acad.Sci.USA, 100:6151-6156,2003; Krishna, J.Acoust.Soc.Am. 120(2):591-593, 2006). Here we present a new model that combines short-term leaky integration and long-term stochastic temporal summation as physiologically inspired building blocks. This leaky integration, temporal summation model (LITS model) explains first spike timing of AN fibres with only three free parameters very accurately. It is shown mathematically that thresholds calculated by the LITS model are approximately proportional to thresholds specified by the temporal integral of sound pressure envelope for a large variety of pure tone stimuli with different temporal envelopes.

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# Differential effects of iontophoretic application of the $GABA_A$-antagonists Bicuculline and Gabazine on tone-evoked local field potentials in primary auditory cortex.

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γ-aminobutric acid (GABA) is one of the main inhibitory transmitters in the central nervous system. In a recent study we have demonstrated differential effects of two iontophoretically applied $GABA_A$-blockers, bicuculline (BIC) and gabazine (SR 95531), on neuronal responses in primary auditory cortex (AI) (Kurt et al., Hear. Res. 212, 224-235, 2006): Whereas the only effect of gabazine was to block $GABA_A$-mediated inhibition, BIC-application additionally induced dose-dependent side-effects, probably on calcium-dependent potassium channels. As both the blocking of $GABA_A$-mediated inhibition as well as the mentioned side-effects lead to an increase in overall neuronal activity and excitability, the increase of neuronal spiking activity was stronger after BIC than after gabazine application. Here we investigated the effects of the two drugs on pure tone evoked local field potentials (LFPs) in AI.

LFPs were recorded from the left AI of anaesthetized and unanaesthetized Mongolian gerbils before, during and after microiontophoretic application of BIC and gabazine using multi-barrel glass electrodes (5-6 μm tip diameter).

After the application of both drugs, a significant (paired t-test) increase of the N1-component of the LFP was observed in both anaesthetized (BIC: control: 480.2 μV; drug: 849.8 μV; P = 1.8E-5; gabazine: control: 333.5 μV; drug: 505.9 μV; P = 0.0002) and unanaesthetized animals (BIC: control: 185.5 μV; drug: 458.4 μV; P = 3.3E-7; gabazine: control: 438.4 μV; drug: 706.0 μV; P = 0.04), but this increase was much more pronounced after BIC than after gabazine application (over all animals: BIC: 310.3 μV; gabazine: 194.6 μV; ANOVA: P = 0.05). Furthermore, after application of BIC a prolongation of LFPs was observed that was not seen after gabazine application.

We conclude from these results that the secondary BIC effects seen in neuronal spiking responses can also be demonstrated in LFP data.

# Two types of neuronal firing in the auditory cortex of monkeys during the categorical task performance

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Category formation allows us to group perceptual objects into meaningful classes and is fundamental to cognition. One of the best-known examples of acoustical categories are frequency contours. The goal of the present study was to examine whether the firing of auditory cortex neurons reflected actually the category membership of frequency contours (rising versus falling) and not merely the physical characteristics of the single tones.

Two monkeys (macaca fascicularis) were trained to categorize frequency contours in variable sequences of 4-10 pure tones. A positive-reinforcement behavioral procedure was used and only the behavioral responses to falling frequency contours were rewarded. After the monkeys had learned this task, recordings of the neuronal firing from the auditory cortex were performed during task performance.

We identified two interrelated types of neuronal firing: increased phasic responses to tones categorically represented the reward-predicting downward frequency steps and not upward steps. Subsequently slow modulations of tonic firing predicted the behavioral decisions of the monkeys, including errors.

Our results on neuronal mechanisms of categorical stimulus identification and of decision making attribute a cognitive role to auditory cortex, in addition to its role in signal processing.

# Activity-dependent changes of the inhibitory MSO input after hearing onset

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The medial nucleus of the trapezoid body (MNTB) provides the main glycinergic input to the medial superior olive (MSO), where it contributes to the processing of interaural time differences (Nature 2002, 417: 546-548). It is known that the glycine receptors on MSO neurons undergo an experience-dependent refinement after hearing onset from a somato-dendritic to a somatic location (Nat Neurosci. 2002, 5: 247-253). These anatomical changes are probably the result of an activity-dependent change in the strength of the glycinergic synapse. The two questions arising are, first, whether the redistribution of postsynaptic glycine receptors is paralleled by a change in the axonal pattern of the MNTB-MSO projection and second, which activity pattern produces long-term plastic changes such as long-term depression or potentiation at these synapses.

To investigate whether glycine receptor redistribution is paralleled by a refinement of the MNTB-MSO projection pattern, MNTB neurons were labeled with an anterograde tracer. Micro-Ruby solution (10 %) was pressure-applied to the lateral MNTB via a glass pipette in acute brainstem slices from P10 and P25 gerbils. Following 3-5 hours incubation brain slices were fixed and counterstained with fluorescent Nissl. The traced MNTB-MSO axonal projections and the Nissl stained MSO neurons were visualized with a confocal microscope and, subsequently, 3D-reconstructed. At P25 the large majority of axonal end-segments (ES) (88 %) were located in the somatic region of the MSO compared to 62 % in P10 animals. In parallel, the total number of ES decreased by more than 60 % and the spread of ES along the dorso-ventral tonotopic axis narrowed by 50 %.

To further examine which activity patterns lead to changes in synaptic strength of MNTB-MSO synapses, whole cell patch-clamp recordings from MSO neurons were carried out in acute brainstem slices before (P11-P12) and after (P14-P15) hearing onset. Stimulation of the afferent MNTB fibers was paired with a postsynaptic spike induced by current injection via the recording pipette. This stimulus (100 Hz, 200 ms, 0.01 Hz, 10 min) resulted in long-term plastic changes of the glycinergic inputs of MSO neurons that lasted at least 30 min after the stimulation. Interestingly, the same stimulus pattern resulted in either substantial depression (the majority) or potentiation (the minority) of the glycinergic input. No change was observed when only the pre- or postsynaptic stimulus was applied.

These data taken together demonstrate a refinement of the MNTB-MSO projection along the tonotopic axis that is driven by acoustic activity. The pruning of the glycinergic inputs to the MSO and the reorganization of the MSO glycine receptors are probably interdependent. Involved in this refinement is possibly an activity-dependent change in the synaptic strength of the glycinergic synapses resulting from simultaneous excitatory and inhibitory activity.

# ITD discrimination ability of the Mongolian Gerbil (*Meriones unguiculatus*) in a six alternative choice setup

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Sound arriving from off the midline impinges upon the ear closer to the sound source earlier than at the more distant ear, generating an Interaural Time Difference (ITD). Animals use ITDs to localize sound in the low frequency range (<2kHz). This ability relies on a temporally very precise interaction of excitation and inhibition in a brainstem auditory nucleus, the Medial Superior Olive (MSO).
Previous studies determined the minimal resolvable angle of mongolian gerbils for localizing sound in a two-alternative-forced-choice (2AFC) procedure (Heffner& Heffner 1988a, Behav. Neurosci 102; Maier& Klump 2006, J. Acoust. Soc. Am).
In this study we are investigating the ITD discrimination ability in a six-alternative-choice setup allowing us to present different frequencies and different angles in a randomized order within one experiment. The experimental stimuli consist of tone, bandpass noise and broadband noise as well as different tone-to-noise levels. In difference to previous 2AFC studies the animals in this six-alternative-choice setup have to resolve angles not just off the midline but also at peripheral locations.

# Auditory size normalization in the auditory pathway of the Mongolian Gerbil *(Meriones unguiculatus)*

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The spectral information in speech signals produced by either a child or an adult varies dramatically. The vocal tract, which scales with body size, acts as a filter on the laryngeal signals. In vowels, the spectral distance between the formants decreases with increasing body size. As shown in psychoacoustic studies, listeners are able to make fine judgments about relative speaker size even beyond the physiologically normal range *(Smith et al., 2005).*Therefore, it is hypothesized that the auditory system automatically normalizes for size information. The auditory system segregates information about object size (vocal tract length) from information about object structure (vowel type).

Because of its superior low-frequency hearing, the Mongolian gerbil *(Meriones unguiculatus)* is a good animal model for human hearing. We looked for evidence for this segregation of size- and structure information in the gerbil auditory brainstem, midbrain and cortex.

Extracellular, single-unit recordings were made in the Lateral Superior Olive (LSO), the Inferior Colliculus (IC), and Auditory Cortex (AC). After determining the frequency response area (FRA) of a neuron, five voiced human vowels with a fixed fundamental frequency of 100 Hz and of five different scales, representing different speaker sizes, were presented.

The responses to the different vowels were compared to predictions of these responses based on the vowel spectra and the FRA. The quality of the prediction is expressed as the two-dimensional correlation coefficient between the predicted and recorded response matrices.

In LSO neurons, the predictability of the vowel-response matrices was high (correlation coefficient above 0.5 for 84% of the neurons). This confirms the validity of the simulation approach. In the IC and AC, predictability decreased to 32 and 18 %, respectively. This shows an increasing degree of non-linear spectral processing in the ascending auditory pathway.

In order to assess the extent to which single neurons can compensate for the speaker-size induced variations in the vowel spectra, the vowel-response matrices will be tested for specificity either along the speaker-size axis or along the vowel-type axis.

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# Comodulation masking release determined in the mouse (*Mus musculus*) using a flanking-band paradigm

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Signal detection threshold in noise with correlated amplitude fluctuations over a range of frequencies is generally lower than the threshold for signal detection in noise of the same bandwidth without such correlation. This effect has been termed comodulation masking release (CMR) and was first observed by Hall et al. (1984, JASA 76: 50) in a band-widening paradigm. The CMR effect was initially attributed to the simultaneous processing of cues available in multiple analysis channels of the auditory system (across-channel cues). With a different experimental paradigm, the flanking-band paradigm, CMR can be explained both by information processing across several auditory channels and by processing of cues within a single auditory filter (within-channel cues, e.g., Schooneveldt & Moore 1987, JASA 82: 1944). While the band-widening paradigm employs a single noise masker of varying bandwidth, two separate narrow-band noise maskers are commonly used in the flanking-band experiment. The effect of within-channel and across-channel cues can be separated from each other only when using more than a single masker band. One of the masker bands is centered at the signal frequency (on-frequency masker, OFM) while a second masker band can be centered at a frequency close to or remote from the signal frequency (flanking band, FB; for a review of both types of experiments see Verhey at al. 2003, Exp Brain Res 153: 405).

Previously we have used a band-widening paradigm to demonstrate CMR effects in the NMRI mouse (Weik et al., Proc Göttingen Neurobiol Conference 2005). Masker bandwidth ranged from 0.1 to 20 kHz and the signal frequency was at 10 kHz, i.e., in the range of most sensitive hearing of the mouse. We found considerable CMR of up to 12.8 dB for masker bandwidths of 0.4 kHz and above. Since the bandwidth of the auditory filter at 10 kHz is 3.3 kHz, much of the CMR effect in the band-widening experiment can be attributed to within-channel cues only. To further separate within- and across channel effects in this species, the present follow-up study determines CMR with the flanking-band paradigm. Behavioural thresholds for the detection of 10 kHz test signal (800 ms) were determined in a Go/NoGo procedure. The OFM (centred at 10 kHz) and the FB masker had a bandwidth of 25 Hz and were presented continuously with a spectrum level of 40 dB. The FB was centered at a frequency of between 5 kHz to 15 kHz and was either correlated or uncorrelated with the OFM. In the new experiment, the amount of CMR appeared to be lower than in the band-widening experiment. Comparing the results from both experiments suggests that in the mouse ear within-channel processing is dominant and has a large effect on the amount of CMR, whereas across-channel cues may not be very prominent.

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# Localisation dominance in the Mongolian Gerbil, Meriones Unguiculatus and the effect of stimulus frequency

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In a reverberant environment, a sound reaches a listeners' ear not only directly but also reflected by nearby surfaces, which results in multiple echoes from different directions. Each of these echoes carries its own set of localization cues and provides misleading information about the sound source's position. However the auditory system does not weigh the spatial information of a sound and its reflections equally. Only the directional information of the sound which reaches the ear first dominates the perceived position of a sound source (localization dominance). The spatial information of the echoes is suppressed. The psychophysical phenomenon relevant to sound localization in reverberant environments is commonly known as the 'precedence effect'.

Localization dominance is stimulus- dependent. The detailed influence of frequency on localization dominance is unclear so far. The present study tries to clarify how different frequencies affect localization dominance in the Mongolian Gerbil, Meriones unguiculatus.

Localization dominance in the Gerbil was examined with a two-loudspeaker paradigm designed to simulate a direct source (lead) with one loudspeaker and a single echo (lag) with an additional delay between 0 and 12.8 ms with a second speaker.

In a 2AFC experiment with food reward, pips with different frequencies (centre frequency ranging from 750 Hz up to 24 kHz) were used to train 8 gerbils to move to that side where they perceive a pip (training trials). After they learned this localization task, test trials with lead and lag are randomly interspersed (25% probability). In these test trials, the gerbils always get a food reward; i.e. the spontaneous performance of the gerbils is assessed.

Data for localization performance in these test trials, measured with pips of different frequencies, will be presented.

# Mechanisms for Echo Roughness Encoding in the Inferior Colliculus of the Spearnosed Bat *Phyllostomus Discolor*

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*P. discolor* travels a distance of several miles each night from its sleeping quarter to its hunting grounds. For orientation, the bat uses landmarks, presumably trees. If so, these trees need to be correctly identified by the bat using their echolocation system. However, the acoustic image of a tree is stochastic and very complex and so is the echo perceived by the bat. Nevertheless, behavioural experiments have shown that bats are able to discriminate and classify signals of different roughness. Firzlaff et al. describe a neural correlate of the psychophysical roughness sensitivity encoded as a rate code in the auditory cortex of P. discolors. But where is this rate code generated and what are the key mechanisms behind roughness encoding? The characteristics of the envelope magnitudes of signals with different roughness suggest amplitude-modulation depth sensitivity to be one of the key mechanisms. However, further mechanisms might exist.

In this study, we explored the role of the inferior colliculus in the neural encoding of echo roughness. Units are characterized in terms of their receptive fields, best-frequency tone peristimulus time histogram (PSTH) patterns and their responses to signals of different roughness and different amplitude-modulation frequency and depth. A stereotactic device allows the spatial reconstruction of recording sites.

155 neurons were tested for their sensitivity to signal roughness. About half of these units responded significantly different to signals with different roughness. Unlike in cortex, many roughness-sensitive units were found where the response strength decreases with increasing echo roughness.

Furthermore, we found a clear correlation between a neuron's roughness encoding properties, amplitude-modulation depth sensitivity and its best-frequency tone PSTH patterns. Units responding best to signals with a high roughness preferred strong amplitude-modulation depths and showed strong onset components in their best- frequency tone PSTH patterns while units responding best to signals with a low roughness preferred weak amplitude-modulation depths and showed sustained components in their best-frequency tone PSTH patterns.

# Sonar hyperacuity revisited: echo-acoustic evaluation of a jittering surface in the bat *Glossophaga soricina*

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Echolocating bats determine object distance by measuring the time delay between the emission of their sonar calls and the returning echoes. Many studies dealt with the acuity of this so-called ranging, using different approaches. One famous approach by Simmons et al. (1979, 1990) used virtual targets played back from loudspeakers within an anechoic chamber. Bat sonar calls were picked up with a microphone and played back from one out of two loudspeaker to the bat. The time delay on one side was the same from playback to playback, whereas the time delay on the other side changed by a small fixed amount from playback to playback. This so-called jitter thus simulated an object which was changing its distance to the bat back and forth. The bats were able to discriminate the jittering echo from the stationary one down to a time-jitter of only 10 ns, corresponding to a range-jitter of 1.7 µm. Since those experiments were published, there is an ongoing debate about the implicated perception of echo phase, about the neuronal mechanisms being capable of processing such small time-differences, about the biological reasons for this hyperacuity, and about possible caveats of these studies, especially due to the stimulus manipulation system (e.g. Pollak, 1993; Beedholm & Mohl, 1998).

We aim to investigate the jitter detection threshold of echolocating bats with an approach as simple and straight-forward as possible. Therefore, we do not present the bats with simulated virtual targets, but use two loudspeaker-chassis as objects whose membranes can slowly vary in their distance to the animal. The speaker membranes are 16 cm in diameter and their centres are separated by 30 cm. In a behavioural two-alternative forced-choice paradigm, we train free-ranging bats (*Glossophaga soricina*) to discriminate between the two loudspeakers, one of which is jittering and one of which is motionless. Each speaker is associated with a feeder. Feeders deliver sugar-water as a reward after the bat approached the feeder in front of the jittering membrane. As stimuli we use a sinusoidal displacement of one randomly selected membrane. The training started with a displacement of several millimetres at different frequencies from 10 - 50 Hz. Psychometrical functions, describing the jitter detection threshold, are obtained by reducing the displacement of the membranes.

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# Processing of Complex Activity Patterns at the Calyx of Held Synapse: A Computational Analysis

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The calyx of Held synapse is a very well described system, especially its fundamental parameters like the release probability and recovery from depression, which have been studied extensively in vitro. However, to date, most of these studies have neglected the fact that auditory brainstem neurons show high spontaneous firing rates of up to 100 Hz. Sound evoked bursts of discharges are thus embedded within this background of random firing.

This study presents in vitro experiments combined with a modeling approach to address the question of how the spontaneous activity influences the dynamic properties of the calyx synapse. Therefore we reintroduce physiological random firing to our slice preparation by 'conditioning' the synapse with Poisson distributed activity. After a few minutes of ongoing activity the synapse reached a new steady-state in which the mean EPSC amplitude showed no further decay. Immediately following this period, we stimulated the synapse with complex patterns mimicking natural input and recorded EPSC amplitudes over a period of minutes resulting in several thousand events. These data were then used to optimize the predictions of a vesicle release model and thereby gain insight into the transmission dynamics in this new, conditioned state.

The model consists, in the most simple variant, of a single vesicle pool with a constant release probability and a single exponential recovery time course. It yields good predictions for the experimental data and reveals profound changes in the vesicle release machinery of synapses that are firing constantly over a prolonged period of time. However the basic model shows slight systematic errors: very large EPSC amplitudes are underestimated and vice versa. There are several physiological mechanisms influencing the vesicle release which could be responsible for these deviations. We therefore developed different extensions to our model to account for these effects and to evaluate their influence on the prediction accuracy.

# Reversible Plasticity of Binaural Neurons in the Mammalian Auditory Brainstem

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Plasticity is the ability of an organism to adapt to environmental changes. In the auditory system plasticity had been primarily examined with invasive experimental manipulation such as induced hearing loss. In the present study we examine the plasticity in the adult binaural system using a non-invasive manipulation. The two dominant cues used for sound localization in the horizontal dimension are interaural time differences (ITDs) and interaural intensity differences (IIDs). IIDs are first coded in the lateral superior olive (LSO) via interactions of ipsilateral excitation and contralateral inhibition (E/I). In this study we present the effects of a manipulated acoustic environment to the interaural intensity difference processing.

We compared IID-sensitivity in three different groups of adult Mongolian gerbils (*Meriones unguiculatus*). The control group was electrophysiological examined without being exposed to noise. The second group was tested within 7 days after being exposed to omnidirectional white noise for 14 days (max. peak-to-peak 80dB SPL). The third group was exposed to the same noise but tested 14 days after the end of noise exposure. We measured the IID functions of 109 single neurons in the dorsal nucleus of the lateral lemniscus (DNLL), which inherits its IID sensitivity directly from the LSO.

Our data show an initial shift of the IID functions due to the noise exposure. That indicates a shift of balance of the excitation and inhibition favoring the inhibitory effects. This shift is reversible since two weeks after exposure the distribution of IID functions is indistinguishable from that in control animals.

# Cross-modal integration of sensory information in auditory cortex

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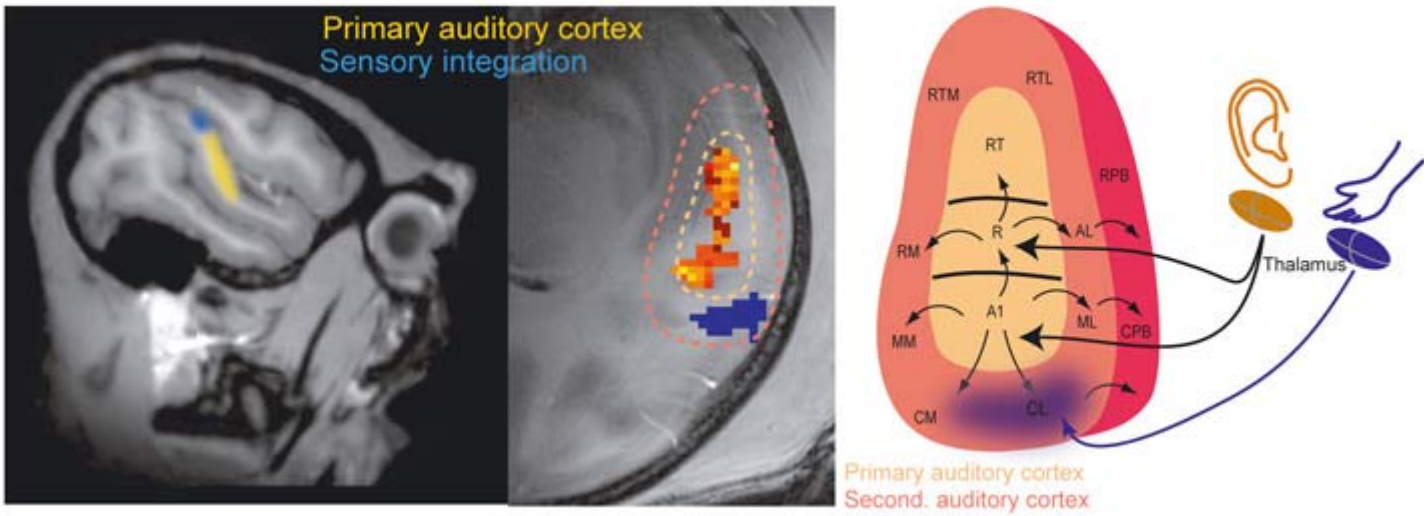
Traditionally it is assumed that information from different sensory systems merges in higher association cortices. Contrasting this belief, we demonstrate cross-modal integration in primary and secondary auditory cortex.

Using a combination of high-resolution functional magnetic resonance imaging (fMRI) and electrophysiological recordings in macaque monkeys, we quantify the integration of visual and tactile stimulation with auditory processing. Integration manifests as enhancement of activity that exceeds a simple linear superposition of responses, i.e. auditory activity is enhanced by the simultaneous presentation of non-auditory stimuli. Audio-somatosensory integration is reliably found at the caudal end and along the lateral side of the secondary auditory cortex. Regions with significant integration respond to auditory but only few to somatosensory stimulation. Yet, combining both stimuli significantly enhances responses. This enhancement obeys the classical rules for cross-modal integration: it occurs only for temporally coincident stimuli and follows the principle of inverse effectiveness; integration is stronger for less effective stimuli. Audio-visual integration is similarly found along the caudal end of the temporal plane in secondary auditory cortex, but also extends into primary auditory fields.

Complementing these results from functional imaging, enhancement of neuronal activity is found in electrophysiological recordings of single neuron and population responses. Hence, we conclude that cross-modal integration can occur very early in the processing hierarchy - at the earliest stage of auditory processing in the cortex. Further, this multisensory integration occurs pre-attentive, as demonstrated in anaesthetized animals. Such early integration might be necessary for quick and consistent interpretation of our world and might explain multisensory illusions where a stimulus perceived by one modality is altered by a stimulus in another modality.

Legend: A-B) fMRI based delineation of primary and secondary auditory cortex (yellow and red colors) and regions showing integration of auditory and tactile signals (blue). C) Functional organization of auditory cortex. Auditory input from the thalamus projects to fields A1 and R and from there to other fields. Cross-modal integration is most prominent in the caudal fields CM and CL.



# Poster Topic

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**T20-7C** Differential reaction of olfactory axons to target tissue: in vitro cultures
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**T20-8C** Cells in the vomeronasal organ express odorant receptors but project to the accessory olfactory bulb
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**T20-9C** Olfactory mixture representation at the level of projection neurons in the honeybee antennal lobe
*N. Deisig, M. Giurfa and JC. Sandoz, Toulouse (F)*

# Olfactory Coding in the Honeybee Brain
# I. Binary Population Code in Projection Neurons

Martin Paul Nawrot[1,2], Sabine Krofczik[1,2], Stefan Stiller[1] and Randolf Menzel[1,2]

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Mushroom bodies (MBs) within the insect brain are higher-order centers performing integration of olfactory, visual and mechano-sensory information. They are known to be involved in neural plasticity underlying associative olfactory learning. Here, and in three companion posters (Yamagata et al., Strube et al., Hähnel et al., this Volume), we address the question of how olfactory stimuli are processed at the input and the output stage of the MBs. The input from the antennal lobe (AL) as provided by the projection neurons (PNs) was studied using intracellular recordings and calcium imaging of PN boutons (Yamagata et al.). Extrinsic neurons (ENs) form the output of the MB and were also studied by means of extra cellular recordings (Strube et al.) and calcium imaging (Hähnel et al.).

In this poster, we concentrate on the mushroom body input neurons, the projection neurons (PNs). We report on the general response characteristics of the PNs, their response pattern to monomolecular stimuli as well as mixtures and their encoding of odour identity.

We performed intracellular recordings from projections neurons within the AL by using multiple trial and one trial presentations of single component odorants and mixtures. Repeated presentation of the same odour were carried out with an inter-trial interval of 60s. One trial presentation of different odorants were accomplished with inter-stimulus interval of 30s. A fixed airflow of 20ml/s (odour diluted 1:1 with control air) and a stimulus duration of 2s was used for all experiments.

Our analyses first concentrated on the reliability with which single neurons responded to the repeated presentation of the same stimulus (reliability index, Ri). We found that all PNs respond reliably to repeated presentation of one and the same olfactory stimulus (Ri = 1). In contrast, ENs responded unreliable with an average reliability index of Gi = 0.44 (see companion poster by Strube et al).

To investigated the encoding of odours in PNs, we first studied the generalisation across odors. We defined the generalisation index Gi as the relative number of odors that evoked significant excitatory or inhibitory responses during the complete 2s interval of odor stimulation. We found that on average PNs responded to 50% (Gi = 0.5) of the odours presented. The ENs, in contrast, responded less specifically (Gi = 0.8). We further tested the simple model of a binary encoding of the presented odour in a population of PNs. For each neuron and each odour we only took into account the presence or absence of a positive response. We found that this was sufficient to represent odour identity in a spatial 0/1 pattern in a pseudopopulation of independent projection neurons.

Supported by the German Federal Ministry of Education and Research (grant 01GQ0413)

# Olfactory Coding in the Honeybee Brain: II. Spatio-Temporal Patterns of Odor Responses in Projection Neuron Boutons in the Mushroom Bodies of the Honeybee

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The mushroom body (MB) is a higher order olfactory processing center as well as a multimodal sensory integration center, which plays critical roles for odor learning in the insect brain. Here, and in three companion posters (Nawrot et al., Strube et al., Hähnel et al., this Volume), we address the question of how olfactory stimuli are processed at the input and the output stage of the MBs of the honeybee. The input stage via projection neurons (PNs) were studied using intracellular recordings (Nawrot et al) and output stage via MB extisic neurons (ENs) were studied using extra cellular recordings (Strube et al) and calcium imaging (Hähnel et al). In this poster, we report about the activity dynamics of PN boutons on the MB which studied by calcium imaging. The lip region of the MB calyces receives input from olfactory projection neurons (PNs), from GABA immunoreactive MB feedback neurons and from modulatory neurons like the VUMmx1. Imaging studies by Szyszka et al. 2005 (J.Neurophysiol. 94: 3303 - 3313 ) revealed differences in the odor responses of l-ACT PNs and Kenyon cells, the intrinsic neurons of the MB, indicating a sparsening of the responses both in the temporal and spatial domain as a consequence of neural processing in the lip region. It is not yet known how much the putative inhibitory input to the presynaptic PN boutons contributes to these sparsening effects.

We used $Ca^{2+}$-imaging to study odor induced responses of boutons of l-ACT and m-ACT PNs after staining them with the mixture of Fura-2 and Rhodamine dextran via their somata. This allowed us to examine both, the neural activity and subsequently the morphology of the imaged neurons with the confocal microscope. The number of activated boutons visible in a measurement ranged from 40 to 120 corresponding to approximately 10 to 20 PNs. Individual boutons showed spontaneous activities and responded both with excitation and inhibition to odors. Boutons that belong to the same axon branch appear to follow the same pattern of excitation and inhibition. 3D reconstructions of boutons and axons that have been imaged will be shown, and the activity map will be compared with the respective morphology. On the population level, odors elicited specific spatio-temporal patterns of activities in boutons that depend on the odor concentration. Principal component analysis separates odor representations at the level of boutons that showed strong responses. Around 10% of the measurements we observed inhibitory responses immediately following excitatory responses, leading to sharper response dynamics than observed in the AL. The inhibition was particularly frequent during stimulation with high odor concentrations indicating that inhibition is concentration dependent. This temporal sharpening may result from a global and delayed inhibitory input onto PN boutons possibly from MB feedback neurons.

# Olfactory Coding in the Honeybee Brain III. Sparseness, Reliability and Reward Conditioning in Alpha-Lobe Extrinsic Neurons

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Mushroom bodies (MBs) within the insect brain are higher-order centers performing integration of olfactory, visual and mechano-sensory information. They are known to be involved in neural plasticity underlying associative olfactory learning. Here, and in three companion posters (Nawrot et al., Yamagata et al., Hähnel et al., this Volume), we address the question of how olfactory stimuli are processed at the input and the output stage of the MBs.

The input stage via projection neurons (PNs) were studied using intracellular recordings (Nawrot et al.) and calcium imaging of boutons that form the axon terminals of PNs (Yamagata et al.). Extrinsic neurons (ENs) form output of the MB and were investigated by calcium imaging (Hähnel et al.), in this poster we present results from means of extra cellular recordings of ENs.

Single-unit activity of typically 2-5 EN´s was measured using extracellular wire electrodes. We report on the general response characteristics, the dynamic encoding of odors and on the response changes associated with reward conditioning of individual ENs.

Our analyses first concentrated on single neuron variability. In particular we analyzed (i) the reliability with which single neurons responded to the repeated presentation of the same stimulus and (ii) the temporal precision of individual response profiles. About 64% of the recorded ENs are showing no reliable resposes (<85% of the trials) to repeated odor presentation (average reliability 0,44). This distinctly differs from the response properties of PNs at the earlier processing stage which exhibited highly reliable responses (see companion poster Nawrot et al.). The average response latency of the ENs was 34ms. We found that those neurons that responded more reliably also responded with higher temporal precision, i.e. smaller latency variability (median 16 ms).

To investigate the encoding of odors in the ENs we calculated the sparseness index Si for each individual neuron as the relative number of odors that evoked transient responses within the first 500ms of stimulus presentation. We found that ENs typically responded to most of the presented odors with a mean Si = 0,79 whereas PNs were comparably sparse (Si <0,5).The strength of EN responses was typically modulated across odors. Time-resolved tuning curves revealed that also onset and duration of the transient response were modulated across odors.

In a reward conditioning experiment we could show that upon repeated presentations of one odor in combination with the reward as reinforcer (CS/US pairing), a large fraction of the neurons (44%) showed either significant enhancement or reduction of their responses. During the subsequent test phase without reinforcer, in some neurons the effect disappeared after only a few trials (extinction), while in other neurons the new activity pattern was retained even after many unrewarded test trials.

Supported by the German Federal Ministry of Education and Research (grant 01GQ0413)

# Olfactory Coding in the Honeybee Brain: IV. Calcium Imaging of Mushroom Body Extrinsic Neurons

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Mushroom bodies (MBs) within the insect brain are higher-order centers performing integration of olfactory, visual and mechano-sensory information. They are known to be involved in neural plasticity underlying associative olfactory learning. Here, and in three companion posters (Nawrot et al., Yamagata et al., Strube et al.), we address the question of how olfactory stimuli are processed at the input and the output stage of the MBs.
The input from antennal lobe (AL) as processed by the projection neurons (PNs) was studied using intracellular recordings (Nawrot et al) and calcium imaging of boutons that form the axon terminals of PNs (Yamagata et al.). Extrinsic neurons (ENs) form output of the MB and were investigated by means of extra cellular recordings (Strube et al.), in this poster we present results from calcium imaging experiments on ENs.

Mushroom body extrinsic neurons that arborize in the vertical lobes with somata organized in clusters in the protocerebrum have been previously identified in anatomical studies (Rybak and Menzel, 1993, J.comp. Neurol. 334, 444-465). A single identified MB output neuron, the PE1, has been shown to respond to different sensory modalities and expresses associative long time potentiation when tetantic stimulation of MB intrinsic neurons is paired with depolarization (Menzel and Manz, 2005, J. Exp.Biol 208, 4317-4332). Neurons with somata localized in the lateral protocerebrum feed back to the calycal input region of the MB, they are GABA immunoreactive and respond to odor stimuli.
To further investigate the role of the MB output neurons in olfactory processing and possibly in odor learning we performed mass stainings of these neurons and investigated their response properties employing calcium imaging. The calcium sensitive dye Fura-2 dextran was injected into the median ventral or lateral rim of the MB vertical lobe together with rhodamine dextran. The anatomy of the recorded neurons was subsequently evaluated under the confocal microscope. The activity of these neurons was recorded during odor, sucrose, water and mechanical stimulation and differential olfactory conditioning. The responsiveness of MB output neurons to odors and rewarding sucrose stimuli was confirmed, making a role in learning likely. In some experiments stained neurons increased their activity in response to the rewarded odor after training; in others the activity of the neurons was reduced. In a number of recordings neurons no longer responded to odor stimuli after sugar had been delivered to the antennae. Overall, the responsiveness to odors and changes in activity possibly related to learning varies considerably across recordings.
One future goal for this study is to match the response behavior to the different somata clusters and possibly identify functional groups of MB extrinsic neurons.

# Activity-dependent expression profiling in the mouse vomeronasal organ

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In addition to the main olfactory system mammals possess an accessory olfactory pathway which has been attributed a predominant role in detection of chemical signals that mediate social recognition and pheromonal communication. Its peripheral structure, the vomeronasal organ (VNO), is build by two bilaterally symmetrical blind-ended tubes at the anterior nasal septum. Given the fundamental functional importance of the VNO in mammalian social and sexual behavior, surprisingly few consolidated experimental data on the basic molecular and cellular mechanisms underlying vomeronasal pheromone sensing are currently available.

In a collaborative effort, we therefore address this issue from a systems-biology perspective. Employing a microarray approach, we analyze activity-dependent mRNA expression patterns in sexually naïve male C57BL/6 mice after continuous exposure to a variety of social stimuli (e.g. female soiled bedding and male urine samples). By contrast, control animals are housed socially isolated in a stimulus-free environment. Purification of total vomeronasal RNA from both groups and subsequent analysis on Affymetrix GeneChip® microarrays allows for detailed analysis of whole genome expression profiles.

Our study will reveal putative correlations between conspecific social recognition and distinct vomeronasal transcription patterns involving interesting candidate signal transduction proteins. This dataset will lay a solid basis for future experiments that will provide new insight into the regulatory mechanisms that govern mammalian social behavior.

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# Is the functional olfactory lateralisation in homing pigeons based on anatomical asymmetries?

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It is well known that pigeons use olfactory cues to navigate over unfamiliar areas and that any impairment of the olfactory system generates remarkable reduction of homing performance. Gagliardo et al. (2005) have shown that the left as well as right piriform cortex (Cpi) are necessary for an intact homing performance, but with a crucial role of the left Cpi in processing olfactory cues needed for determining the direction of displacement. This function seems to be triggered by the right olfactory system as demonstrated by plugging the left or right nostril of homing pigeons (Pecchia et al., 2006). Since the olfactory bulb (OB) projects bilaterally onto Cpi (Bingman et al., 1994) it is conceivable that the contralateral projection from the OB to Cpi might be asymmetrically organized, with a stronger projection from the right OB to the left Cpi. A larger bilateral innervation of the dominant brain structure is also known from visual pathways in birds (Güntürkün et al. 1997; Rogers and Deng, 1999).

To address this question we used the retrograde tracing technique. Choleratoxin subunit B (CtB) was injected unilaterally into either the left or right Cpi of adult pigeons. After immunohistochemical detection of CtB-positive cells, the number of ipsi- and contralaterally projecting neurons located in the olfactory bulb (OB) was estimated. The percentage of contralateral cells served as an index to assess the degree of asymmetry.

Retrogradely labelled cell were found bilaterally in the OB with a higher number of ipsilaterally located cells. We detected a higher percentage of contralaterally projection neurons in the left OB (42.78%) compared to the right one (33.43%). This indicated a larger projection from the left OB to the right Cpi than vice versa (Mann-Whitney U test: $Z=-1.732$ $p=0.083$). These data suggest that the functional dominance of the right OB / left Cpi is not based on a stronger connection between the right OB and the left Cpi compared to that between the left OB and the right Cpi.

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# Adenoviral mediated PI signaling markers unravel $PIP_2$ signal transduction pathways in trigeminal neurons

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Facial sensory innervation is provided by the trigeminal (V cranial) nerve, which comprises neurons that transduce mechanical, thermal and chemical stimuli probably including odorant molecules. The signal transduction mechanism in sensory nerve endings is extremely complex and details remain unclear.
We were interested in the role of phosphoinositol (PI)-signaling in trigeminal chemosensation, and focused on Phospholipase C (PLC) and PI3-kinase pathways.
We used a pleckstrin homology domain of phosphplipase Cδ1 fused to GFP (referred to as PH-GFP) as a $PIP_2$ signaling marker and tested its properties in transiently transfected HEK293 cells heterologously expressing various thermo- and chemosensitive TRP ion channels. In addition, an adenoviral vectorsystem is used to deliver PH-GFP to primary cultured trigeminal neurons. Live confocal laser scanning microscopy of transfected HEK cells and infected neurons is performed to observe the subcellular distribution and stimulus-induced translocation of the fluorescent $PIP_2$ binding marker.
The application of TRP receptor agonists to transiently transfected HEK cells lead to a decrease of the PH-GFP signal at the plasma membrane and translocation of PH-GFP to the cytoplasm, indicating a decrease in membrane $PIP_2$.
The results obtained in the recombinant system will be compared to data obtained in trigeminal neurons. We will present data indicating that chemical substances known to activate TRP channels on trigeminal neurons (trigeminal "odorants") could use and modulate PI-signaling pathways in chemosensory signal transduction.

# Comprehensive Maps of *Drosophila* Higher Olfactory Centres: Spatial Segregation of Fruit and Pheromone Representation

Gregory S. X. E. Jefferis[1], Christopher J. Potter[2], Alexander M. Chan[2], Elizabeth C. Marin[2], Torsten Rohlfing[3], Calvin R. Maurer[4] and Liqun Luo[2]

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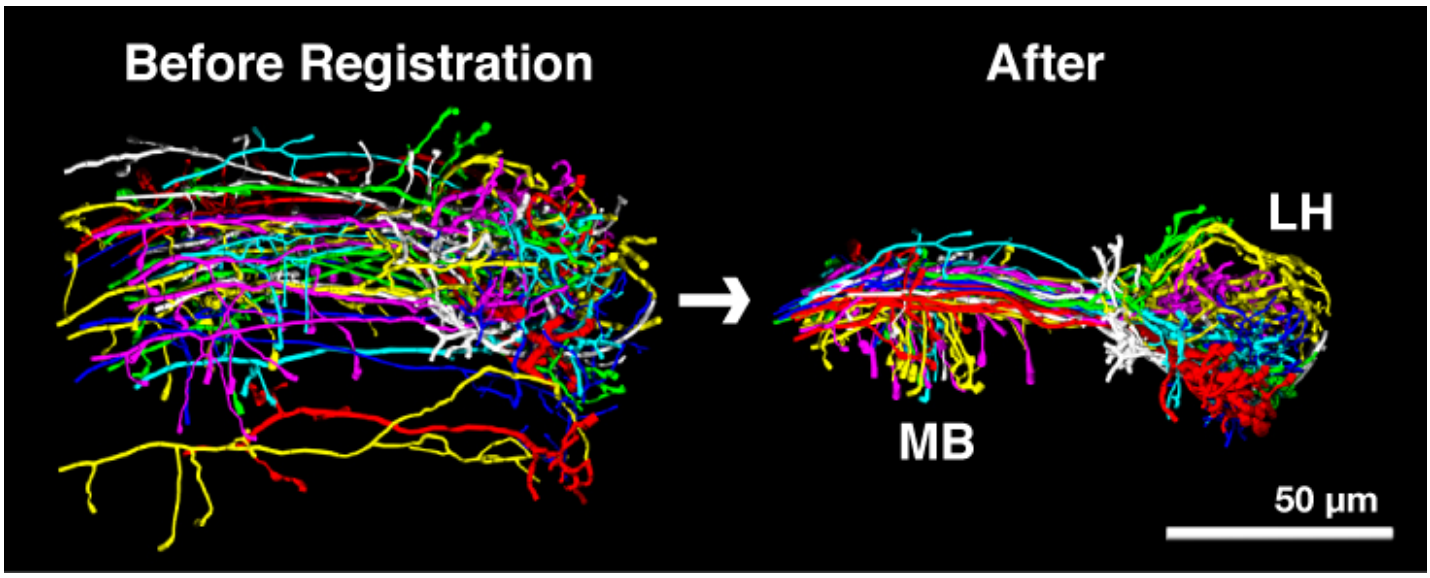
In *Drosophila*, ~50 classes of olfactory receptor neurons have convergent axonal projections to 50 glomeruli in the antennal lobe. Uniglomerular projection neurons (**PN**s) then relay olfactory information to the mushroom body (**MB**) and lateral horn of the protocerebrum (**LH**). Here we combine single cell labelling and nonrigid image registration methods to create high-resolution, quantitative maps of the MB and LH with respect to 35 input PN channels and several groups of LH neurons. We find 1) PN inputs to MB exhibit a certain degree of stereotypy, as previously shown for LH projections; 2) PN inputs are clustered according to a combination of sensillar class, glomerular position and axonal pathways; 3) fruit odours are represented mostly in the posterior-dorsal LH whereas candidate pheromone-responsive neurons project to the anterior-ventral LH; 4) dendrites of single LH neurons each overlap with a specific and reproducible subset of PN axons. Our analysis suggests that the lateral horn is organised according to biological values of the olfactory input.

Figure: **Registration Example**. This figure shows the result of co-registering 35 brains each containing a single labelled projection neuron. Traced neurons of 7 different classes are shown in 7 different colours; after registration it can be seen that neurons of the same class project to the same region of the lateral horn and mushroom body.



# Improved imaging of olfactory bulb neuropil by blocking multidrug resistance transporters

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ATP-binding cassette (ABC) transporters are a family of transmembrane proteins that, also known as multidrug resistance proteins can transport a wide variety of substrates across biological membranes in an energy-dependent manner. Recently it has been shown that members of this protein family interfere with fluorescent (calcium indicator) dye uptake in taste buds of rat and in cells in the olfactory epithelium of larval *Xenopus laevis*, including peripheral olfactory receptor neurons. Here we show that also central neurons, namely neurons in the olfactory bulb of larval *Xenopus laevis*, express multidrug resistance transporters. Blocking these transporters increases the uptake of fluorescent (calcium indicator) dyes, improving the outcome of calcium imaging experiments. Especially the imaging of fine structures such as individual dendrites or olfactory glomeruli consisting of a feltwork of fine neuronal processes becomes feasible. The expression of multidrug resistance proteins could be a frequent feature of cells in the central nervous system. Application of specific transport inhibitors could therefore generally improve calcium imaging in various regions of the central nervous system revealing otherwise overlooked structures.
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# Strength and similarity of intrinsic activation patterns define olfactory discrimination time in mice

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Mice discriminate the simple odors amylacetate (AA) and ethylbutyrate (EB) on a fast time scale, in the range of 200 - 250 ms, while requiring additional time to discriminate binary mixtures of the same odors with the same accuracy level (Abraham et al, 2004). To investigate further if speed of the odor discrimination and extra discrimination time (DT) needed for mixtures are general properties of the olfactory system we trained mice to discriminate additional odor pairs including alcohols and enantiomers. Furthermore, we examined the relationship of DTs and the properties of odor representation patterns in the olfactory bulb (OB) in vivo.

DTs were examined using a go/no-go operant conditioning paradigm for the following odor pairs: 1% AA vs. EB, cineol (C) vs. eugenol (E), (+)carvone (C+) vs. (-)carvone (C-) and (+)octanol (O+) vs. (-)octanol (O-) and binary mixtures of these odor pairs (e.g. 0.6AA+0.4EB vs. 0.4AA+0.6EB). Mice discriminated the esters (AA vs EB) in 243±8 ms (all numbers mean±SEM, n of 6 to 28) whereas the discrimination of C vs. E took significantly longer (300±8 ms, $p<0.01$). DTs for the enantiomers were 345±9 ms for C+ vs. C- and 342±13 ms for O+ vs. O-, respectively. Hence, DT of simple odors covers a range of 240 to 350 ms. Examining the DT for binary mixtures revealed an increase relative to simple odor DT of 95 ms, 78 ms, 32 ms, and 35 ms for the odor pairs AA vs. EB, C vs. E, C+ vs. C-, and O+ vs. O-, respectively. Thus, compared to simple odors mice consistently took more time to discriminate the binary mixtures, suggesting that the increased DT for more similar stimuli might be a general property of the olfactory system. Interestingly, the increase in DT for binary mixtures was large for simple odor pairs that were discriminated rapidly (e.g. AA/EB), but small for those requiring more time (e.g. O+/O-).

In order to determine physiological parameters of the odor representation in the OB that correlate with the differences in DTs we imaged intrinsic signals evoked by the same set of odors in anesthetized mice. Spatial patterns of odor pairs with longer DTs appeared to be more similar on visual inspection. As a measure of similarity of the spatial odor representations we calculated the Pearson's correlation coefficient between responses to two odors. This similarity value increased with DT ($r^2 = 0.36$) however not significantly ($p=0.4$). Maps of the odor pair AA vs. EB contained more activated glomeruli and the higher Pearson's correlation values were higher than e.g. maps of the odor pair C/E, but DT was significantly shorter for AA vs EB. So we tested if the general strength of activation correlates with the DT. Overall activation was anti-correlated with DT ($r^2 = 0.5$) however this anti-correlation was also not significant ($p=0.2$). To incorporate both similarity and strength of activation, we calculated the Euclidean distances between pairs of maps. Distance values were significantly anti-correlated with the DTs ($r^2 > 0.9$, $p<0.05$). Therefore, strength and similarity of the activation patterns on the dorsal OB may define odor discrimination time in mice.

# Testing a model of the olfactory bulb function with dynamic input patterns

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Several models of olfactory bulb function have been proposed. Some of these models can generate stimulus specific dynamic output of the olfactory bulb, however to our knowledge all models published so far use only static input patterns to the olfactory bulb. Since selective imaging of olfactory bulb input has demonstrated that input patterns to the olfactory bulb are not static but change in an odour-specific way [1], we tested how dynamic input changes the behaviour of a model similar to the one suggested by Margrie and Schaefer [2].

Our model consists of two layers of integrate and fire neurons. The first layer corresponds to olfactory bulb mitral cells, each representing input from one olfactory receptor type only. Independent of the 'odour stimulus' the membrane potential of the mitral cells is modulated with a sub-threshold oscillation at respiration frequency (4 Hz). Each mitral cell is connected to two thirds of all second layer neurons. Connection strength and connection delay (maximal 17 ms) are drawn from a uniform distribution. The membrane potential of the second layer neurons (analysis cells) was used to analyze the behaviour of the model. In order to calculate response times, the membrane potential of the analysis cells was calculated for pairs of stimuli and compared.

As odour stimuli we implemented oscillating input signals with different amplitudes and latencies for each glomerulus. The width of the distribution from which the latencies were taken and the relation between latency and input amplitude of the glomeruli were varied systematically and explored.

Response times were reduced by increasing the input amplitude (i.e. strength of input, or odor concentration). Increasing the range of latencies of otherwise identical pairs of input patterns could reduce the differences of response times between strong and weak stimulus pairs. This demonstrates that dynamic input patterns can in deed change the performance of a model of olfactory information processing.

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[2] Margrie,T.W. and Schaefer,A.T. (2003) Theta oscillation coupled spike latencies yield computational vigour in a mammalian sensory system. J Physiol, 546, 363-374.

# Fluorimetric measurement of calcium signals in intact palaemonid shrimps following chemical stimulation with amino acids and bromophenol

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Chemoreception is an important sensory modality for marine organisms. Because vision is often limited and acoustic information processing needs a resonant body for sound perception, olfaction/chemosensory information is very important for localization of food and sex partners in aquatic invertebrates.
Especially in shrimp larvae there are no physiological data on chemoreception available so far and only limited information from behavioural studies exist in the literature. We used optical techniques to record cellular responses to olfactory stimuli in intact animals using a fluorescent calcium dye. Responses of larval shrimps (Palaemonetes argentinus), which were reared at the marine biological station of Helgoland, to bath application of different amino acids in differing concentrations were measured by focusing on the brain using an imaging system. Among amino acids best responses were obtained using glutamate.
Brominated phenols are synthesized by algae or stem from anthropogenic sources and are transmitted via the food web to all trophic levels. The ecological role of brominated phenols is not clear yet, but they seem to be responsible for the sea-like taste of many marine organisms such as fish (Ma et al. 2005). We tested the marine 2,4-dibromophenol, which disturbs cellular calcium signaling in endocrine cells (Hassenklöver et al. 2006, Hassenköver and Bickmeyer 2006), as a chemical stimulus in larvae of the shrimp Palaemonetes argentinus and registered responses after application of varying concentrations down to the picomolar range.

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Hassenklöver, T., Predehl, S., Pilli, J., Ledwolorz, J., Assmann, M., Bickmeyer, U., 2006. Bromophenols, both present in marine organisms and in industrial flame retardants, disturb cellular Ca2+ signaling in neuroendocrine cells (PC12). Aquat. Toxicol. 76, 37-45.
Hassenklöver, T., Bickmeyer, U., 2006. The marine secondary metabolites 2,4-dibromophenol and 2,4,6-tribromophenol differentially modulate voltage dependent ion currents in neuroendocrine (PC12) cells. Aquatic Toxicol. 79, 384-90.

# Odour stimulation of the Drosophila olfactory receptors Or22a/Or83b heterologously expressed in HEK293 cells produces an ion conductance in concentration-dependent manner which does not involve $G_q$ protein activation

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The classical view considering olfactory receptors as G protein coupled receptors is - especially in the case of *Drosophila* - not commonly accepted (e.g. Benton et al. PloS Biol. 2006 4:e20.). *Drosophila* olfactory receptors are composed of a conventional olfactory receptor protein such as dOr22a and another protein called dOr83b with a topology untypical for a receptor protein. Similar as in vivo, dOr83b is essential for functional expression of dOr22a in HEK293 cells indicating a chaperone-like function of dOr83b (Neuhaus et al. Nat Neurosci. 2005 8:15). Alternatively, dOr22a and dOr83b might couple to form a functional unit with dOr22a as ligand acceptor and dOr83b as signal transducer.

We have performed a Patch clamp study on HEK293 cells expressing dOr22a and dOr83b. Application of the dOr22a agonist ethyl butyrate caused an increase in membrane conductance in those HEK293 cells expressing the receptors as visualized by GFP fluorescence but not in cells without fluorescence. Using bath and pipette solutions for whole cell experiments reflecting the physiological ion distribution the odour-induced current with a reversal potential around 0 mV indication a non-selective conductance. The size of the current was dependent on odour concentration up to 100 nM; the $EC_{50}$ was 475 pM.

Coexpression of dOr22a and dOr83b together with the voltage-gated potassium channel hKCNQ4 in HEK293 cells was then performed to test for a possible role of $G_q$ proteins in the transduction of odour signals. An odour-induced $G_q$ protein activation would attenuate the KCNQ current (Wicher et al. J Neurophysiol. 2006 95:2314). Although stimulation with ethyl butyrate again gave rise to an ion current, there was no effect on KCNQ current indicating that $G_q$ proteins are not involved in enhancing the membrane conductance.

# Physiological Properties of Kenyon Cells Recorded in an Intact Brain Preparation

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Olfactory information is processed in multiple stages. In insects the first stage of central processing occurs in the primary olfactory centres, or antennal lobes. Important neuropiles in which second stage olfactory and other sensory information processing follows are the mushroom bodies. These are bilaterally symmetrical neuropiles in the insect brain that are thought to be essential in learning and memory and integrating multimodal information. The mushroom bodies receive input from different sensory systems including the olfactory - and visual system. Each mushroom body of *Periplaneta americana* consists of about 200,000 intrinsic neurons, the Kenyon cells.

To better understand their role during odor processing and generally in central sensory information processing we have begun to study the physiological properties of the mushroom bodies' Kenyon cells. We are using an intact brain preparation that allows stable patch clamp recordings of Kenyon cells and physiological relevant stimulations and a subsequent morphological characterization of the recorded neuron.

All recorded Kenyon cells, despite strong excitatory input, had no or a very low spontaneous activity. However, in every recorded neuron we could elicit action potentials by injecting depolarising current. In a subset of Kenyon cells application of odors to the antennae evoked strong, stimulus locked excitatory synaptic input that could reach threshold and elicit one or a small number of action potentials at the beginning of the stimulus. In parallel to investigating the intrinsic firing properties and the responses to sensory stimulations we have started to investigate the underlying ionic currents such as $I_A$, $I_{K(V)}$, $I_{Na}$ and $I_{Ca}$.

# Pharmacological Characterization of Voltage-gated Calcium Currents in Olfactory Interneurons of *Periplaneta americana*

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Voltage-activated calcium currents in olfactory interneurons from *Periplaneta americana* were analysed in short term cell culture and in an intact brain preparation. We started to study the effect of anorganic calcium channel blockers (cadmium, nickel, cobalt) and organic calcium channel blockers of three distinct chemical classes (Phenylalkylamines (PAA), Benzothiazepines (BZT), 1,4-Dihydropyridines (DHP)), which are known to block preferentially a specific subtype (L-type) of voltage-gated calcium channels in vertebrate preparations. Our long-term goal is to better understand the cellular and biophysical mechanisms underlying the calcium signal in olfactory neurons.

The somata were voltage-clamped under conditions, in which other voltage-gated currents ($I_{Na}$, $I_K$, $I_h$) were pharmacologically blocked. Depolarising voltage steps from -60 mV generated voltage dependent inward currents. The currents consisted of a transient, fast inactivating component and a sustained, non inactivating component. Reduction of the extracellular calcium concentration reduced the currents reversibly. When calcium was substituted with barium the currents were enhanced and the inactivation during a sustained voltage step was reduced, suggesting a calcium dependent inactivation of the current. *In vitro*, $I_{Ca}$ started to activate above -35 mV to -30 mV, with a maximum around -5 mV. The inactivation of the calcium currents started with pre-pulse potentials above -50 mV to -45 mV. In the intact brain these parameters were shifted to more depolarised membrane potentials. *In situ* $I_{Ca}$ began to activate above -30 mV to -25 mV, with a maximum around +20 mV. The inactivation in intact neurons of the antennal lobes started at pre-pulse levels above -35 mV to -30 mV.

The investigated anorganic calcium channel blockers reduced $I_{Ca}$ reversibly in a dose-dependent manner. $I_{Ca}$ was effectively blocked by cadmium but less affected by Nickel and Cobalt. Verapamil, a PAA, showed half-maximal block ($IC_{50}$ ) of ICa at 150 µM wheras Diltiazem (BZT) has an $IC_{50}$ of 287 µM. The tested organic compounds modified $I_{Ca}$ had different effects on parameters such as voltage-dependence of steady-state activation and inactivation as well as the time constant of the decay. We plan to use these and other blockers to better characterize the different components of calcium influx and their roles in intracellular calcium dynamics.

# Electrophysiological and Morphological Characterization of Spiking and Nonspiking Local Interneurons in the Antennal Lobe of *Periplaneta americana*

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Inside the insect antennal lobe each olfactory receptor cell projects to one glomerulus and many receptor axons converge in each glomerulus, where they provide synaptic input to local interneurons and projection (output) neurons. The arborizations of the local interneurons are confined to the antennal lobe. In contrast, the projection neurons extend axons to higher order neuropiles of the protocerebrum, including the mushroom bodies. In particular the projection neurons have been the focus of intensive studies because they serve multiple functions and play a key role in the central olfactory pathway. However, the role and functional properties of the local interneurons are less clear. Using whole cell patch clamp recordings in current and voltage clamp mode in combination with single cell staining, we studied 3 different types of local interneurons of the ventrolateral cluster of somata in a physiological intact brain preparation of the cockroach Periplaneta americana.

So far, we were able to distinguish 2 types of nonspiking local interneurons (nspLN) and one type of spiking local interneurons (spLN). In spLNs odor stimulation and current injection elicited overshooting TTX sensitive action potentials. Voltage clamp recordings revealed a voltage activated sodium current, a transient and a sustained potassium current and a calcium current. In nspLNs odor stimulation and depolarizing current injections induced depolarization of the membrane potential but no TTX sensitive overshooting action potentials. In most recordings small (ca. 2mV) spikes were riding on the odor evoked depolarization. In these neurons voltage activated sodium currents were not detected. Voltage activated calcium currents, however, were significantly larger compared to spLNs. These results suggest that the odor-evoked depolarization in nspLNs is carried by calcium currents. In addition to their physiological properties spiking and non-spiking LNs were clearly distinguishable by the location of their somata, the soma size and the branching pattern within the glomeruli. One type of nspLNs was characterized by a homogeneous innervation of all glomeruli. The other type showed a zonal, cup like, innervation of the glomeruli. The spLN that we studied had arborizations in many, but not all glomeruli. However, there was a difference in density of innervation between glomeruli. The physiological and morphological differences between different types of local interneurons might suggest functional differences for specific types of local interneurons.

# Quantitative Analysis of Insect Olfactory Interneuron Responses to Odors in the First Olfactory Relay, the Antennal Lobe

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The olfactory system of insects has to detect large variety of different odor molecules and to convert chemical signals into a pattern of nervous signals. In the antennal lobe the information from different receptor neurons leads to activation of several neurons of a population encoding the olfactory information. To study how the modulation of neurons affects the network activity of olfactory interneurons in insects we inserted multichannel microelectrodes (tetrodes, Center for Neural Communication Technology, Ann Arbor, Mi) and wolfram electrodes into the antennal lobe of Periplaneta americana. These Multi-Electrode Array (MEA) extracellular recordings allow to monitor routinely neuronal populations activities and to analyze aspects of information processing, such as synchronization in sensory coding.

Here, we present single-unit and population recordings from the first olfactory relay of insects, the antennal lobe. We are using these population recordings to investigate the potential role of highly interconnected neurons to stimulus representation. A prerequisite for such correlation studies is an accurate model to characterize the responses of single neurons to odor application. The developed quantitative model based on MEA recordings of projection neurons activity in the antennal lobe. After acquisition of spike data, we utilized a custom-made R-based analyzing program (open source software, R-project site: www.r-project.org; SpikeOMatic: www.biomedicale.univ-paris5.fr/SpikeOMatic/) for spike detection, spike sorting and clustering, i.e. semi-automatic classification and separation of spikes. Once the events are separated and sorted into clusters, each cluster represents the activity of a single unit. After discarding the clustering errors (e.g. superpositions, amplitude-modulated spikes in bursting neurons) adjustment of the classification enables us to assign the units to the activity of single neurons. Finally, we exploit the obtained model to classify all the detected events during the long time data collection in an experiment e.g. with application of different odors or neuromodulators. Results of this classification will be useful to reveal information about cellular interactions in this complex neuronal network.

# Calcium Dynamics in Olfactory Interneurons

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Information processing in single neurons is at least in part dependent on highly localized calcium gradients. Our long-term goal is to better understand how intracellular calcium dynamics contribute to olfactory information processing in the insect olfactory system that has served as an excellent model to study general mechanisms of olfaction. The spatial and temporal dynamics of these calcium gradients are determined by several cellular parameters including the calcium influx, calcium buffering and locally changing diffusion coefficients.

We have estimated these parameters that are related to calcium handling in cockroaches (*Periplaneta americana*) first olfactory relay neurons. We have done that based on a model for calcium handling and a model for our imaging and detection apparatus. Including the latter is essential to get unbiased parameter estimates as well as meaningful confidence intervals. Once equipped with such a model we could simulate ideal data, upon which our statistical inference (i.e. parameter estimates and confidence intervals) are based.

We used patch-clamp recordings and fast optical imaging in combination with the 'added buffer approach' (*J Physiol, 1992, 450, pp 273-301*) to quantitatively analyze the dynamics of internal calcium signaling. Recordings were performed *in vitro* on olfactory interneurons from the antennal lobe of adult *Periplaneta americana* and in the intact brain.

During experiments calcium currents were pharmacologically isolated, cells were voltage-clamped to -60 mV and loading of the ratiometric indicator fura-2, serving as the 'added buffer', was monitored quantitatively at 360 nm excitation. From this loading curve absolute fura-2 concentrations could be determined for any time point. During dye loading voltage steps from -60 mV to +5 mV (for 50 ms) were used to generate voltage-activated calcium influx. Intracellular fluorescence was measured ratiometrically and converted to absolute calcium kinetics using the approach from Grynkiewicz (Grynkiewicz, Poenie et al. 1985). Values for dye concentration, absolute free intracellular calcium concentrations and decay constants of the signals were used to compute the endogenous buffering capacity, the extrapolated decay constants (without fura-2) and the rate of calcium removal *in vitro* and *in situ*.

# Pheromones of the cricket *Gryllus bimaculatus*: Do male larvae have the female pheromone?

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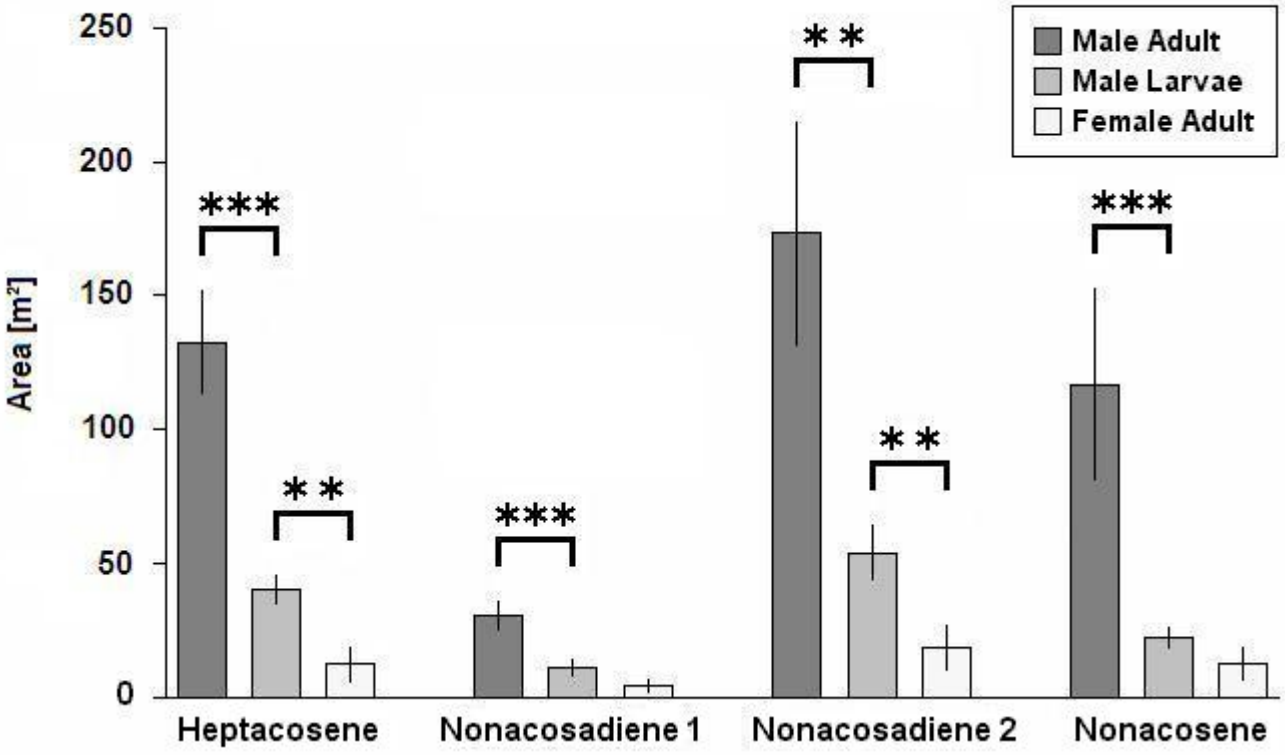
Pheromones are essential for sex recognition in the cricket *Gryllus bimaculatus* (Adamo and Hoy, 1994 Anim. Behav. 47:857 - 868; Tregenza and Wedell, 1997 Anim. Behav. 54:979 - 984). In combination with tactile and acoustic signals, they incite adult males to court females and aggression between adult males. Interestingly, adult males show both courtship and aggressive behaviour towards larval males, suggesting that the latter may have the pheromones of both sexes. To test this idea, we evaluated the behavioural response of adult males towards cuticular extracts of male larvae, and subsequently analyzed the chemical composition of these extracts by gas chromatography-mass spectrometry (GC-MS). The behavioural assay indicates that male larval-extracts contain the male, but not the female pheromone. Our GC-MS analysis revealed that male larval-extracts contain the same compounds as male adult-extracts, but in lesser amounts. However, the relative concentrations of identified substances are almost identical. Contrasting this, female adult- and male larval-extracts contain the same compounds in similar amounts, but in different relative concentrations. We do not know yet which compounds act as pheromones; possible candidates are heptacosene, two nonacosadienes and nonacosene. Their extract-concentration is far higher in adult males than in females, and male larvae have intermediate amounts (Fig. 1). We suggest that adult male crickets confuse the sex of male larvae, because the latter have an insufficient adult-male pheromone, rather than the female pheromone.

Fig. 1: Comparison of putative pheromonal components



# Daytime-dependent modulation of the pheromone transduction in olfactory sensilla of the hawkmoth *Manduca sexta*

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Pheromone-dependent mate search is under strict circadian control in different moth species. But it remains unknown whether daytime-dependent changes in the pheromone sensitivity already occur at the periphery. To investigate the modulation of insect pheromone transduction, the membrane-permeable cyclic nucleotide analogues 8bcGMP, 8bcAMP and the biogenic amines octopamine (OA) and tyramine (TY) were applied in long-term tip recordings from pheromone-sensitive trichoid sensilla by perfusion of the sensillar lymph. To search for daytime-dependent differences recordings were performed either from ZT 22-1, ZT 1-4 or from ZT 8-11 (ZT 0 = lights on). Using a non-adapting pheromone-stimulation protocol in control recordings the responses to a bombykal stimulus of 10 µg remained constant over the recording duration of 180 min. The perfusion of the sensillar lymph with 8bcGMP adapted the action potential (AP) generator by decreasing the initial AP frequency and the numbers of the APs elicited in the first 100 ms of the response. The sensillar potential (SP) response was not affected. In contrast, 8bcAMP increased the SP amplitude continuously, but had no effect on the AP frequency. The application of OA and TY also increased the sensillar potential amplitude. In addition, OA also increased the initial action potential frequency in recordings from ZT 8-11. Several daytime-dependent effects were found. Perfusion with 8bcGMP decreased the initial AP frequency stronger in recordings from ZT 8-11 as compared to ZT 1-4. The OA- and TY-dependent increase of the sensillar potential amplitude was apparent both at ZT 1-4 and ZT 8-11. However, the increase was statistically significant at ZT 8-11 only. At ZT 22-1 no effects of OA and TY were found. In contrast, the sensitizing effect of 8bcAMP did not show any time-dependency. Furthermore, daytime-dependent differences in the AP distribution in control recordings were observed: during the middle of the day the pheromone responses became increasingly tonic and less phasic. This decrease in the temporal resolution of the pheromone response was enhanced by 8bcGMP application and antagonized by application of 8bcAMP, OA and TY at ZT 8-11. To summarize: We hypothesize that during the photophase elevated cGMP levels underlie a daytime-dependent decrease in pheromone sensitivity and a decline in the temporal resolution of pheromone pulses. We also hypothesize that OA activates an adenylyl cyclase and that the sensitivity of the OA-receptor is controlled in a daytime-dependent manner. [Supported by DFG grant STE531/13-1]

# Activation of the Wnt-βcatenin pathway in a cell population on the surface of the forebrain is essential for the establishment of olfactory axon connections

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A variety of signals governing early extension, guidance and connectivity of olfactory receptor neurons (ORN) axon have been identified, however little is known about axon-mesoderm and forebrain-mesoderm signals. Using Wnt-βcatenin reporter mice we have identified a novel Wnt-responsive resident cell population, located at the surface of the embryonic forebrain, along the trajectory of incoming ORN axons. Organotypic slice cultures that recapitulate olfactory-associated Wnt-βcatenin activation showed that the Bcatenin response depends on placode-derived signal(s). Blocking this canonical Wnt pathway with the Wnt-Frizzled antagonists Dikkopf-1 or secreted-Frizzled-Receptor Protein-2 prevented ORN axon contact to the forebrain. This effect resembles the phenotype of Dlx5-/- embryos, in which axons and migratory cells are present but the primary connections fail to form due to cell-autonomous defects in ORN. In Dlx5-/- embryos Wnt-βcatenin response on the surface of the OB is strongly reduced, accompanied by reduced expression of Wnt5b and Wnt7b around the OB. These data reveal a novel function for the Wnt morphogens in establishement of periphery-CNS olfactory connections, and highlight a complex interplay between cells of different embryonic origin for ORN axons guidance and connectivity.

# The Grueneberg ganglion - a novel chemosensory organ in the nose

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The detection of odors and pheromones in mammals is mediated by chemosensory neurons of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), which generally express the olfactory marker protein OMP. We have found that OMP is also expressed in cells of the so-called Grueneberg ganglion (GG), a cluster of neuronal cells in the vestibule of the anterior nasal cavity. Chemosensory responsiveness of olfactory neurons is based on the expression of distinct receptors: odorant receptors in the MOE or pheromone receptors in the VNO, respectively. To scrutinize whether neurons in the GG may indeed be chemosensory cells, they were subjected to molecular phenotyping. It was found that a distinct vomeronasal receptor type was expressed in the majority of GG neurons which were concomitantly endowed with the G proteins Go and Gi; both are also present in sensory neurons of the VNO. Expression of odorant receptors was only observed in very few cells during perinatal stages; a similar number of cells expressed adenylyl cyclase type III and Golf/s. These findings demonstrate that the GG mainly comprises cells with a VNO-like phenotype. The GG neurons extend axonal processes which fasciculate to form nerve bundles that project caudally along the roof of the nasal cavity and through the cribriform plate, finally terminating in the olfactory bulb of the brain. In summary, the expression of olfactory signaling proteins as well as the axonal projection to the olfactory bulb strongly support the notion that the GG may indeed have a chemosensory function.

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# Co-localisation of a pheromone receptor and PBPs in pheromone-sensitive hairs of H. virescens

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Due to the crucial role of pheromones for insect life, highly specialized pheromone detection systems have evolved, capable to detect volatile compounds with extreme sensitivity and selectivity. The process of pheromone detection in moth antennae begins when the molecules enter sensory hairs (sensilla trichodea) through pores in the cuticle. The hydrophobic molecules are shuttled through the aqueous sensillum lymph, a process probably mediated by pheromone binding proteins (PBPs). PBPs are supposed to ferry the pheromonal compounds towards receptors in the chemosensory membrane of the receptor cells. We have identified several candidate pheromone receptors in the tobacco budworm Heliothis virescens and generated antibodies directed against the receptor-type HR13. In immunohistochemical studies the antibodies specifically labeled multiple cell bodies housed in male-specific pheromone-sensitive sensilla. In addition, the dendrites as well as the axonal processes of immunoreactive cells were labeled. To explore which PBP-type may be present in sensilla hairs housing HR13-neurons, we set out to evaluate how many PBP-subtypes may exist in Heliothis virescens. Presently only one PBP-subtype has been described (HvirPBP1); by screening of an antennal cDNA library from Heliothis we have now identified two novel PBP-subtypes, designated as HvirPBP2 and HvirPBP3. The sequences of the three Heliothis PBPs were found highly related to each other and to pheromone binding proteins from other moth species. In situ hybridization on male antenna in combination with laser scanning microscopy revealed that HvirPBP1 and HvirPBP2 are expressed in cells below long pheromone-sensitive sensilla trichodea. Double-labelling studies indicated that the two PBPs are co-expressed in the same cells. Moreover, the PBP-expressing cells were found to surround the HR13-neurons indicating that the three proteins are present in the same sensory hair. These results suggest a possible interplay of HR13 with one of the two PBPs in the detection of a distinct pheromone component.

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# A distinct binding protein and receptor type of H. virescens mediate the reaction to the major sex-pheromone component

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Mate finding behavior in moths relies on sex-pheromones released by the females and detected by the highly specialized antennae of males. The remarkable responsiveness of male moths to female-released pheromones is based on the sensitive and selective reaction of specialized sensory neurons. These cells extend a dendrite with specific receptor proteins into the trichoid sensillar hairs on the antenna. To reach the chemosensory dendrites the hydrophobic pheromone molecules have to cross the aqueous sensillum lymph, a transport which is supposed to be mediated by distinct pheromone binding proteins (PBPs). We have identified several candidate pheromone receptors and two PBPs from the tobacco budworm Heliothis virescens. Expression of the male-specific receptor type HR13 was visualized by means of a receptor-specific antiserum in the dendrites of neurons located in a high number of pheromone-sensitive sensilla trichodea. The HR13 neurons were found to be surrounded by cells which co-express the two binding proteins PBP1 and PBP2. These findings suggest that one of the PBPs may act together with HR13 in detecting a distinct component of the sex-pheromone blend. To determine the ligand specificity of HR13 and to assess a possible role of the PBPs, the receptor encoding cDNA was stably integrated into the genome of HEK293/Gα15 cells. The responsiveness of receptor-expressing cells to pheromonal compounds of H. virescens was monitored by Ca2+-imaging analyses. These studies showed that expression of HR13 rendered HEK cells solely sensitive to the pheromone component Z11-16:Al solubilized by DMSO. Experiments with either one of the PBP subtypes replacing DMSO revealed that PBP2, but not PBP1 can mediate a response of HR13 expressing cells to Z11-16:Al. The results support the notion that both a distinct PBP and distinct pheromone receptor are involved in the detection of a pheromone component; this specific combination may contribute to the remarkable ligand specificity of the moths´ pheromone detection system.

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# Gut hormone receptors in chemosensory systems - a chemosensory system in the gut?

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Peptidergic hormones secreted by cells of the gut convey information about the nutritional state of the body to the brain thus governing feeding behaviour. Appetite and satiety are strongly affected through the action of the gustatory and olfactory system; in addition palatability and the smell of food vary with the metabolic situation. It has been suggested that the 'appetite hormones' may also affect the chemosensory systems. We have performed RT-PCR studies indicating that receptors for these hormones appear to be expressed in both the olfactory epithelium as well as taste buds. This was confirmed using immunohistochemical techniques for the leptin receptor Ob-R and the receptor GPR39. In the gut, a variety of intestinal processes, such as secretion, resorption and motility, are affected by the chemical composition of the gut contents. Thus, precise chemosensory monitoring of the luminal contents is of great importance, however, the relevant cell types and the molecular mechanisms are still elusive. The so-called "brush cells" in the intestinal mucosa are considered as candidate chemosensory cells due to their morphological similarity to taste receptor cells of the tongue. We have confirmed that some of these cells express the G-protein alpha-gustducin which is known to be involved in sweet and bitter taste transduction. Alpha-gustducin expressing cells were found to be scattered throughout the intestines; however, they are especially abundant in the cardia of the stomach where they are arranged in large cell clusters. In RT-PCR approaches, we have found that several members of taste receptor families are expressed in the intestine, which might act as chemosensors not only in the tongue, but also in the intestinal system.

# Candidate pheromone binding proteins of the silkmoth Bombyx mori

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Candidate pheromone binding proteins of the silkmoth Bombyx mori

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Throughout the animal kingdom pheromone-induced behaviour plays a critical role for survival and reproduction. In particular, moths rely strongly on distinct chemical cues for intraspecific communication; sex-pheromone signaling in the silkmoth Bombyx mori represents one of the most remarkable examples. Female moths release a blend of sex-pheromones to attract males over long distances and males detect the released pheromones with extremely high sensitivity and selectivity. In male moths pheromone detection is mediated by specialized sensory neurons in long trichoid sensilla on the antennae. Soluble pheromone binding proteins (PBPs) in the sensillum lymph surrounding the dendrites of the pheromone-sensitive cells are supposed to transfer the usually hydrophobic pheromone molecules to the dendritic membrane of the sensory neurons. Recent studies have shown that the known PBP of Bombyx mori (BmorPBP1) appears to be specifically tuned to bombykol but not to bombykal raising the question if additional subtypes may exist. Combining BLAST analysis of the Bombyx genome and screening of an antennal cDNA library with BmorPBP1 we have identified two novel genes, which encode candidate PBPs (BmorPBP2, BmorPBP3). All three B. mori PBP genes are located in close proximity in the genome and share the same exon / intron structure. Comparison with PBPs from various moth species revealed a high degree of sequence identity and assigned the three BmorPBP-subtypes to distinct groups within the moths' PBP family. In RT-PCR experiments the novel BmorPBPs were found to be expressed in the antenna of males and females. In situ hybridization revealed that BmorPBP2 and BmorPBP3 are expressed only in a rather low number of cells along the antennal side branches. Double-labeling experiments uncovered that the two novel BmorPBPs are expressed in the same cells but are not co-expressed with BmorPBP1. Furthermore, unlike BmorPBP1, cells with the newly identified PBPs did not surround neurons expressing the BmOR-1 receptor. The results indicate that BmorPBP2 and BmorPBP3 are located in sensilla types which are different from the long sensilla trichodea.

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# Differential reaction of olfactory axons to target tissue: in vitro cultures

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The main olfactory system is characterised by a unique topographical organisation; thousands of olfactory sensory neurons (OSN) scattered within the olfactory epithelium (OE) project into a few glomeruli in the olfactory bulb (OB). The mechanisms for establishing the precise wiring pattern, notably for the guidance of axons and for the fine targeting within distinct glomeruli are still elusive. Recent findings suggest that the odorant receptor (OR) protein itself may contribute to targeting processes, but nothing is known about its functional role. To study the outgrowth of olfactory axons we have established in vitro cultures of explants from the OE of prenatal OMP-GFP mice, which allows visualising axons by intrinsic fluorescence. Monitoring the outgrowing fibers in a co-culture of explants from the OE and the OB revealed that most of the GFP positive axons showed a repulsive reaction when contacting neurites originating from the bulb. However, some of the axons fasciculated on their way towards and into the OB explant, where loose neuropil structures and/or dense bundles were formed. In the presence of explants from mesenchyme - located directly beneath the OE - the axons were attracted without making direct contact; as they reached the explant, the axons grew along the tissue border. The use of transgenic mice, which express GFP in distinct receptor-specific OSN, allows to monitor specific axon populations and its interaction with other cells, especially cells with OR proteins in the plasma membrane. For this purpose, several cell lines were used to express various OR-EGFP chimeras in combination with proteins, such as ß2AR, RTP1/2 and Reep1, which are supposed to improve membrane targeting of heterologous expressed OR.

# Cells in the vomeronasal organ express odorant receptors but project to the accessory olfactory bulb

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In addition to the main olfactory system, most mammals possess a well-developed vomeronasal system that is considered to be specialized for detecting pheromones. Within the vomeronasal organ (VNO), two populations of sensory neurons are distinguished; cells in the apical layer expressing V1R-receptors and cells in the basal layer expressing V2R receptors. Recent evidence indicates that the VNO of mice not only responds to pheromonal compounds but also to general odorants. RT-PCR analyses and in situ hybridization studies indicated that certain members of the OR gene repertoire are expressed in the VNO. These genes expressed in the VNO were found to be scattered over several genomic clusters and were concomitantly expressed in cells of the MOE. Gene-targeted mice which co-express histological markers with the receptor mOR18-2 allowed to unravel characteristic morphological differences between OR-expressing cells in the VNO compared to the MOE. Experiments towards a molecular phenotyping of these cells revealed that the OR-cells in the VNO did not share elements of the signal transduction machinery typical for MOE neurons, such as ACIII or Golf, but rather expressed TRPC2 and G?i. Visualizing the axonal processes of VNO cells expressing distinct ORs revealed that they projected to the accessory olfactory bulb (AOB). Axon fibers were visible in the anterior AOB where they converged into glomerular-like structures. The finding that distinct OR-types are expressed in cells which are located in the apical layer of the VNO and have the typical features of VNO sensory neurons including the projection to the AOB suggest that this population of sensory cells may be a novel facet in the complexity of the chemosensory system.

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# Olfactory mixture representation at the level of projection neurons in the honeybee antennal lobe

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Natural olfactory stimuli occur as mixtures of many single odorants. We studied whether the representation of a mixture in the brain retains single-odor information (elemental rule) and how much mixture-specific information it includes (configural rule). To understand mixture representation in the honeybee brain, we used in vivo calcium imaging at the level of the antennal lobe, and systematically measured odor-evoked activity in 24 identified glomeruli in response to four single odorants and all their possible binary, ternary and quaternary mixtures.

Our previous work used bath-application of the calcium sensitive dye (calcium green AM) to record signals emphasizing activity from the olfactory receptor neurons (input to the antennal lobe). We found that although a gain control system prevents saturation of the olfactory system with wide-spread inhibition, mixture representation follows essentially elemental rules. We now continued our search for mixture unique properties, specifically staining a population of projection neurons, using a retrograde calcium sensitive dye (Fura dextran). This staining allows the study of mixture representation, after processing by antennal lobe local networks, and before it is conveyed to higher-order brain centers, like the mushroom bodies or the lateral horn.

# Poster Topic

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# Magnetic Field Perception in Teleost Fish

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Magnetic field perception is shown in many vertebrates and was discussed as an orientation cue in long distance navigation. The receptor structure is largely unknown. A number of diverse hypotheses have been proposed that might provide the basis for a mechanism to detect the Earth magnetic field. Recent research has focused on three potential mechanisms. Electromagnetic induction, magnetic field dependent chemical reactions and magnetite based magnetoreception. In previous studies magnetite was found in the ethmoid region of the rainbow trout Oncorhynchus mykiss and juvenile trouts were found able to discriminate fields with different intensity. To verify these behaviorial experiments and to test magnetic field perception, discrimination tests and electrocardiographic conditioning experiments in the same species were done. In the discriminaton test trouts discriminate between two artificial magnetic fields superimposed to the earth field. The measure for magnetic field perception during the application of a magnetic field strength of 100 µT was the rate at which the trout hit a target in anticipation of a food reward in comparison with the response rate during the application of a magnetic field strength of 10 µT in non-reinforced trials. Three juvenile rainbow trouts showed significant higher response rates in reinforced trials.

Magnetosensation in rainbow trouts was examined in adult and juvenile animals also by conditioning and electrocardiography. The method depended on establishing a conditioned response, in this case a longer heart beat interval of the trout, when exposed to an intensity shift (the conditioning stimulus) in the artificial magnetic field accompanied by short light flashes (the unconditioned stimulus). The tested rainbow trouts showed the conditioned response after 5-11 Sessions with pairings of unconditioned and conditioned stimuli. The trouts showed significant longer interbeat interval times in comparison to the ongoing heart rate frequency. These results unequivocally show that trouts are able to detect and respond to changes (100 µT) in the intensity of an artificial magnetic field.

# Kármán vortex street detection by the lateral line

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We stimulated the lateral line of goldfish, Carassius auratus, with running water (10 cm*s-1) and with a Kármán vortex street. While stimulating the lateral line we recorded the neural activity from anterior lateral line nerve fibers. Compared to still water most fibers increased their discharge rate under both running water and Kármán vortex street condition. In terms of spike rate there was no difference between the Kármán vortex street and the running water condition. However, if exposed to a vortex street, spike train frequency spectra showed a reproducible peak at the vortex shedding frequency. This peak was also apparent if the relative position between the fish and the cylinder that generated the Kármán vortex street was altered. Any change in the vortex shedding frequency evoked by a change in either cylinder diameter or water flow velocity shifted the reproducible peak of the neuronal data into the expected direction. So far, only an increase or no change in mean firing rate of lateral line afferent fibers was reported as a response to unidirectional water flow. Our data show that the vortex shedding frequency can be retrieved from the spike trains of single lateral line afferent fibers. Fish might use this information to analyze hydrodynamic trails of other fish or to detect stationary objects in running water.

# Distribution of Connexine in a sensory system that relies on temporal coding - the active electrosensory system.

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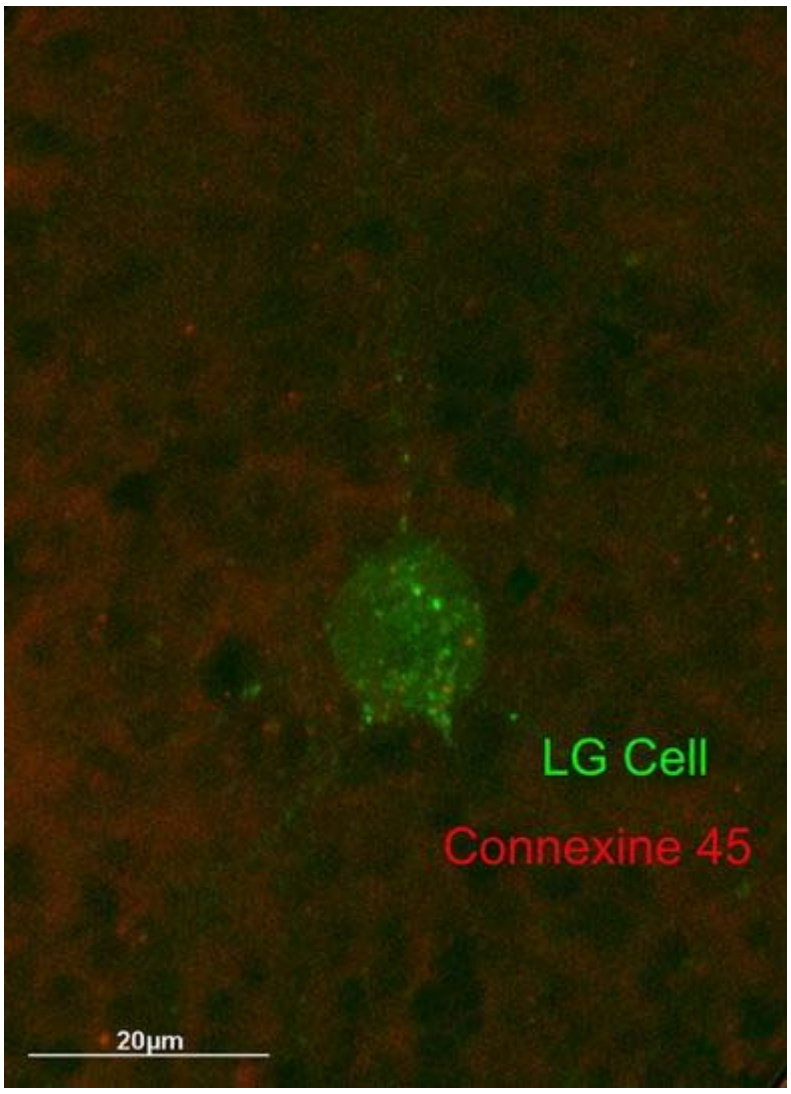
Gnathonemus petersii, a member of the pulse-type weakly electric fish, are well studied animals with respect to their unique electric sense. They possess three different peripheral receptors devoted to separate aspects of their environment. These aspects include active/passive electro location and electro-communication. Active electrolocation is based on the generation of a short electrical pulse (EOD) that builds up a field around the fishes body. The currents associated with the EOD flow through the skin of the fish and are sensed by specialised electroreceptors. These so-called mormyromasts respond either to changes in the amplitude of the EOD and/or the temporal structure of the EOD.




Two sub modalities of the electric sense, the mormyromasts and the knollenorgans encode sensory stimuli using a temporal code and the sensory input couples directly to the motor system responsible for the generation of the electric field. Hence we were interested in the presence of gap junctions as a means to reliably and quickly transmit temporal information in this system.

Gap junctions are present at many synapses in the electrosensory system of mormyrid electric fish, and physiology shows that many of these synapses are electrical. The goals of the present project were to use antibodies to determine the proteins present at known gap junction synapses and to identify previously unrecognized gap junctions in the system. Antibodies against mammalian connexin 36 (the teleost ortholog to Cx 35), 43 and 45 were used. We confirmed known electrical synapses including: terminals of knollenorgan afferents in the nucleus of the electrosensory lobe (nELL); synapses on EOD command and medullary relay nuclei; and synapses of nELL axons on large and small cells of the exterolateral nucleus of the mesencephalon. Co-expression of the antigenes, especially 45 and 36 was found in these regions.

# Responses of lateral line brainstem neurons to dipole stimuli of different frequencies in Goldfish, Carrasius auratus

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The medial octavolateralis nucleus (MON) is the first site of sensory processing in the ascending lateral line pathway of fish. Whereas the frequency response characteristics of primary lateral line afferent fibres have been described at least in some species, nothing is known about the characteristics of brainstem lateral line neurons. In this study, we investigated how MON neurons in goldfish, Carassius auratus, respond to sine wave stimuli of different frequencies.

Extracellular recordings were made from single MON neurons while the lateral line was stimulated with sinusoidal water motions (20, 50 and 100 Hz, 0.5 s duration, displacement amplitude 400 µm) generated by a stationary vibrating sphere (8 mm diameter). Thus far, we recorded from 54 neurons in 23 fish. As expected (Mogdans and Goenechea 2000), not all MON neurons responded to sine wave stimuli: 42 neurons exhibited an increase or a decrease in discharge rate and, 12 neurons did not change discharge rates in response to the stimulus.

Different MON neurons exhibited different frequency sensitivities. Depending on their responses, neurons were grouped as follows. (i) Eight neurons responded to only one of the applied frequencies. Two neuron increased rate to 50 Hz and, six neurons decreased rate, two of them to 50 Hz and four to 100 Hz. (ii) Sixteen neurons responded with a change in discharge rate to two of the three frequencies. Of these, eleven responded always with an increase in discharge rate (nine to 50 and 100 Hz, one to 20 and 50 Hz and one to 20 and 100 Hz) and five with a decrease in discharge rates (two to 20 and 100 Hz, three to 50 and 100 Hz). (iii) Fifteen neurons responded to all three frequencies, 14 with an increase and one with a decrease in discharge rate. (iv) In three neurons discharge rate increase or decrease depended on stimulus frequency. One neuron increased rate in response to 20 Hz and decreased rate to 50 and 100 Hz. Another neurons decreased rate to 20 and 100 Hz and increased rate to 50 Hz. A third neuron did not respond to 20 Hz, but increased rate to 50 Hz and decreased rate to 100 Hz. In summary, independent of stimulus frequency 53% of the neurons responded with an increase in discharge rate, 23.5 % with a decrease and 23.5 % did not respond to the applied stimuli (neurons from group (iv) were excluded from this analysis).

Primary lateral line afferents respond to a sine wave stimulus always with an increase in discharge rate. In terms of displacement, they have highest sensitivity in the frequency range 20 to 60 Hz (superficial neuromasts) or 60 to 120 Hz (canal neuromasts) (review Bleckmann 1994). Our findings demonstrate that response behaviors, patterns of discharge and frequency response characteristics of brainstem lateral line neurons are much more diverse than those of primary afferents. This is indicative of the fact that MON neurons integrate information across a large number of afferent fibres that may innervate widely distributed neuromasts.

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# Differential characteristic curves of infrared processing single units in the Tectum opticum of rattlesnakes

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Snakes like boids and pit vipers have the ability to detect minute temperature differences of objects by sensing the emitted thermal radiation. This sensory system is involved in the prey capture and thermoregulating behaviour of these homoeothermic animals. In pit vipers, infrared signals are mediated from the loreal pits via the trigeminal nerve and specific nuclei within the hindbrain to the Tectum opticum and forebrain. The Tectum opticum of pit vipers integrates infrared and visual input.

We investigated the response characteristics of infrared processing tectal neurons by means of electrophysiological recordings in the anaesthetised western diamondback rattlesnake (*Crotalus atrox*). By stimulating the pit organs with an IR-emitter (λmax = 2,500 nm) at varying distances, we applied different stimulus intensities ranging from 20 μW/cm$^2$ to 9 mW/cm$^2$. A white LED-lamp was used to test for visual sensitivity of the infrared sensitive units.

Extracellular recordings of single units were performed with NaCl filled glass micropipettes (R = 15-60 MΩ). Recordings were carried out within the stratum griseum centrale of the contralateral Tectum opticum of the snakes.

A total of 22 single-units were recorded, 13 of which were spontaneously active. Single unit-responses were either phasic (n= 18) or phasic - tonic (n = 4). No bimodal single units were found.

The response latencies at the highest stimulus intensity varied between 7ms and 33ms. With increasing stimulus intensity the latency of the single units decreased.

The spike frequency of single units increased with stimulus intensity and varied considerably among the single units. The spike frequency of action potentials as function of stimulus intensity was analysed for 11 single units (Fig.1). The characteristic curves of spike frequencies revealed a distinct dynamic range of the individual single units with specific threshold (0.3 mW/cm$^2$ - 2.3 mW/cm$^2$) and saturation levels (2.4 mW/cm$^2$ - 7.1 mW/cm$^2$) of stimulus intensities. These characteristic curves have very different slopes and thus separated dynamic ranges for infrared stimuli.

The differences in dynamic range of the single units presented in this study indicate the existence of optimal tuned tectal single units for any infrared stimulus within the full range of detection sensitivity.

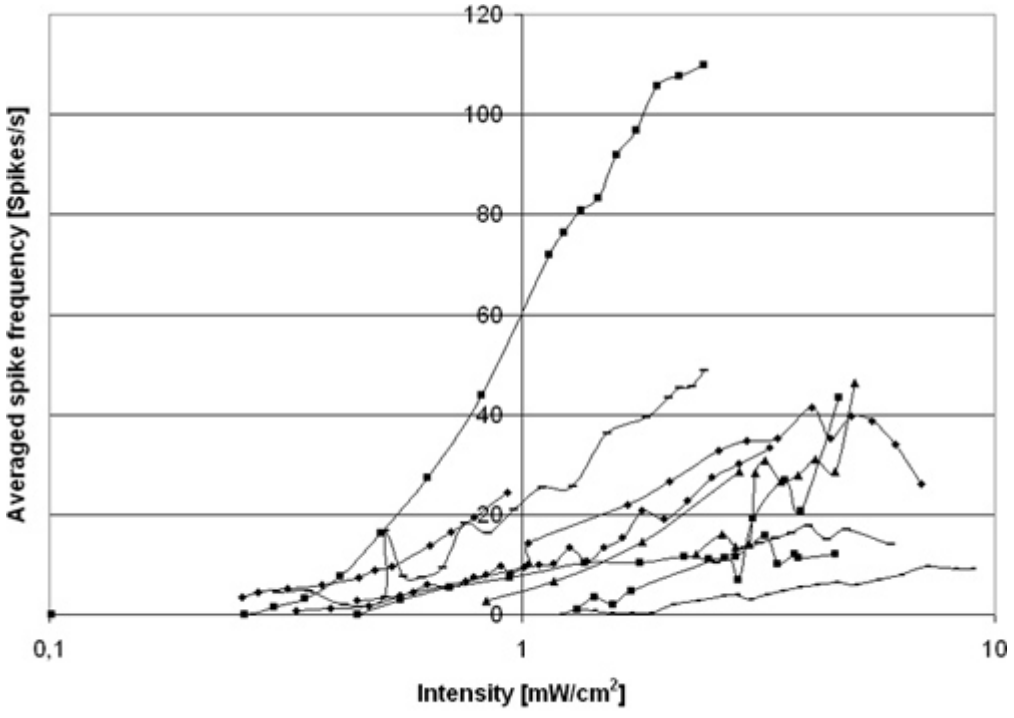


Fig.1: Characteristic curves of 11 infrared sensitive single units.

# Neuroanatomy and electrophysiology of the infrared sensilla of the Australian pyrophilous "little ash beetle" *Acanthocnemus nigricans*

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The Australian "little ash beetle" *Acanthocnemus nigricans* is attracted by forest fires. It has one pair of unique prothoracic sensory organs which respond to thermal stimulation. Each organ mainly consists of a small cuticular disc which contains about 90 cuticular sensilla on its outer surface [1, 2]. Retrograde dye labelling of some of these sensilla revealed that they only sparsely ramified in the ipsilateral hemisphere of the prothoracic ganglion. Axons ascended into the ipsilateral neck connective, passed through the suboesophageal ganglion without any notable side branches to terminate in the region of the trito- and deutocerebrum with extensive ramifications.

To further characterise the thermal responses of the disc sensilla, we recorded extracellularly from single sensilla whilst irradiating the organ with pulses of red laser light ($\lambda$ = 632.8 nm). The sensilla spiked at rest with firing rates between 8 and 19 spikes/s. Laser irradiation elicited phasic-tonic discharges with peak spike rates of approximately 80 spikes/s at the highest irradiation intensity (549 mW/cm²). Phasic responses did not saturate at the highest irradiation intensities used in this study. Tonic response portions adapted within one second to reach steady state-firing rates between 50% and 90% of the corresponding peak rates in an intensity-dependant manner. Switching off the laser irradiation caused distinct inhibitions of the spiking activity, which lasted several hundreds of milliseconds at the highest irradiation levels. These findings proof that the disc sensilla are thermal sensors which are able to provide behaviourally relevant information for the pyrophilous *Acanthocnemus* beetles. In particular, we hypothesise that the trailing inhibition of the spiking responses might assist the beetle to detect temperature gradients on the ground.

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# How electrosensitive neurons of the ELL of *Gnathonemus petersii* encode information about shape and material of nearby objects.

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The weakly electric fish *Gnathonemus petersii* is able to explore its environment with an extraordinary sensory system which is called active elektrolocation. For this it elicits brief electrical pulses (*e*lectric *o*rgan *d*ischrarge, EOD) with specialised muscelcells situated in the tail. The properties of the electric field are perceived via cutaneous electroreceptors which contain different sensory cells (A- and B-cells). In the context of active electrolocation these electroreceptors are the so-called mormyromasts. Whereas A-cells respond in proportion to amplitude-modulations of the EOD, B-cells are sensitive to the shape of it. The afferent fibres from A-cells code the EOD-amplitude within the range of amplitude-modulations that occur due to small objects (2-10%) in form of a latency-code. This was figured out by former investigations by Bell (1989) and Engelmann (2005). They concluded that primary afferents use first spike latency to encode the objects resistive properties. Primary afferents of the sensory cells responded to a local increase of the electric field amplitude with a decrease of spike latency, while the rate of the responses usually remained constant. The main question for this study is how information about objects is being processed in the first central stage of the ascending electrosensory pathway (ELL). Thus we recorded extracellularly with NaCl-filled (3 mol/L) glass microelectrodes (resistance: 1-3 MΩ) from electrosensitive neurons in the medial zone of the ELL. Curarized fish were stimulated with an artificial whole-body stimulus. We then passed either a metal-cube (4x4 mm, brass) or a plastic-cube (4x4 mm, acrylic) through the receptive field (RF) and determined discharge rates and first spike latencies. At the same time we recorded the peak-to-peak (p-p) amplitude of the EOD in the centre of the receptive field. This allows analysing the neuronal responses with respect to the actual modulation of the electric field due to the presence of the objects. For good conductors the local p-p-amplitude increases while it decreases for non-contuctors. For analysing we divided between inhibitory (I) and excitatory (E) neurons. I-units showed a decrease in firing and an increase in first spike latency in the center of their receptive field when a metal-cube was passed through it. The plastic-cube provoked a more or less increase in firing in the center of the RF than the good conductor whereas the periphery was more affected by it. E-units responded with an increase in firing and a decrease in first spike latency when the metal-cube was presented. The plastic-cube seems to have the lowest influence to E-units. To sum up, we can say that the local field geometry caused by the material properties of an object has a very strong influence to the responses of electrosensitive neurons. In contrast to the primary afferents, which use only the latency for encoding object properties, electrosensitive neurons in the ELL of *Gnathonemus petersii* seem to use both, latency *and* rate-coding.

# Active Electrolocation of Objects: Pre-Receptor Mechanisms, Spatial Resolution and Electric Images of Objects in the weakly electric fish, *Gnathonemus petersii*

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The weakly electric fish *Gnathonemus petersii* explores its environment by emitting electric organ discharges (EOD) with an electric organ located in its tail. Each EOD builds up a 3-dimensional electric field around the fish. Nearby objects project electric images onto the fish's skin, which are scanned by epidermal electroreceptor organs, so called mormyromasts. 'Active electrolocation' of objects is used by the fish during prey search and for object detection during spatial navigation. We previously have hypothesized that *G. petersii* possesses two 'electric foveae' on its head: one at the chin appendix ("Schnauzenorgan") and another one in the nasal region. Our hypothesis has been supported by several findings, e.g., by the voltage distribution of the electric field, the density of electroreceptors, and the behavioural use of the Schnauzenorgan. In the experiments reported here, we further tested the foveal hypothesis (1) by measuring the shape of the electric field produced by *G. petersii* and (2) by recording the electric images of defined objects within the nasal region and comparing them to those in non-foveal regions.

The biphasic electric field produced by *G. petersii* showed the typical properties of a complex dipole field. The voltage distribution at the caudal pole around the fish's tail was concentric, while the rostral pole around the trunk and head was enlarged. The voltage drop along the side of the fish was rather high, whereas the voltage distribution in the head region was homogeneous.

Objects of different shapes and/or material (cubes, 2 x 2 cm, and spheres, Ø 2 cm, made of metal or plastic) were tested. Their electric images were recorded with a silver electrode on several levels in the head region of the fish. Both, shape and material of an object effected the properties of the electric images in the head region. The local EOD amplitude at the skin changed when objects with a different resistance than the surrounding water were presented. Local EOD amplitude increased in presence of a good conductor, while non-conductors decreased it. The shape of an object also had an effect on the properties of the electric image. We found significant differences of electric images evoked by cubes and spheres. Images of differently shaped objects differed mainly in their slopes, i.e. at the rim area of the image. Spheres projected images with steeper slopes compared to cubes. The values of the image slopes were constant for objects of the same shape and did not depend on the material of the object. This is in contrast to electric images outside the head region, which did not show this constant dependency of object shape and image slope. Thus, image slope in the head region might be one of the cues for the fish to recognize an object's shape.

The homogenous voltage distribution and the shape-dependency of electric images in the nasal region support our hypothesis that *G. petersii* has a specialized rostral electroreceptive skin area. Our results show that this region serves special functions during active electrolocation and thus might be called an electroreceptive fovea.

# Responses of trout anterior lateral line nerve fibres to sine wave stimuli in still and running water

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The lateral line of fish is comprised of superficial neuromasts (SN) and canal neuromasts (CN). In goldfish and trout, two types of posterior lateral line nerve (PLLN) fibres were distinguished. Type I fibers were sensitive to water flow and, consequently, their responses to sinusoidal water motions were masked by a background flow. In contrast, type II fibres were insensitive to water flow and responded about equally well to sine wave stimuli in still and running water (Engelmann et al. 2000, 2002). We investigated how fibres in the anterior lateral line nerve (ALLN) of trout, Oncorhynchus mykiss, respond to running water and whether different fibre types comparable to those found in the PLLN can be distinguished.

Using glass micropipettes filled with 3 mol/l KCl (impedance 50-90 MOhm) we recorded the ALLN fibre responses to sinusoidal water motions (50 Hz, displacement amplitudes 2-63 µm) generated by a small (8 mm diameter) oscillating sphere in both still and running water (10 cm/s). As in previous studies, we used running water to distinguish flow-sensitive and flow-insensitive fibres. Thus far we recorded from 76 fibres in 17 trout. Ongoing rates in still water were surprisingly high (mean 83.21 Hz ± 44.42 Hz, range 4.2 Hz to 200 Hz). Out of a total of 19 ALLN recorded under running water conditions, 17 were defined as flow-sensitive, i.e. ongoing rates in running water differed from those in still water (Mann Whitney U-test $p<0.01$). In most of these fibres, the change in discharge rate was fairly small. In 11 fibres, ongoing rates changed by less than 10% under running water conditions whereas in 6 fibres ongoing rates changed by 12% or more. Two units were defined as flow-insensitive, i.e. ongoing rates were comparable in still and running water (Mann Whitney U-test $p=0.57$ and 0.65). Level-response functions under both still and running water conditions were obtained from 10 flow-sensitive fibres. Discharge rates and the degree of phase-locking increased with increasing displacement of the vibrating sphere. Fibres frequently responded with multiple spikes per stimulus cycle even at displacement amplitudes close to threshold. In none of the fibres were the responses to sine wave stimuli affected by water flow, i.e. none of the responses was masked by water flow in terms of discharge rate or phase-locking.

Our findings are in contrast to previous data from PLLN fibres in goldfish and trout (Engelmann et al. 2000, 2002). A possible explanation could be the already high discharge rates of the recorded fibres and the fairly small changes in ongoing discharge rates to a background water flow. On the other hand, the head lateral line of trout consists predominantly of CNs and therefore, we expect that most if not all fibres respond at best weakly to a background water flow. Consequently, their responses to sine wave stimuli should not or only barely be affected by running water. Most of the data collected from trout ALLN afferents are in agreement with these assumptions.

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# Spatio-temporal structure of spontaneous state transitions in the neocortex

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Spontaneous state transitions of neocortical activity, as observed during slow-wave sleep or under certain anesthetics, are characterized by fast changes between epochs of intense network activity (up-states), and silent periods, where spiking activity is virtually absent (down-states). In single cells, up-states are characterized by massive synaptic input, leading to a strongly fluctuating, depolarized membrane potential, while in down-states, the membrane potential shows only few fluctuations at a hyperpolarized level. The strong synchronization between cells during this kind of activity is reflected in population signals, such as the local field potential and the EEG. Waves of synchronized activity have been demonstrated to travel from varying initiation points over the human cortex during slow-wave sleep (Massimini et al. 2004). In animal experiments it was shown that only a small, time-varying fraction of the cells in a given cortical volume takes part in the oscillatory activity (Kerr et al. 2005).

We investigated the spatio-temporal structure of slow-wave activity in vivo in the rat neocortex under ketamine/xylazine anesthesia, combining extracellular recordings with up to 12 electrodes with one simultaneous intracellular recording, which was used to detect state transitions. Triggered on these, we extracted episodes of spike activity from the extracellular electrodes and determined the distribution of times of the state transition for each of the electrodes from the individual episodes. The emerging spatio-temporal pattern was then tested for pattern consistency and variability across repeated activity waves employing single trial rate estimates (Nawrot et al. 2003).

In a plane parallel to the cortical surface, we found that in most recordings there was considerable variability with respect to the precise spatio-temporal structure of activity waves. However, in many cases a preferred direction of activity spread could be identified during limited recording periods. By contrast, recordings from different cortical layers revealed that state transitions occurred much more simultaneously throughout the depth of the cortex. These findings indicate that under ketamine/xylazine anesthesia, activity waves may travel in a stereotypic manner across the surface of the neocortical tissue, recruiting cells in all cortical layers synchronously. Such stereotypic patterns of activity might lead to selective strengthening of active synapses, linking slow-wave activity to learning-related phenomena like memory consolidation during slow-wave sleep.

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# Transcranial theta-burst stimulation diminishes experimentally induced pain perception in human subjects

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The administration of repetitive transcranial magnetic stimulation (rTMS) to the primary motor cortex (M1) has long been recognised its role in the alleviation of chronic central and neuropathic pain, but its overall efficacy is still controversial. We applied three new recently reported methods using rTMS based on theta-burst patterns of neuronal firing to the hand area in the left primary somatosensory cortex (SI) of 8 healthy subjects in order to produce an inhibitory effect on pain perception. The site of stimulation was individually identified using a frameless stereotactic system. In the first paradigm, a three-pulse burst at 50 Hz was given every 200 ms for 2 s, repeated every 20 s for 20 cycles (intermittent theta burst stimulation - iTBS). In the second paradigm, we gave a three-pulse burst at 50 Hz every 200 ms for 40 s (continuous stimulation, - cTBS). In the third paradigm (intermediate stimulation - imTBS) a 5 s train of TBS was repeated every 15 s for a total of 110 s. Additionally, sham rTMS was also introduced using a sham coil and the cTBS paradigm. Subjective pain perception measured by nummerical analogue scale (NAS) and the N2-P2 component of the laser evoked potentials (LEPs) were evaluated before and after stimulation. All of the stimulation conditions resulted in diminished A-delta fiber mediated pain perception, mainly when the contralateral hand was stimulated with a laser beam. Consistent with the psychophysical results, the amplitude of the N2-P2 component also decreased. The most significant pain reduction was observed after imTBS and iTBS stimulation. TBS over the SI cortex may contribute to the understanding of the mechanisms underlying pain relief and pave the way towards a therapeutic benefit.

# The taste of wind - wind sensitive hairs on the palps of Phasmatodea (Insecta)

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Wind sensitive filiform hairs are located on the cerci and the antennae of many insects in general but also on the body wall of certain caterpillars (Keil 1997), and on the head and prosternum of locusts (Bacon & Tyrer 1979, Pflüger & Tautz 1982). They can detect air currents of the wind, of approaching predators and of the insect's own movements, especially when flying. In winged phasmids the filiform hairs of the cerci do not contribute to flight control but surprisingly, we found 10-15 wind sensitive hairs located laterally on each maxillary and labial palp. These are described for the first time in Phasmatodea (stick-and leaf- insects).

The newly discovered hairs differ from that of other such air current mechanoreceptors, because the elevated base is narrow and does not allow strong deflection. Longer hairs are pliable in stronger wind currents.

Electrophysiological investigations of Sipyloidea sipylus show that the stimulation of the hairs contributes to the control of flight via descending interneurons.

When these hairs are immobilized flying ability decreases to near zero. Immobilization of the wind sensitive hairs on the antennae had no such effect.

A new hypothesis concerning the evolution of flight in phasmids has emerged recently. The analysis of molecular data led to the conclusion that the ancestral phasmid lacked wings and that they originated de novo in several subordinate taxa (Whiting et al. 2003).

The wind sensitive hairs on the palps may be a corresponding evolutionary novelty of Phasmatodea.

The results of the present study lead to the conclusion that a new sensory pathway of flight is required for any of the volant Phasmatodea.

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# Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced pain perception

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Multiple cortical areas including the primary somatosensory cortex (SI) are involved in nociception. However, pain processing related to this area is still largely unknown in comparison to tactile processing. The aim of our study was to investigate the effect of transcranial direct current stimulation (tDCS) over the SI on acute pain perception induced with Tm:YAG laser. tDCS modulates the cortical excitability painlessly and non-invasively. Generally, cathodal stimulation decreases, while anodal stimulation increases cortical excitability. Subjective pain rating scores and amplitude changes of the N2 peak that is related to the physical properties of the stimulus, and P2 component is related to the cognitive-emotional aspects of stimulation, of laser evoked potentials (LEPs) of 10 healthy subjects were analyzed prior to and following anodal, cathodal and sham tDCS. Our results demonstrate that cathodal tDCS significantly diminished pain perception when the contralateral hand to the side of tDCS was laser-stimulated while anodal and sham stimulation conditions had no such an effect. In accordance with the psychophysical results, cathodal tDCS significantly reduced the amplitude of the N2 component for the contralateral hand stimulation. Our study may contribute to the understanding of the mechanisms underlying pain relief and extend the application of tDCS in human subjects.

# Dopaminergic modulation of cathodal direct current stimulation decreases experimentally induced acute pain perception

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Transcranial direct current stimulation (tDCS) was recently reintroduced as a tool for inducing relatively long-lasting changes of cortical excitability and activity in focal brain regions reversibly, painlessly and safely. It causes polarity dependent shifts of the resting membrane potentials which consequently change the firing rates of neurons beneath the electrodes and connected cortical areas. Generally it has been found that anodal stimulation over the motor cortex enhances cortical excitability while cathodal stimulation decreases it. It was observed that cathodal stimulation diminishes experimentally induced pain sensation and in parallel reduces the N2 and P2 amplitudes of laser-evoked potentials immediately after the end of stimulation. Furthermore, it is known, that the enhancement of D2 - and to a lesser degree - of D1 receptors by pergolide consolidated tDCS-generated excitability diminution up until the morning post stimulation.

In the present study we have investigated the effect of pergolide and cathodal tDCS on acute pain perception induced with Tm:YAG laser in a double-blind, placebo-controlled study. Subjective pain rating scores and amplitude changes of the N2 (stimulus-related) and P2 (cognitive-emotional) of laser evoked potentials (LEPs) of 10 healthy subjects were analyzed prior to and following 15 minutes cathodal tDCS. Our results demonstrate that cathodal tDCS significantly lowered pain sensation, and the changes in motor cortical excitability stable for up to two hours after stimulation. In accordance with the psychophysical results, the amplitude of N2 component and N2-P2 peaks were reduced. In addition to this, pergolide prolonged the effect of the cathodal tDCS until the next morning. Our study highlights the antinociceptive effect of cathodal tDCS technique and underscores the importance of the dopaminergic system for human neuroplasticity. Furthermore, the data suggests a first pharmacological add-on mechanism to prolong the excitability-diminishing effects of cathodal tDCS for up to 24 hours post stimulation.

# Dynamics of context-specific sensorimotor transformations in the parietal reach region of monkeys

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Sensorimotor transformations do not only depend on sensory input, but also on internal behavioral goals. We can flexibly associate different motor actions with the same sensory stimulus, e.g., reach towards an object or avoid it. How do we integrate decisions about a movement with sensorimotor transformations to achieve goal-directed behavior? The parietal reach region (PRR) in the medial intraparietal cortex of rhesus monkeys specifically is involved in visuospatial planning of arm reaches. What are the neural dynamics of the visuomotor transformations in PRR when two context-specific motor response alternatives are associated with identical spatial cue information? To investigate context-specific sensorimotor transformations we recorded single neuron activity and local field potentials (LFP) while monkeys performed a memory-guided anti-reach task. Monkeys either had to reach to a memorized peripheral target position (PRO-reach) or to a diametrically opposed position (ANTI) while keeping central ocular fixation.

We have shown previously that the ensemble spiking activity encoded the sensory information on the cue position weakly and only during a short period of cue visibility. From the end of the visual cue presentation and during the memory period the representation of the reach goal was increasingly prevalent in the ensemble spiking activity. The representation of the reach goal evolves later in the ANTI- than in the PRO-condition. The delay corresponds well to reaction times being increased by 40-50ms in the ANTI- as compared to the PRO-reaches, when the monkeys performed a reaction-time task instead of a memory task. We now show that LFPs in PRR also are spatially tuned, mainly in the frequency range above 15 Hz. Tuning in this frequency range reliably represents the reach goal during the memory period, regardless of whether the monkeys are planning a PRO or ANTI reach.

The results indicate that spiking activity as well as LFPs in PRR encode context-specific sensorimotor transformations. Due to their superior properties for stable long-term recordings, this confirms the suitability of LFPs for the use in neuroprosthetic devices.

# BROAD-BAND FREQUENCY CODING IN RESPONSES TO FREQUENCY-MODULATED (30-210 Hz) WHISKER VIBRATIONS IN THE BARREL CORTEX OF THE RAT.

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Rats can perform subtle texture discriminations with only very short contacts of a few whiskers with an object. This requires a sensory system which can identify small differences in roughness, i.e., frequency components of textures. We have demonstrated that neurons of the primary somatosensory barrel cortex (S1) of the rat encode high frequency (>100 Hz) whisker vibrations in a precisely phase-locked manner (Ewert et al. 2006). We now investigated if also a broad band of vibratory frequencies is represented in responses of S1 neurons.

Extracellular single- and multi-unit activities as well as the electrocorticogram were recorded in mechanically ventilated isoflurane-anesthetized rats, and end-tidal pCO2, heart rate and body temperature were controlled. Single whiskers were attached to the probe of a feedback-controlled electromechanical stimulator and sinusoidal vibratory stimuli were applied ten times pseudo-randomly at either constant or linearly modulated frequencies from 30 to 210 Hz at amplitudes of 0-280 µm for 1 s with interstimulus intervals of 5 s. The phase-locking to the cycles of the sinusoidal whisker movements was analyzed in phase histograms and the entrainment to the vibratory frequencies was analyzed in time-frequency plots which were both generated from time stamps of the spikes.

Significant phase-locking of the responses (Rayleigh test, $p<0.01$) was found for every stimulus with constant frequencies of whisker movement at 60, 110 and 180 Hz. When the three frequencies were presented with different amplitudes either maintained during the 1 s stimulus epoch or linearly modulated with increasing or decreasing values, the spikes were elicited at earlier phases both for stimuli with higher frequencies and higher amplitudes. Thus, the spike activity was correlated to the acceleration of the whisker movement. Stimuli with constant amplitudes modulated with either increasing or decreasing frequencies from 30-210 Hz elicited phase-locked responses with spikes occurring at a distinct phase of the vibratory cycle for each frequency. For all presented stimuli, whether constant or modulated in amplitude or frequency, the time-frequency analyses showed that the frequencies of whisker movements were encoded by the spike discharges in a one to one manner.

The findings show, that some S1 neurons are not only tuned to a specific frequency of whisker vibration, but can also follow constant or modulated stimuli within a broad range of frequencies with precisely phase-locked responses. Since the spikes were elicited at earlier phases with increasing acceleration of the sinusoidal waveform, the acceleration or speed of the whisker movement appears to be the determining factor for elicitation of spikes at precise time points.

Ewert TAS, Vahle-Hinz, C, Engel, AK. FENS Abstr., vol.3, A003.6, 2006
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# OSCILLATORY GAMMA BAND ACTIVITY MAY MEDIATE INTEGRATION OF VIBRATORY SIGNALS FROM NEIGHBORING WHISKERS IN THE BARREL CORTEX OF THE RAT.

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The whisker-to-barrel system is used by the rat to detect surface textures usually by contact with several whiskers of one or both sides. How the signals from mechanoreceptors of the different whiskers are integrated is still a puzzle. To study these interactions in the primary somatosensory (SI) barrel cortex, sinusoidal stimuli reflecting frequency components of textures were used which were applied independently to two whiskers and modified with respect to the whiskers involved, frequency and phase.

Extracellular single- and multi-unit activities as well as local field potentials (LFPs) were recorded in mechanically ventilated isoflurane-anesthetized rats, and end-tidal pCO2, heart rate and body temperature were controlled. Two feedback-controlled electromechanical stimulators were used for independent in-phase or out-of-phase vibration of the principle and a second whisker within the same row or arc. Sinusoidal vibratory stimuli were applied at frequencies of 100-240 Hz for 0.5 or 1 s with interstimulus intervals of 5 s. Fifty consecutive responses to each kind of stimulus were analyzed. The phase-locking to the cycles of the sinusoidal whisker movements was analyzed in phase histograms generated from time stamps of LFPs and spikes. The frequency components of evoked and induced activities were analyzed from time-frequency plots obtained by applying wavelet transform to the averaged responses and to each single response before averaging and subtraction of the evoked activity, respectively.

High frequency vibrations evoked sustained responses of both LFPs and spikes throughout the stimulus epoch. LFP and spike responses occurred tightly correlated and with dominant components at the stimulus frequency as well as lower and higher harmonics. The entrainment of the vibratory responses to stimulation of the principal whisker (evoked activity) was increased by in-phase vibration of a neighbor within the same row and by vibration of the same whisker on the ipsilateral side. This was accompanied by oscillatory activity in the low (35-45 Hz) and high (60-80 Hz) gamma band (induced activity) which was not present during out-of-phase vibration of neighboring whiskers from the same row or vibration of neighboring whiskers from the same arc.

The results show that duration and periodicity of sustained vibration of whiskers in the high frequency range is encoded by LFPs and spikes of SI neurons. Additional information about the pattern of movement of neighboring whiskers and whiskers from both sides may be signaled by oscillatory activity in the gamma band.

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# Acetylcholine effects in layer 4 of primary sensory cortices

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Acetylcholine (ACH) postsynaptic effect on the excitatory component of the neocortex is classically considered as excitatory. Indeed, ACH directly depolarizes pyramidal cells of the neocortex through M1 muscarinic receptors activation, even though a brief transient hyperpolarizing component may be seen under certain conditions. Here we show that layer 4 (L4) spiny neurons of the primary somatosensory cortex are persistently and monophasically hyperpolarized by ACH through activation of M4 muscarinic receptors. This hyperpolarization, correlated with a decrease in membrane resistance, increases the current threshold to evoke action potential in L4 spiny neurons. In parallel, pair recordings of spiny neurons indicate that ACH decreases synaptic efficacy through a diminution of release probability. We propose that this combination of pre- and post- synaptic effects contributes to an increase in signal-to-noise ratio in the L4 recurrent microcircuit. We then demonstrate that through reduction of the slow afterhyperpolarization (sAHP) present in spiny neurons, ACH improves the temporal fidelity of the relevant sensory inputs in L4. Finally, we suggest that these effects of ACH may be a general feature of primary sensory cortices, since L4 spiny neurons of the primary visual cortex and of the primary auditory cortex are also persistently hyperpolarized by ACH.

# Control of isometric forces in humans during different gravity-levels

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Alterations of gravitational forces can affect the sensorimotor coordination and might result in deficits for the performance of motor skills. In the present study we investigated isometric force production in different gravity (G)-levels during parabolic flight and the contribution of proprioception to modifications in force production.

Subjects were tested in three sessions: pre (1G), during (0G, 1G, 1.8G), and after (1G) the flight. Holding an isometric joystick in their dominant hand, they had to reproduce prescribed force vectors in the four cardinal directions and three different magnitudes (5,15,25 N) presented on a laptop screen. The contribution of proprioception was scrutinized by applying bilateral tendon vibration to the wrist during some parabolas.

We found that subjects produced substantially more force in 0G compared to 1G and 1.8 G. No significant increase in force production could be observed during 1.8 G. A similar pattern of augmented force during 0G could be observed for the initial force after 100 ms of response onset, when no proprioceptive feedback should be available. During exposure to vibration subjects produced less force, but consistent through all G-levels.

We confirm that isometric force production is increased during exposure to 0G and therefore might affect accurate motor control during changes of gravitational forces. The increased initial force and the lack of G-related modifications during vibration suggest that the exaggerated forces during microgravity might not be explained only by changes of proprioception.

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# Single and dual whole-cell recordings from the barrel cortex of awake mice during quantified whisker behaviour.

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Active processing of sensory information is thought to be of fundamental importance in perception. The central nervous system must extract relevant sensory information from the mix of external and self-generated sensory input during behaviour. Active sensory processing is an obvious feature of the mouse whisker sensorimotor system, since mice make large rhythmic whisker movements while exploring their environment. In order to investigate subthreshold and suprathreshold membrane potential dynamics during active sensory processing, we made whole-cell recordings of barrel cortex neurons in awake, head-fixed mice whilst recording and quantifying whisker movements. Spontaneous activity in barrel cortex neurons is characterised by large amplitude, low frequency changes in membrane potential when the animal is resting. During active whisking the membrane potential depolarises and fluctuates with high frequency and small amplitude. These fast membrane potential changes during whisking are correlated with the whisker position (Crochet and Petersen, 2006). In order to determine the relationship between membrane potential dynamics in different individual neurons, we made dual whole-cell recordings in awake mice. The slow large-amplitude membrane potential changes observed during rest were found to be correlated in nearby neurons. Simultaneously recorded neurons, however, had different phase relations with respect to the fast correlation with whisker position during whisking. To investigate the source of the input during whisking we made an acute bilateral cut of the infraorbital nerves that contain the whisker primary sensory neurons immediately before the recording session. We found that the overall depolarisation during whisking remained prominent after nerve cut, but not the fast correlation with the whisker position. The depolarisation during whisking is therefore generated centrally within the central nervous system. We propose that this depolarisation during whisking might prepare the cortex for the expected self-generated sensory input and also contribute to active processing of tactile information.

# A potential decision-making circuit: Synaptic transmission between antennal proprioceptors and giant descending brain neurones in crickets and its differential modulation by octopamine

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Crickets depend on tactile antennal information to decide on the appropriate response towards a conspecific (e.g. fight, flight or courtship). To unravel the neural mechanisms underlying such decision-making, we investigated the synaptic activation of identified giant descending brain neurones (DBN) by mechanical antennal stimulation and its modulation by octopamine in *Gryllus bimaculatus*. Touching an antenna activates two contralaterally descending giant interneurones (DBNc2-2, DBNc1-2) that have similar wide-field arborizations in antennal mechanosensory neuropils of the brain. Simultaneous antennal nerve and neurone recordings indicate that the afferent connection to both neurones must be monosynaptic, and may even be electrical with DBNc2-2. Ortho- and retrograde fluorescent nerve-staining revealed a population of 16 large campaniform sensilla (oval cuticular dome: ø 16 x 9 µm) which form (together with 26 small campaniform sensilla: ø 9 x 5 µm) a band round the distal pedicel, to monitor deflections of the flagellum. The large campaniform sensilla possess relative thick axons (ca. 8 µm) in the antennal nerve which give rise to varicose terminals in close association with the presumptive spike initiating zone of DBNc2-2 and DBNc1-2. The large diameter descending axons (20 - 25 µm) of these neurones conduct spikes at comparatively high velocity (DBNc2-2: 5,0 m/s; DBNc1-2: 4,5 m/s) that arrive in the metathoracic ganglion less than 10 ms after the touch stimulus. In this ganglion, DBNc2-2 has bilateral projections, whereas DBNc1-2 has only ipsilateral projections, as in the other ganglia. Preliminary data suggest that these interneurones excite leg motoneurones, and we suspect that they may be involved in mediating turning responses. Systemic application of the tissue-permeable octopamine agonist chlordimeform greatly enhances the responsiveness of DBNc1-2 to mechanical antennal stimulation, while drastically diminishing the responsiveness of DBNc2-2 in a reversible and dose-dependent fashion. Since the two investigated giant interneurones receive rapid suprathreshold monosynaptic inputs from the same mechanoreceptors, but are differentially modulated by octopamine and have different terminal projections in the thoracic ganglia, the circuit has potentially decision-making properties. We hypothesize that neuromodulation of this system may provide a cricket with the behavioural plasticity to turn towards or away from a conspecific depending on previous experience and current internal drive.

# Interneurons participating in the processing of directionality information of vibrational signals in the southern green stinkbug *Nezara viridula* (L.) (Heteroptera: Pentatomidae)

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Localization of mating partners by southern green stink bug (*Nezara viridula*) is mediated by substrate-borne vibrations. Male emits his own signals and while female is replying, he is trying to localise her on the plant. It has been observed that while moving on the plant, male usually pauses at branching and spreads legs across the branching as to compare inputs. Measuring and comparing signal intensity across the branching showed us that intensity difference is not a reliable cue. Measuring latency of signal arrival across the branching, however, assured us in the opinion that latency, not amplitude difference is the cue, important for the orientation.

In attempt of understanding the directionality coding in CNS we set up neurophysiological experiment based on these findings. We used two minishakers to deliver vibrational stimuli to the legs of the stinkbug attached to the minishaker heads in two different configurations. In the first configuration we stimulated only the front legs, each with one minishaker, while in the second configuration we stimulated one front leg with one minishaker and contralateral second and third leg with the other. The same stimuli was delivered by both minishakers with increasing time-delay in onset between one and the other. At the same time we performed intracellular recording in PTG or CG from cells responding to vibrational stimulation.

The most important findings were interneurons which were exhibiting excitation when legs attached to the first minishaker were stimulated and responding with inhibition when legs attached to the second minishaker were stimulated. Interneurons which code time delay with different spike timings have also been found. Other interesting group of cells are local interneurons with branching restricted to one side of the ganglion, responding to the vibrations delivered to that side only.

# Anatomical and functional organisation of the vibratory system in *Troglophilus neglectus* (Orthoptera, Rhaphidophoridae)

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All insects can detect substrate vibrations; however, central processing of these stimuli has been poorly investigated at the level of identified neurones. We have examined vibration-sensitive interneurones in *Troglophilus neglectus*, a primitive deaf ensiferan [Jeram et al. 1995, J Morp 223:109; Dessuter-Grandcolas 2003, Zool Scr 32:525] for two reasons; to provide evidence on the hypothesised homology of the auditory / vibratory sense in Ensifera at the level of central processing [Stritih et al. 2003, Proc. 29th Goett. Conf. p.415] and, as is presented here, to extend our knowledge on anatomical and functional organisation of the vibratory systems in insects.

Four local and twenty-six intersegmetnal interneurones with the soma and the main dendritic segment in the prothoracic ganglion were identified by the intracellular recording and staining technique and vibration of front legs with sinusoidal pulses (50-5000 Hz). Based on serial tissue sectioning, position of the neurones was related to the identified fibre tracts and association areas of the neuropile.

Neurones appear predominantly in clusters of morphologically similar cells. Their somata are arranged in five anterior and five posterior locations in the cortex. Most neurones cross the midline of the ganglion at the level between the anterior ring tract and the supramedian commissure and their axons project almost exclusively via the intermediate longitudinal tracts. Surprisingly, only seven of the neurones are tuned to frequencies between 400 Hz and 2000 Hz, thus matching the range of the tibial organ (TO) response [Čolk et al. 1995, J Exp Zool 273:376]. The rest respond best to vibrations of 50-200 Hz, including the most sensitive units with thresholds highly exceeding that of the TO receptors. A class of these low-frequency neurones is characterised by a sharp tuning and inhibitions received in the side high-frequency band. The high- and most of the low-frequency neurones with short response latencies, which suggest a direct excitation by receptors, have dendritic segments in the ring tract neuropile and/or the root iii of the leg nerve. Besides the TO, a subgroup of vibration-sensitive femoral chordotonal organ receptors is known to terminate there [Fielf and Pflueger 1989, Comp Biochem Physiol 68:99; Nishino 2000, Cell Tissue Res 299:145] and apparently provides this low-frequency input. Dendritic branches of the high-order interneurones are located in the most ventral neuropiles and dorso-medially between the longitudinal tracts. Several of these neurones send large axonal collaterals into the dorso-lateral, sensory-motor areas. The highest thresholds were measured in these units, suggesting their potential inputs from vibration receptors of the other legs and/or their multimodality. There are two likely homologues to the cave cricket neurones among the vibratory cells identified in a bushcricket [Sickmann 1996, Dissertation, Marburg] and a grasshopper [Bickmeier 1991, Dissertation, Marburg]. The highly conserved morphology, anatomical position and response properties of corresponding neurones suggest that all Orthoptera might possess a common plesiomorphic vibratory system that is comparable to that found in Troglophilus.

# ZEBRA FINCHES CAN BE TRAINED TO USE THE EARTH MAGNETIC FIELD FOR ORIENTATION

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In the last decade the use of the earth magnetic field for orientation and navigation has been demonstrated for an increasing number of animals. In avian species notably migratory songbirds and homing pigeons are well investigated. However, it is contrary discussed whether the sense for magnetic fields is well established in non migratory birds and for short distance spatial orientation.

We tested non migratory zebra finches from the institutes breeding colony for the ability to use the earth magnetic field for finding food in a special direction. Ten adult birds were trained in a cubic box of 80 x 80 x 80 cm to search for food trays. Preliminary experiments indicated that the birds were able to perceive the axis of the magnetic field, but not the polarity. Thus, food was available at both poles of one correct axis, either in north/south direction, or in east/west direction. The setup was designed that no visual or other cue (sound, smell, etc.) could be used for orientation.

Each bird was adapted to the situation in a center cage and then trained to search for food in either north/south or east/west direction. The direction of the first chosen food tray was recorded. At the end of the training session nine out of ten birds were significantly oriented at the appropriate axis. The magnetic field was then artificially shifted by 90° in declination, using a Helmholtz coil system. The trained birds were tested in the new field as described above for training sessions. Statistical analysis of the first directional choices in the test session revealed that the birds again significantly searched for food at the correct directions. The study shows that zebra finches as non migratory birds also possess a sense for magnetic fields and that they can be trained to use it for short distance spatial orientation.

# Do migratory birds "see" the magnetic field? A visual pathway as neuronal substrate for magnetoreception visualised by neuronal tracing

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**Long distance orientation** in migrating animals has fascinated mankind for centuries; within the last three decades hints about how birds sense the Earth´s magnetic field emerged from scientific studies which can be grouped into either **magnetite-mediated** or **photo-induced quantum-chemical compasses**. Physiological evidence favourises the first theory, whereas behavioural studies suggest that the sensing component is likely to involve vision. Recently, it was suggested that birds detect the magnetic compass direction through modulation of radical-pair processes based on photo-excitation of photo-sensitive molecules, the **cryptochromes**. Recent studies show that, in birds migrating at night, **cryptochromes are expressed in the retina (Mouritsen et al., 2004).**

Furthermore, both the **cryptochrome-containing neurons in the retina as well as a newly found region ("Cluster N") in the anterior forebrain show strongly increased neuronal activity** when birds are performing magnetic orientation at night **(Mouritsen et al., 2004; 2005).**

Information from the retina needs to be processed in the brain. Cluster N, due to its location, putatively resembles part of a known region of visual procession, the **"visual wulst"**, as shown in other bird species. Proving this as well as showing a functional neuronal connection between the eye and Cluster N would be an important step towards understanding if/how birds perceive the magnetic field through vision. The present study addresses two main goals:

**1) Anatomical and biochemical characterisation of Cluster N to put this hitherto unrecognised area within the framework of the known telencephalic pathways.**

**2) Mapping of a putative neuronal pathway linking the mentioned regions being active during magnetic orientation (retina/Cluster N) using a combination of neuronal tracing techniques together with behavioural experiments and immunohistochemical analysis.**

# Lateralised activation of Cluster N: a putative magnetic processing station in the brains of migratory songbirds

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Cluster N is a cluster of forebrain regions found in night-migratory songbirds that shows high activation of activity-dependent gene expression during night-time vision. We have suggested that Cluster N may function as a specialised night-vision area in night-migratory birds and that it may be involved in processing light-mediated magnetic compass information. Here, we investigated these ideas. We found a significant lateralised dominance of Cluster N activation in the right hemisphere of European robins (*Erithacus rubecula*). Activation predominantly originated from the contralateral (left) eye. Garden warblers (*Sylvia borin*) tested under different magnetic field conditions and under monochromatic red light did not show significant differences in Cluster N activation. In the fairly sedentary Sardinian warbler (*Sylvia melanocephala*), which belongs to the same phyolgenetic clad, Cluster N showed prominent ZENK expression levels, similar to that observed in garden warblers and European robins. Thus, it seems that Cluster N activation occurs at night in all species within predominantly migratory groups of birds probably because such birds have the capability to switch fast between migratory and sedentary life styles. The activation studies suggest that although Cluster N is lateralised as is the dependence on magnetic compass orientation, either Cluster N is not involved in magnetic processing or the magnetic modulations of the primary visual signal forming the basis for the currently supported light-dependent magnetic compass mechanism are either relatively small and/or directed in opposite directions such that activity dependent gene expression changes are not sensitive enough to pick them up.

# Virtual echoacoustic space for echolocating bats

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Bats can recognize three-dimensional objects by analyzing the echoes of their ultrasonic emissions. Acoustically, information about an object is encoded in its impulse response. However, an impulse response of a three-dimensional object will only reflect the depth dimension unambiguously. Bats acquire more detailed information about an object by moving around the object and scanning it with a series of echolocation calls. When moving around the object, the impulse responses and therefore the echoes change dependent from which angle the object is ensonified. Stringing together object related information from the echoes obtained from different observation angles will produce a three-dimensional echoacoustic image of the object. Our goal is to analyze the basic mechanisms with which the bat, *Megaderma lyra*, is able to reconstruct the three-dimensional shape of complex objects by perceptual integration of the information acquired through sequences of echoes. To analyze this reconstruction we created two different virtual 3D objects defined exclusively in terms of their impulse responses. One object, made up of 36 different impulse responses, changes in dependence of the observation angle and the other, consisting of 36 identical impulse responses, is independent of the bat's observation angle. The bats are trained to fly around two units each consisting of a microphone, loudspeaker and feeder. A high-speed video camera above these units determines the bat's position in space. The bat's echolocation calls are recorded with the microphone, filtered with one of the 36 impulse responses dependent on the bat's position and played back over the loudspeaker. While flying around both units the bat will experience echoes, as it would experience when moving around two real objects. This virtual echoacoustic space technique allows us to study echolocation behaviour of a bat exploring objects echoacoustically under highly controlled laboratory conditions.

# Multiple multi-sensory networks involve the claustrum

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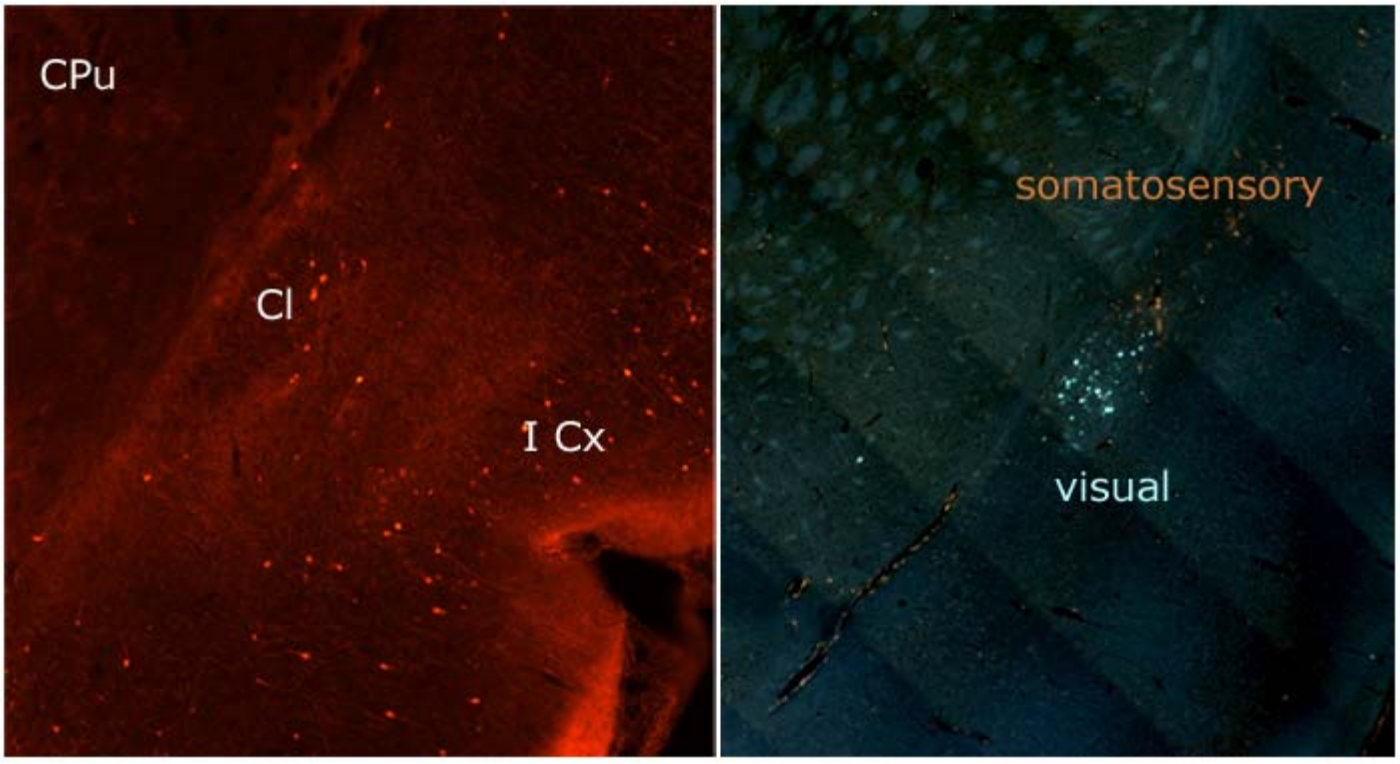
Recent reports propose a role for the claustrum in multi-sensory integration. Since afferent and efferent projections subdivide the claustrum into sensory specific regions, this poses the question of how this structure could participate in multi-sensory integration. In the rat, particular modality specific regions have been shown to overlap, while others remain aloof. Using retrograde tracers such as FluoroGold and Diamidino Yellow we visualize the connections of the rat claustrum with primary and higher sensory areas. This allows us to quantify the topographic arrangement of projections between these cortical areas and the claustrum with respect to the known sensory map within the claustrum.

Our results not only confirm previous reports of arealization within the claustrum, but also suggest new routes of multi-sensory interaction involving this structure. Of interest is our observation of significant overlap between somatosensory and frontal claustral zones. Tracer injections made at subcortical loci too reveal connectivity to somatosensory zones. However, multi-sensory integration could also rely on the intrinsic connectivity between different sensory zones. In this regard, using calcium binding proteins as markers, we investigate the spatial arrangement of different cell populations and the relation of their dendritic trees with respect to the different sensory zones in the claustrum.

The extrinsic and intrinsic connectivity of the claustrum reveals a complex network with the claustrum as a node between different cortical sensory and association areas and suggests a prominent role of this structure in combining and modulating sensory information.

Figure legend: Left) The claustrum (Cl) can be identified surrounded by Calretinin expressing fibers. Right) Retrograde labelling of neurons projecting to somatosensory and visual cortex.



# Serotonin action on the locust femoral chordotonal organ

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Serotonin (5HT) is an important neurotransmitter and neuromodulator in the arthropod central nervous system. 5HT exhibits a broad spectrum of actions, for instance, it participates in the control of aggressive behaviour in lobster (Livingston et al. (1980) Science 208: 76), modulates escape behaviour in crayfish (Yeh et al. (1996) Science 271: 366), and inactivates the flight system in moths (Claassen & Kammer (1986) J Neurobiol. 17: 1). While such central nervous effects of 5HT are well-studied, actions in the periphery are enigmatic and sometimes indeed controversial (compare e.g. Reichert, B. and Hustert, R. (1992) In Neurobiology Conference, Vol. 20; and Ramirez & Orchard (1990) J Exp Biol 149: 255).

The femoral chordotonal organ (fCO) in locusts and stick insects is a particularly well-studied mechanosensor, with regard to peripheral signalling, central nervous processing, and behavioural relevance (review e.g. in Bässler (1993) Brain Res Reviews 18: 207). It thus lends itself well for the examination of both, central and peripheral actions of neuroactive compounds (e.g. Ausborn et al. (2005) J Exp Biol 208: 4451). The goal of our present experiments was examination of a possible modulation of chordotonal organ signalling by 5HT, especially in view of recent reports suggesting an interaction of 5HT and neuroactive insecticides on the peripheral level (Kaufmann et al. (2004) Comp Biochem Physiol C 138: 469; Ausborn et al. ibid). We therefore recorded the response of the fCO in the locust middle leg to imposed ramp-and-hold stimulation, during the application of 5HT at concentrations ranging from $10^{-4}$ to $10^{-2}$ M.

Unexpectedly, stimulus-related spike discharges in the fCO nerve were virtually identical over the whole range of 5HT concentrations, as well as for saline controls. This held true for all aspects of the fCO response, and in particular for position and velocity signalling. Since it is notoriously difficult to prove the absence of an effect, a particularly large number of stimulus presentations was given in each trial ($n \geq 11$), many animals were tested ($n=14$), and several statistical tests were performed. However, all tests were unable to resolve any significant differences. P-values ranged from $P=0.21$ to $P=0.77$.

In conclusion, the spike activity of the locust fCO appears to remain completely unaffected by 5HT, up to (physiologically irrelevant) concentrations of $10^{-2}$ M. Effects of 5HT on leg movement and joint control, where present, would thus appear to be due to central nervous effects of serotonin.

# Distance estimation in desert ants, Cataglyphis fortis, is unexpectedly resistant to disturbance of walking conditions

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Desert ants, *Cataglyphis fortis*, perform extensive foraging trips in mostly featureless landscape. Path integration is employed by the foragers to reliably return to their nest, a navigation feat that requires continuous calculation and updating of a home vector. The angular component of this vector is provided by the ants' celestial compass (review in Wehner (2003) J Comp Physiol A 189: 579), and distance estimation is performed by a stride integrator (Wittlinger et al. (2006) Science 312: 1965). Distance information thus probably derives from monitoring of leg movement. The ants' meandering outbound search trajectories and their rather straight homebound journeys are usually very different, not just in length but also in surface structure (e.g. dense clay versus loose sand) and substrate undulation (e.g. flat versus rippled terrain). This raises the problem of how resistant distance estimation in desert ants may be with regard to substrate structure - the question addressed in this study.

Individually marked ants were trained to walk from their nest to a feeder, over a distance of 10m and in a linear channel. The channel floor was supplied with corrugated walking substrates of different wavelengths and correspondingly different amplitudes (wavelength/amplitude relationship was about 2.5). Trained individuals were caught at the feeder and transferred to a test channel with an even floor. The test channel was otherwise identical to the training channel, and aligned in parallel, although it was longer (total of 25m). Once transferred to this test channel, the ants performed their homebound journeys. Having covered their homing distance, the ants switched from a steady return to their typical nest searching behaviour, characterised by a conspicuous U-turn and a subsequent a run pacing back and forth around the anticipated nest location. This search behaviour was evaluated to determine the ants' estimation of homing distance (Wittlinger et al. ibid), and high-speed video analysis was used to examine walking behaviour and kinematics.

Walking performance was more or less normal at corrugation wavelengths of 50mm, but it was noticeably compromised at 25 and 15mm. The animals stumbled frequently, often missed ground contact when stepping into the undulation troughs, and bumped into the ascending slope of the oncoming hill. A corrugation wavelength of 10mm tended to entrain the animals' footfall pattern, affecting (and usually increasing) stride length, stride frequency, and walking speed. Despite these changes in walking performance, estimation of homing distance was still precise after training on corrugated sheets of all wavelengths.

The results demonstrate that C. fortis is able to cope with small-scale substrate undulations and still gauge travel distance correctly. The data further extent previous findings and show that desert ants are able to assess the ground distance of their travels on a broad range of substrates, ranging from large-scale hills (Wohlgemuth et al. (2001) Nature 411: 795) to small-scale undulations that significantly affect walking performance.

# Leg amputation does not affect homing distance in desert ants, *Cataglyphis fortis*, irrespective of residual sensory feedback from mechanosensory hair fields

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Desert ants, *Cataglyphis fortis*, achieve notable navigation feats (review in Wehner (2003) J Comp Physiol A 189: 579), and they exhibit considerable accuracy when returning from foraging trips to their nest. This homing performance is remarkably resistant to disturbance, for example, injury and even leg loss *en route* - as may result from attacks by predators or foreign conspecifics. This suggests that sensory feedback monitors actual locomotor performance and adjusts input to the path integrator accordingly. Here we study to what extent altered sensory input from leg mechanoreceptors affects homing distance.

Individually marked ants were trained to walk from their nest to a feeder, over a distance of 10 m and in a linear channel. After training, individuals were caught at the feeder, manipulated, and transferred to a test channel. The parallel aligned test channel was identical to the training channel except for its exceeding length of 24 m. Once transferred into this test channel, the ants performed their homebound runs. Having covered their homing distance, the animals switched from a straight and steady return to their typical nest searching behaviour, characterised by a conspicuous U-turn and a subsequent run pacing back and forth around the anticipated nest location. Each ant was tested twice, first, right after the experimental manipulation, and second, after having visited the feeder again after re-emerging from the nest.

Normal, marked ants were caught at the feeder and subjected to experimental manipulation. There were three experimental groups (each n=25).

(i) Control ants were put into the test channel without manipulation. Their homing distance was 10.2 m (IQR = 2.4).

(ii) One middle leg and the contralateral hind leg were severed at mid-femur level. This prevented the remaining leg stumps to reach the substrate and participate in walking. The animals' normal tripod gate was thus severely disrupted, and the ants stumbled on almost every other stride. The leg stumps nevertheless moved vigorously in the walking rhythm, and the mechanosensory hair plates associated with the coxa must have provided rhythmic input to the nervous system. Homing distance of these animals was 10.8 m (IQR = 1.4), and thus unaffected by the operation. This held true for the second test, when the ants had reached the feeder again on four legs (10.4 m, IQR = 2.9).

(iii) The same two legs were removed completely, including the coxae and any associated hair plates. Again, homing distance remained unaffected, although mechanosensory feedback from the two legs was now completely absent on the homebound journey (10.6 m, IQR = 2.3; test after outbound journey on four legs, 10.9 m, IQR = 3.1)

In conclusion, leg amputation does not affect homing distance in desert ants, irrespective of whether or not residual mechanosensory feedback from coxal hairs is present. This result is intriguing in view of the recent finding that distance estimation in desert ants relies on a stride integrator that considers stride length (Wittlinger et al. (2006) Science 312: 1965).

# Structure and function of a cold receptor in the leaf-cutting ant *Atta vollenweideri*

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Leaf-cutting ants respond very sensitive to microclimatic conditions inside the nest by showing strict thermal preferences which are expected to maximize fungus growth and brood development. Besides this in-nest behavior, which requires the assessment of air temperature, we showed in a recent study that leaf-cutting ants are able to use infrared radiation as an orientation cue outside the nest. Thus, the ants are able to sense convective as well as radiant heat. Peg in pit sensilla located at the tip of the antenna (S. coeloconica and/or S. ampullacea) are known to house thermoreceptive neurons.

We investigated the S. coeloconica of the leaf-cutting ant *Atta vollenweideri* with extracellular recordings of the associated receptor neurons. The activity of one to three neurons was recorded simultaneously from single sensilla. In most of the sensilla investigated, one of the associated neurons responded to a drop in air temperature with a phasic-tonic response but not to an increase in relative humidity. From this result, we conclude that the neuron recorded from function as a cold receptor. Using a heated thermistor (40°C), placed at a distance of 2cm from the antenna, and an air current with constant temperature to reduce convective influences, we showed that this type of neuron also responds to radiant heat. In addition, following a temperature change of 6°C, the neuron showed complete adaptation after 10 min.

In addition to our physiological experiments, we investigated the ultrastructure of the S. coeloconica. Three neurons with unbranched outer dendritic segments innervate the grooved peg. Within the pit the peg is separated from the outside by a small pore. This aperture might shield the peg from radiation from the side, resulting in reduction of the receptive field and therefore in directional sensitivity of the cold cell. We tested this hypothesis and found strong direction sensitivity to the radiant heat emitted by the thermistor. It is suggested that ants might use S. coeloconica as a sensory system to orient outside their nest. Thermal orientation may help ants, for instance, to locate sun-exposed leaves, which were shown to be highly attractive.

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# Representation of odor intensity along the olfactory pathway of the *Drosophila* brain

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Animals are able to differentiate a large variety of odours. To perform such a task a brain must be able to differentiate odours of different chemical identities, but also of different concentrations. We ask how different odour intensities are represented in the brain of the fruit fly *Drosophila melanogaster*. To address this question we presented several odours at different concentrations to the antennae of the fruit fly while monitoring calcium activity at different levels of the olfactory pathway. We have used the genetically encoded, FRET-based calcium sensor Yellow Cameleon 2.1 because of the minimization of movement artefacts due to its ratiometric properties, a good signal-to-noise ratio and high fluorescence intensity in the non-activated state. The expression of this calcium sensor in three different neuropils by specific Gal4-driver lines enabled us to trace the processing of the presented odor concentrations along a part of the olfactory pathway: in olfactory receptor neurons terminating in the antennal lobe, in olfactory projection neurons arborising in the antennal lobe, in terminal arborisations of projection neurons in higher brain regions, and in Kenyon cells, the intrinsic neurons of the mushroom body. We find that receptor neurons show odour specific calcium responses which increase with increasing odour intensity. Investigating odour representations at higher order levels of processing we ask whether this principle is maintained throughout the olfactory pathway. First results will be presented.

# Cells and networks involving layer 6 of the rat barrel cortex

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Layer 6 (L6) of primary sensory cortices receive feedforward sensory signals from the thalamus while also forming the primary source of excitatory input to both, the principal thalamic targets in L4 and, as feedback projections, the thalamic nuclei themselves. Cells in this layer are therefore in a position to profoundly influence sensory signals reaching the cortex. In this study we have investigated, in vitro, cell types in L6 of the barrel subfield in the rat primary somatosensory cortex.

At least three excitatory (L6e) cell types are identified based on axonal and dendritic morphology, each exhibiting a distinct electrophysiological response pattern. Retrograde labelling from the thalamus identified one morphological type of L6e neuron, the corticothalamic (CT) cell. CT cells have characteristic axonal profiles with a thick, subcortically projecting principal axon, and collaterals that ascend in a columnar manner towards L4. Most unlabelled cells show contrasting axonal arbours, with no obvious subcortical projection and collaterals that arborise widely in the infragranular regions. Unlabelled cells matching this profile are labelled corticocortical (CC) cells.

Analysis of dendritic structure distinguishes CT from CC cells and further differentiates the latter into CC1 and CC2 types. CC1 cells have narrower and shorter apical dendrites compared with CC2 cells.

Electrically, CT cells have short membrane time-constants, a high rheobase, and fire regular trains of action potentials. In contrast, CC cells fire trains initiated by a short burst of action potentials. CC2 cells are distinguishable by their higher intraburst frequencies.

Inhibitory cells in layer 6 (L6i) were also recorded and their dendritic and axonal trees reconstructed. Morphologically, at least two types of L6i cells can be identified, one with axons arborising specifically in layers 6 & 4, and another with axon restricted to infragranular laminae. Preliminary data show that these cells can also be distinguished by their firing patterns.

L6 therefore contains multiple excitatory and inhibitory cell types that innervate contrasting regions of cortical space and have correspondingly different physiological responses. CT cells, with narrower dendritic and axonal fields, higher rheobase and shorter latencies are likely to respond to strong, and spatially & temporally specific stimuli, and relay them in a similar manner. CC cells, on the other hand, have wider fields of input and output; coupled with a low rheobase and bursting behaviour, these cells are likely to signal non-specific whisker deflections.

# Multimodal sensory integration for groundspeed control in *Drosophila*

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A current challenge in neurobiology is to understand how multimodal sensory input is processed and integrated to produce robust behavioral output in a realistic behavioral context. We address this problem in the context of visual groundspeed control in *Drosophila*, whose underlying neuromotor control circuits we explore under free flight conditions in a 'virtual reality' wind tunnel. It is well known that flies rely on perceived translational optic flow to modulate their groundspeed via changes in body pitch, however, the sensory-motor computations and pathways pertaining to this behavior remain largely unknown.

The fly's mechanosensory balance organs, the halteres, provide essential reafferent feedback for body stabilization. Body pitch is further modulated by inputs from a velocity dependent visual pathway (Rohrseitz et al., in this issue), which ultimately results in a change of forward speed.

We perform brief tests in the wind tunnel, in which the 'virtual reality' stimuli are used to precisely control the optic flow perceived by the fly, while the body pitch angle and 3D position are measured using high-speed video techniques. In a first step, we perform a control systems analysis of the measured behavioral responses based on detailed biomechanical simulations. In a second step, we estimate the mechanical stimulation experienced by the halteres to explore the mechanosensory transduction processes that lead to robust feedback control. This well defined and amenable behavioral paradigm allows relevant components of the underlying neuromotor control system to be measured and the control principles to be identified.

# Poster Topic

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# Neuronal connections from the ventral nerve cord to the visceral nervous system in the locust

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In contrast to the stomatogastric nervous system of decapod crustacea the visceral nervous system of insects is still poorly understood. Earlier work showed connections between the frontal ganglion and the head ganglia. It was also shown that the frontal ganglion (and perhaps other ganglia of the visceral nervous system) play a role in growth and moulting. Recently it was demonstrated that frontal ganglion output is synchronized with that of respiratory networks that reside in thoracic and abdominal ganglia. Therefore it was of interest to see how many neurons in the ventral nerve cord are connected to the visceral nervous system. Filling the frontal connectives towards the central nervous system as expected showed numerous somata in the tritocerebral lobes of the brain, but also a few in the deuto- and protocerebral lobes. Some 70 neurons were labeled in the suboesophageal ganglion. Surprisingly there are also two neurons in the prothoracic and two in the metathoracic ganglion. The latter two are prime candidates for relaying respiration-correlated rhythmic input to the frontal ganglion and perhaps other visceral ganglia.

# Effects of antennal stimulation on premotor and motor neurons in the prothoracic ganglion of the stick insect, *Carausius morosus*

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Walking stick insects explore their environment ahead through coordinated movements of their antennae. Antennal contacts during walking cause fast adaptive movements of the front legs towards the detected object, whereas a manually applied tension to the antennae induces backward walking. Thus, different tactile information has to be processed and conveyed to the motoneurons of the prothoracic ganglion.

For the investigation of this sensory-motor system we studied the response of the front leg premotor and motor neurons in restrained animals to induced movements of the antennal scape-pedicel joint. Movement of this joint is known to be monitored by hair fields. However, neither motoneurones nor premotor interneurones showed a reaction to step changes in antennal joint angle.

In a second experimental session, tactile stimuli were applied to the flagellum of the immobilized antenna by means of a small paint brush or using two piezoelectric elements.

We examined coxo-trochanteral and femoro-tibial motoneurons as well as the nonspiking premotor interneuron E4. From 12 levator trochanteris motoneurons tested, only 2 showed a depolarization response when touching the ipsilateral flagellum with the brush. From the extensor tibiae motoneurons neither FETi nor the spontaneously active SETi showed changes in their activity. Also flexor tibiae motoneurons did not show reactions to this tactile stimulation. The premotor interneuron E4 was identified physiologically and morphologically in 9 cases and in two different morphological varieties. In no case, tactile stimulation did elicit reproducible responses.

However, touching and holding the antennae between two fingers resulted in a clear-cut response. 6 levator trochanteris motoneurons were depolarized to the ipsilateral antennal stimulus. 5 neurons also responded to the stimulus at the contralateral flagellum. One neuron showed a hyperpolarization to the ipsi- and contralateral stimulus. When repeating this stimulation with gloves or tweezers, only one neuron was slightly depolarized. All tested extensor motoneurons (n=4) showed a depolarization when holding the ipsi- and contralateral flagellum between two fingers. No response was found after stimulation with gloves or tweezers. Two of three tested flexor motoneurons showed a depolarization in response to the ipsi- and contralateral stimulation. The third neuron was hyperpolarized. Similar results were found for the premotor interneuron E4. Three neurons were depolarized while one neuron was hyperpolarized. There was no response to the stimulation with gloves or tweezers.

We conclude that the clear-cut response to touching and holding the antennae between two fingers appears to be due to contact-chemosensory stimulation because we mostly found no response to the stimulation with gloves or tweezers. As most of the neurons showed no response on the antennal stimulation, we assume that tactile sensory information is gated according to the state of activity of the animal.

The next step in this study will be to repeat all experiments in activated animals in order to elucidate how the walking pattern of the front legs is changed in response to the various stimuli.

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# A simple mechanism for swing trajectory adaptation during walking

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Walking in natural terrain requires continual adjustments of leg movements to different substrate shapes. For instance, a stick insect walking on a small branch shows a laterally directed swing trajectory. However, the trajectory is more upward directed when walking on flat ground. *Carausius morosus* is able to react in the course of only few step cycles to abrupt changes in the substrate shapes (Schumm & Cruse, J Comp Physiol A, in press). Cruse (Biol Cybern 86, 2002) suggested a concept which we use to explain how *C. morosus* might adapt the swing trajectory to the substrate condition experienced during the preceding stance phase. He proposed one half-center oscillator (Brown, J Physiol Lond 48, 1914) for each leg joint. Each oscillator comprises two interneuron pools which are connected via motoneurons to the antagonistic muscles. According to the model, the interneuron pools inhibit each other and show an increase in excitability after release from inhibition, which might cause a rebound effect. Stronger stance activity should cause stronger inhibition of the units driving the swing muscles. This should lead to an increased rebound effect and, thus, to an increased activity during swing. According to our hypothesis this change of the relative activation of the antagonistic muscles contributes to the adjustment of the leg trajectory during swing.

In this study we test this mechanism of adaptation to different substrates in *C. morosus* by experimentally inducing changes of the activity of the stance unit of the oscillator of a joint. For this purpose we videotaped *C. morosus* walking tethered on a treadwheel. By means of a DC motor we were able to add a constant torque either assisting or resisting the retraction movement. This setup was chosen to mainly affect the protractor-retractor systems of the legs. The data evaluation comprised the analysis of the swing-stance transition points (AEP; PEP), the highest reached point during swing (DEP), and the swing velocity within the first 60 ms of the swing phase.

We found a shift of the whole step in the direction of the applied load in the middle and the hind leg. Also the form of swing trajectory was changed significantly: front and middle legs were moved significantly closer to the body in the decelerated than in the accelerated condition. Both results indicate an adaptation of the swing movement in response to the changed situation during the stance phase.

However, according to our hypothesis a resisting load which increases the activity of the retractor system should cause an increased swing velocity. In contrast to our expectations in most cases we found that decelerating the animal led to a decrease in swing velocity. The accelerated animals did not show the expected decrease in swing velocity but mostly revealed no significant difference to the controls.

A possible explanation for these unexpected results might be that the animals, when accelerated, actively use their protractor muscles to brake the treadwheel. Thus, no switch between protractor and retractor unit of the oscillator would occur during stepping in this experimental set up. With force measurements and simultaneous EMG recordings during the stance phase this point has to be further elucidated.

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# Programmable Array Microscopy: Applying a novel confocal technique to the study of neural information processing

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Cairn Research Ltd has developed a Programmable Array Microscope (PAM), a confocal microscope in which a liquid crystal display simulates an aperture plane to give birth to confocality. The PAM should allow high-speed high-quality confocal imaging, with unprecedented versatility. Our aim is to use the PAM to analyse sensory integration in locust flight motoneurones using calcium-sensitive dyes, with simultaneous electrophysiological recordings. We should be able to obtain sufficient temporal and spatial resolutions to observe in vivo how cross-modal integration (wind, vision, audition, proprioception) activates different neurites, leading to the generation of spikes and ultimately eliciting twitches in the corresponding muscle.

# Sensory Feedback Action Depends on Walking Direction in the Stick Insect Walking System

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In terrestrial locomotion sensory feedback from load sensors is important for adapting the ongoing motor output on a step-by-step basis. Here we investigated in the walking system of the stick insect how load signals from the leg exert their influence onto motoneuron pools of the thorax-coxa (TC-) joint. We stimulated load sensors during rhythmic, alternating activity in protractor coxae (ProCx-) and retractor coxae (RetCx-) motoneuron pools. Alternating activity in the examined segment was induced by mechanical stimulation of the animal or by pharmacological activation of the isolated thoracic segment with the muscarinic ACh-agonist pilocarpine. Load signals from the leg affected the timing of TC-motoneuron activity by affecting the central rhythm generating network of this joint: Load signals were able to reset and to entrain TC-motoneuron activity. In front and middle legs load signals induced or promoted RetCx-activity and terminated ProCx-activity. In the hind leg, reverse transitions were elicited with increasing load terminating RetCx and initiating ProCx activity. Studying the influence of load signals in intact walking animals showed that the execution of the load influence on the TC-joint motoneurons depended on walking direction, with increased load promoting the functional stance phase motoneuron pool. In forward walking this is RetCx activity, in backward walking ProCx activity. Thus, we show for the first time that modifications of sensory feedback action in a locomotor system are related to walking direction. Ablation experiments showed that the three groups of trochanteral campaniform sensilla provide the underlying load signals.
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# Phasing of subsets of motoneurons in the lamprey spinal cord is gated by segmental activation

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Motoneurons are the arbitrating neural units transmitting evoked motor patterns from the central nervous system to the executive organs, i.e. the muscles of wings, legs or the trunk. The activation pattern of subsets of motoneurons (MNs) can vary substantially within the locomotory cycle between various sets of motoneurons as well as for the same set of motoneurons during different forms of a given locomotion. The mechanisms mediating alterations in phasing of MNs during locomotion are unknown. In the lamprey spinal cord are two subsets of MNs whose activity is coordinated during swimming; myotomal motoneurons (mMN) activating the trunk muscles of the left and right body side in alternating fashion and fin motoneurons (fMN) activating the muscles of the caudal dorsal fins. This second group of MNs is active out of phase compared to myotomal motoneurons of the same ipsilateral hemi-segment during swimming in vivo and during fictive locomotion in the isolated spinal cord upon activation by NMDA. Sagittal lesions applied to the segment of a finMN in the isolated spinal cord demonstrate that inputs from the contralateral side of the own segment are not necessary for the out of phase activity of finMNs (Mentel et al., 2006, Europ. J. Neurosci. 23:2012). With a midline lesion between 50-70%, however, out of phase activity of fMNs ceased indicating that contralateral signalling underlies out of phase activation in fMNs (Mentel et al. 2006, Abstr. Soc. for Neurosci.). What are the necessary prerequisits for phasing fMN activity in the locomotor cycle? We conducted various combinations of lesion and "split-bath" expriments to clarify this issue. Extending a midline lesion up to six segments rostrally and 3-5 segments caudally from the recorded segment does not change out-of-phase activity of finMNs, excluding short range projecting and midline crossing interneurons to mediate phasing of finMN activity. In "split-bath" configuration fMNs receive an in phase drive from the rostral spinal cord, when the rostral pool was perfused with NMDA, and the caudal pool with the recorded finMN with ringers solution. The same was found with the reversed setup for caudal inputs. Only when NMDA was present in the segment recorded out of phase synaptic drive to fMNs was established. Our results stongly suggest that the contralateral side of the spinal cord is necessary for the phasing of fin motoneuron activity during fictive locomotion, but that gating of this influence upon fMNs relies on activation of the own segment.

# Oscillatory synchronization in voluntary tremor and Parkinson´s disease resting tremor

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It has recently been demonstrated that resting tremor in Parkinson's disease (PD) is associated with oscillatory synchronization in a cerebello-thalamo-cortical network. These data raise the question about the specific pathological nature of the demonstrated network. Does it reflect a pathological phenomenon per se or does it represent a functional network underlying physiological motor behaviour which may be specifically altered in PD? To answer this question in a first experiment we investigated cerebro-cerebral coupling in eleven healthy subjects voluntarily imitating the typical antagonistic resting tremor. Neuromagnetic activity was recorded by using a 122-channel whole-head magnetoencephalography (MEG) system. For data analysis, we used the analysis tool Dynamic Imaging of Coherent Sources (DICS) which allows the investigation of functional connectivity as a measure of coherence. Our results demonstrate that, indeed, the same oscillatory network subserves voluntary tremor as well as involuntary resting tremor. However, differences of coupling strength were evident between groups: First, coupling between thalamus and primary sensorimotor cortex (S1/M1) was increased in PD patients, indicating a stronger influence of the thalamus on motor cortical activity most likely resulting in involuntary tremor. Second, coupling between premotor cortex (PMC) and S1/M1 was stronger in healthy controls most likely indicating that PMC drives M1 resulting in voluntary tremor.

In a second study, we aimed at further elucidating the significance of functional coupling between thalamus and motor cortical areas for the genesis of PD resting tremor. To this end, we investigated the effect of L-Dopa on the demonstrated network in ten PD patients suffering from resting tremor. We compared coupling strength after overnight withdrawal of PD medication and 30 minutes after intake of 200 mg of fast acting L-Dopa. Analysis of MEG data revealed that L-Dopa selectively influences functional coupling within a thalamus-premotor-motor network.

Taken together, both studies indicate that (i) PD tremor results from alterations within a physiologically pre-existing oscillatory network and that (ii) L-DOPA selectively reduces oscillatory synchronization in a thalamo-motor-cortical network and, thereby improves PD motor symptoms.

# CONTRIBUTION OF NONSPIKING INTERNEURONS TO THE LOCAL CONTROL OF SINGLE LEG WALKING IN THE STICK INSECT

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In the stick insect leg muscle control system the motor pattern driving leg motoneurons (MNs) results from three different synaptic actions: upon activation of the locomotor system motoneurons are depolarized tonically for the duration of a locomotor bout (Ludwar et al., 2005; J. Neurophysiol.93:1255). Alternating activation and inactivation of MNs in the stepping cycle is mediated by additional phasic excitatory and inhibitory synaptic inputs (Schmidt et al., 2001; J. Neurophysiol.85:354). In the present study we addressed the role identified premotor neurons play in the generation of MN activity. In our study we focussed on those identified premotor nonspiking interneurons (NSIs) that provide synaptic drive to the tibial MNs. Their membrane potential is integrating inputs from central rhythm generating networks, from leg sense organs as well as from intersegmental pathways (see Bässler & Büschges, 1998; Brain Res. Rev.27:65). Previous investigations suggested for a few NSIs (I1 and E4) a prominent role in generating the motor output for stepping in the intact animal (e.g. Büschges et al., 1994; JCP A174:685). We were interested to unravel how identified NSIs contribute to the local control of walking, especially for certain aspects as e.g. changes in walking velocity.

To do so coordinating influences from other legs were excluded by amputation of all legs except the middle leg studied. Identified NSIs (E1-E9; I1-I4) were recorded in the mesothoracic segment in the single-leg preparation (Fischer et al., 2001; J. Neurophysiol.85:341) and stained by dye injection (TMR-D) following physiological characterization. Activity of postsynaptic MNs was recorded extracellularly from lateral nerve nl3 (extensor) and as EMG-recording from the flexor tibiae muscle.

Our study yielded the following results:

1. All NSIs recorded exhibited marked phasic membrane potential oscillations related to the walking cycle suggesting that none of them is contributing solely to the tonic depolarization in tibial motoneurons.
2. Different types of NSIs seem to serve different functions in the control of walking. While some of them exhibited for example phasic depolarizations only at the step phase transitions, e.g. NSIs E2 and E9, other NSIs expressed membrane potential modulations that were closely correlated with the activity of tibial motoneurons, e.g. NSIs I1, I2.
3. Associated with changes in stepping velocity up to now only few types of NSIs were recorded that exhibited alterations in the magnitude of membrane potential modulation, e.g. NSI E6. Therefore we are currently concentrating on NSIs that provide synaptic drive to flexor tibia MNs.

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# Evidence for a role of 2nd messengers in the control of motoneuron activity in the walking stick insect

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In a single legged preparation of the stick insect *C. morosus* middle leg motoneuron (MN) membrane potentials are phasically modulated and tonically depolarized by ~5mV during front leg stepping movements on a treadmill. Tonic depolarizations are based on a membrane conductance increase (Ludwar et al., J. Neurophys. 94:2772, 2005).

Repolarization time constants of up to ~30s indicate that 2nd messengers might play a role in mediating the tonic depolarization.

For a pharmacological analysis of the tonic depolarization intracellular recordings from mesothoracic flexor MNs were performed while drugs were bath applied. Silicon gel barriers were used to restrict application of drugs to the mesothoracic ganglion. Atropine (500µM), which blocks metabotropic ACh receptors, reduced the tonic depolarization by about 30%. In addition, serotonin (1mM) was found to enhance the tonic depolarization in 3 of 5 experiments. 8-Br-cAMP (500µM) increased the tonic depolarization up to 40%. These results indicate a role of ACh and serotonin in mediating the tonic depolarization and a role of cAMP as a 2nd messenger.

However, the role of cAMP needs further analysis because application of the adenylyl cyclase inhibitor SQ22536 (250µM) led to an increase in the tonic depolarization. A decrease in the tonic depolarization would have been expected because adenylyl cyclase catalyzes the synthesis of cAMP.

Inhibition of phospholipase C by application of neomycin increased the amplitude of the tonic depolarization up to 50%. The latter result appears to indicate a reduction of inhibitory input to middle leg motoneurons mediated by interneurons that employ phospholipase C in a 2nd messenger pathway.

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# Walking on the Slippery Surface: Kinematic Analysis of Straight and Curve Walking in the Stick Insect

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In stick insects there is no centrally located control unit for all legs and walking is the result of the co-action of different pattern generators for the single legs as well as coordinating intersegmental influences. The pattern generator for each leg consists of central pattern generators (CPGs) for each leg joint. Intrasegmental sensory information appears to be crucial for the coordination, and the femoral chordotonal organ as well as the campaniform sensillae have been shown to exert such influence.
The slippery surface setup implemented in this study permits the legs of a tethered stick insect to move freely and with very little surface resistance (1). It allows the study of the coordination of a single insect leg in the intact animal without the effect of substrate coupling. We analyzed the walking pattern of the front middle and hind legs of the stick insect walking on the slippery surface and compared the kinematics of the straight walking legs with those of the curve walking inside and outside legs. The walking pattern was monitored electrically through tarsal contact measurement, and optically using synchronized high speed video. The vectors of leg movement are presented for the intact and the reduced preparation. Animals walking on the slippery surface are capable of coordinated walking activity between the legs. Upon change from straight to curve walking, the stride length for the inside legs shortens and the vector of movement of the inner legs changes to pull the animal into the curve, while the outer legs act to push it into the curve. We then reduced the animal to the two legged and single-leg preparation. The behavior of the legs remained largely unchanged in all behavioral contexts of straight or curve walking. The three different movement patterns can even be observed in the single-leg preparation, with only small increases in the extreme positions.
This suggests that the stepping behavior of the single leg in a given motor program is highly independent of the other legs.

# An in vitro platform for and from Actuators to Animation to sensing and back; using a co-culture of DRG, Muscle Cells, and Motor Neurons.

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Experiments using animals have the disadvantage that by interfering with the system multiple effects are evoked which can hardly be separated and investigated on-line. However, in order to manipulate and monitor different parts of the somatic-nervous system in a defined way we develop an in vitro model with which the signal transmission between cells can be studied. In other words, by co-culturing motor neurons, dorsal root ganglion neurons (DRG) and skeletal muscle cells we recreate the loop of actuation (realized with motor neurons), animation (twitches' obtained from skeletal muscle cells) and sensing (represented by sensory neurons from DRG).

These cells are grown on a CMOS micro electrode array chip (MEA) which makes them accessible to electrical, chemical as well as mechanical stimulation (which is can be obtained either by agitation of the device or poking the cells) [1, 2]. The substratum for motor neurons, muscle cells and DRG should be adapted according to the specific cell needs i.e. in terms of attachment and proliferation. The muscle cells are grown on collagen ensuring that they are able to striate while nerve cells are kept on laminin which is known to favour the proliferation of neural cells [3]. Under these conditions we show that motor neurons are able to form neuromuscular junctions with striated myotubes.

Furthermore, we show that the presence of motor neurons affects the twitching rate of the muscle cells. We obtain extracellular electrical signals from the muscle cells and neural cells. These recordings are shown to be susceptible to electrical manipulation and mechanical influence.

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# Proprioceptive feedback enables frequency regulation of pattern generators

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The locust flight motor output is produced by a central pattern generator (cpg). Interactions between this cpg and proprioceptive feedback play an important role in the functionally adequate patterning of motor activity (Wolf & Pearson, 1988, J Neurophysiol 59: 1831). A particularly important proprioceptor, the tegula, is associated with the wing base. It signals the downstroke movement of the wing and speeds up the wing beat rhythm (to >20Hz, compared to ~15Hz without tegula; Büschges & Pearson, 1991, J Exp Biol 157: 313).
Although the effect of the tegula on the motor pattern is profound, terminals of the tegula afferents are subject to presynaptic modulation (Büschges & Wolf, 1999, J Neurophysiol 81: 959), which fine-tunes the strength of tegula input to postsynaptic moto- and interneurons. We investigated the functional relevance of this modulation by modeling the locust flight cpg, including tegula feedback, and by testing the predictions of this model in tethered flying locusts.
In the model, neurons with Hodgkin-Huxley-like active and passive properties were used to reconstruct the actual connectivity of the flight motor rhythm generator. As expected, implementing tegula feedback into the model cpg sped up the rhythm. However, the model predicted an as yet unknown, and initially counter-intuitive, characteristic: Increasing the strength of tegula feedback slowed down the rhythm.
To scrutinize this prediction, we replaced tegula feedback in tethered flying locusts with artificial feedback through computer-controlled electric stimulation of the respective afferent nerves. These experiments verified both, speeding of the rhythm by enabling tegula feedback, and slowing of the rhythm by increasing feedback strength.
Our study thus provides a physiological test for modeling-based predictions, and it yields a general idea on the function of sensory feedback, namely, in frequency regulation of cyclic motor patterns. In particular, we showed that negative feedback with strong resetting characteristics not only resets and entrains a centrally generated motor pattern, but also controls the frequency of this rhythm.
Significance of these results in flight performance (e.g. Lehmann & Dickinson, 2000, J Exp Biol 204: 627) will have to be examined in future studies.

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# Synaptic inputs to modulatory projection neurons

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We are using the stomatogastric nervous system (STNS) of the crab, *Cancer pagurus*, to study the effects of higher neural centres and sensory input on motor pattern generation. Two central pattern generators in the stomatogastric ganglion (STG) generate the spontaneously active, rapid pyloric rhythm (filtering of food) and the slow gastric mill rhythm (chewing of food). Both motor patterns are modulated by upstream projection neurons, the somata of which are located in the bilaterally symmetric commissural ganglia. As yet, four of about 20 projection neurons descending to the STG have been identified in each ganglion. While the transmitters used by these projection neurons as well as their actions on the STG circuits have been characterized, little is known about their inputs. It has been shown that some of these neurons are involved in the processing of sensory information from mechanoreceptors and proprioceptors (Beenhakker, DeLong, Saideman, Nadim, Nusbaum, *J Neurosci* 21, 2005).

Here, we recorded from the somata of four identified projection neurons, the modulatory commissural neuron 1, 5 and 7 (MCN1, Nusbaum, Weimann, Golowasch, Marder, *J Neurosci* 12, 1992; MCN5, Norris, Coleman, Nusbaum, *J Neurophysiol* 75, 1996; MCN7; Blitz, Christie, Coleman, Norris, Marder, Nusbaum, *J Neurosci* 19. 1999) and the commissural projection neuron 2 (CPN2, Norris, Coleman, Nusbaum, *J Neurophysiol* 72, 1994). We tested whether they received input from a sensory pathway (the anterior gastric receptor AGR, Combes, Meyrand, Simmers, *J Neurosci* 19, 1999) and a neuronal pathway descending from the brain (the inferior ventricular (IV, Christie, Stein, Quinlan, Beenhakker, Marder, Nusbaum., *J Comp Neurol* 469, 2004) neurons).

MCN1 depolarized when the IV neurons were activated. However, no clear EPSPs were visible. Further tests showed that the connection between IV neurons and MCN1 is polysynaptic. When AGR was activated, MCN1 activity decreased. Similar to IV Neuron stimulation, no obvious PSPs could be detected.

CPN2 received a direct electrical postsynaptic potential with every AGR spike. In contrast, no obvious PSPs were detected when the IV neurons were active. Rather, CPN2 was slowly depolarized. This slow excitation proved to be polysynaptic.

MCN5 received direct excitation from AGR and a direct electrical postsynaptic potential from the IV neurons.

MCN7 received distinct EPSPs and concomitant inhibition which diminished MCN7 activity, indicating a parallel and antagonizing information processing in MCN7. The IV neurons slowly depolarised MCN7; no discrete PSPs were observed.

We conclude that information from sensory pathways and pathways descending from the brain converge on the level of modulatory projection neurons which, in turn, control the activity of motor pattern generating networks.

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# Poster Topic

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# Role of catecholaminergic cell groups in spontaneous, 'undirected' singing in zebra finches

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Song production in song birds is controlled by a set of song system nuclei, which include the major forebrain nuclei HVC and its projections to the robust nucleus of the arcopallium (RA) and Area X of the striatum. These three major song nuclei are innervated by mesencephalic cell groups of the catecholaminergic system. The ventral tegmental area (VTA) mainly projects to Area X, which is part of the anterior forebrain pathway which plays a key role in song learning, and has minor projections to HVC and RA, which are part of the caudal motor pathway involved in song production. The catecholaminergic cells groups in the periaqueductal gray (PAG) and locus coeruleus (LC) project to HVC and RA.

It has been shown that catecholaminerig actions in song nuclei play a role in singing behavior. Evidence is based on electrophysiological, biochemical and behavioral data. Recent studies also show that singing drives the expression of IEGs not only in the song nuclei but also in VTA and PAG, as shown during territorial song production in song sparrows (Maney and Ball, J Neurobiol. 2003, 56). However, more data are needed to show that these cell groups are activated more generally in singing behavior. It is also not clear whether catecholaminergic neurons in particular are activated during singing behavior, which would indicate that catecholamines are directly involved in singing behavior.

We studied these questions in the context of spontaneous, 'undirected' singing behavior in male zebra finches (Taeniopygia guttata). We investigated whether the neurons exhibiting ZENK expression in the VTA, PAG, and LC during singing are catecholaminergic neurons and whether these neurons are active during spontaneous song production. Singing behavior of individually housed zebra finches was recorded over a 30 min period. According to their rate of song production, birds were placed into three groups: no singers, low singers (30-90 sec), high singers (>120 sec). Immunocytochemistry was performed to examine the expression of ZENK, an IEG used as activity marker in song production, and tyrosine hydroxylase (TH), the rate limiting enzyme necessary for catecholamine production. Our results show that ZENK expression is significantly elevated in the VTA during spontaneous song production. TH-ZENK double labeled cells were found in the PAG, in particular in caudal regions, and in smaller numbers in AVT and LOC. More double labeled cells were found in singing than in non-singing birds in all three areas. This difference was significant in AVT and highly significant in PAG.

The results suggest that catecholaminergic neurons in PAG and AVT, participate in singing behavior, as these regions show an elevated ZENK expression in TH-immunopositive cells. AVT also plays a role in song production, as evident from the up-regulation of ZENK expression. Overall, our findings support the emerging concept, that singing behavior is directly modulated by the catecholaminergic system.

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# Structural organization of locust metathoracic DUM neurone types in relation to the ganglionic architecture.

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Neuromodulatory, octopaminergic dorsal unpaired median (DUM) neurones with their midline somata and bilaterally symmetrical axons form a unique class of insect neuromodulatory cells which innervate peripheral target organs such as skeletal and visceral muscles, glands and some mechanoreceptive sense organs. They were divided into different types according to which peripheral nerve their axons exit. These neurones are involved in the control of local networks in the locust metathoracic ganglion including peripheral control of contraction kinetics and metabolic rate of skeletal muscle but their connectivity with central circuits is still unknown. 3D-reconstruction techniques (Schmitt et al. 2004, Evers et al. 2005) combined with anti-tubulin and intracellular staining of DUM neurones in whole mount preparations and vibratome sections were used to study their central dendritic projections. The whole population is divided into sub-populations that are recruited in parallel to motor rhythms and that respond very differently to mechanosensory stimulation. DUM5 and DUM345 neurones, which innervate leg and non-flight muscles, exhibit similar branching patterns, having extensive branching around all major longitudinal tracts with many branches extending ventrally to multimodal ventral association centers and ventral fibre tracts, which is consistent with the described multimodality of these neurones. In contrast, DUM34 and DUM1 neurones, which innervate flight-muscles, may only respond to antennal stimulation. These types of neurones ramify consistently within dorsal regions of the metathoracic ganglion with their dendritic branches located between the dorsal and ventral intermediate tracts, whereas ventral tracts or ventral association centers are never contacted. However, an interesting feature of all metathoracic DUM34 neurones is collateral projections of lateral neurites into the lateral association centres, which are mixed neuropilar areas containing mechanosensory, motor, interneuronal and neurosecretory fibres. All DUM neurones, except DUM3, have branches making contacts with all types of dorsal longitudinal tracts, which extend the entire length of the ganglion and form the anterior and posterior connectives. DUM3 neurones did not respond to any mechanosensory stimulation, and their branches are restricted to very dorsal areas. Our neuroanatomical data suggest that within the metathoracic ganglion specific DUM subpopulations are targeted by specific presynaptic pathways that provide different activation of DUM neurones during principal motor programmes.

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# Focal TMS hand muscle representation in the awake monkey

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Background: rTMS- or tDCS-induced neuroplastic changes of cortical excitability may be of therapeutic value in several neuro-psychiatric disorders. However, more potent stimulation paradigms leading to therapeutically relevant, i.e. long lasting, after-effects are required. In order to enable the shaping of advanced stimulation protocols without taking human subjects at risk we developed a primate model of transcranial magnetic stimulation.

Methods: In a first step, we trained a male rhesus monkey to tolerate the fixation of the forearm and to relax the hand muscles. Surface EMG was recorded either from the right abductor pollicis brevis (ABP) or first dorsal interosseous (FDI) muscle. Motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation with a Magstim double small coil 25mm were recorded in both muscles. The animal was trained to maintain a relaxed EMG-monitored muscle activity during the stimulation procedure. The coil was placed over the left motor cortex and moved according to a grid of 25 $cm^2$ with 0.5 cm intersection lines. At each point 20 stimuli were applied at 1.1 and 1.2 times the resting motor threshold and the mean peak to peak amplitude was calculated. The area of the scalp from which responses were evoked was estimated counting the points at which the amplitude was greater than 10% of the maximal MEP recorded for that muscle.

Results: After a training period of 3 month the monkey was able to tolerate a 2 hours session of 400 TMS-single-pulses, which allowed mapping 8 different points daily. Resting motor thresholds were 25% and 27% of maximal stimulator output for FDI and ABP. The area of representation for FDI and ABP was 5.01$cm^2$ and 4.26$cm^2$ respectively, a surface 5 times smaller than that previously reported by human subjects. The centre of gravity (CoG) for each map was 1.1cm ant-post, 0.3 cm lateral and 1.5cm ant-post, 0.1 cm lateral for FDI and APB respectively. The measured hot-spot was 2mm away from the CoG in both muscles.

Conclusions: This first TMS mapping in the awake monkey demonstrates that TMS is practicable, reliable and well tolerated in a properly trained monkey. The TMS-assessed primate motor representation is comparable to that of humans, being also in accordance with the current neuroanatomical data. Particular because of its remarkable low TMS thresholds in combination with the high focality of the commercial TMS coil, the rhesus monkey represents a promising model for the investigation of long lasting TMS assessable neuroplastic changes.

# Do anurans possess a subthalamic nucleus?

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The mammalian subthalamic nucleus (STN) is associated with the indirect pathway of the basal ganglia and plays a critical role in motor and cognitive processes. It is a glutamatergic cell group localized in the prosomere 4 of the diencephalon. The STN receives input from the globus pallidus externus and in turn projects to the globus pallidus internus and to a midbrain area, the substantia nigra pars reticulata (SNr). While the direct pathway from striatum to globus pallidus internus and substantia nigra pars reticulata is relatively well established in anurans, it is unknown whether anurans possess an indirect pathway which includes structures homologue to a subthalamic nucleus.

The exact boundaries of the anuran prosomere 4 are not clarified in detail yet, but most likely the dorsal part of the caudal suprachiasmatic nucleus (dcSN) and the posterior entopeduncular nucleus (pEP) are derived from this area. Therefore we investigated whether the connections and transmitters of dcSN and/or pEP match those of the mammalian STN.

We used double fluorescence labeling (n=3) with tracer injections sites in areas homologous to the globus pallidus in anurans (dextranamine tetramethylrhodamine, red) and the SNr (dextranamine Alexa Fluor 488, green). Confocal laser scan analysis revealed that both dcSN, contacted by red anterogradely labeled terminals arising in the globus pallidus. Furthermore, immunohistochemical stainings (n=10) using an antibody against the transmitter glutamate demonstrated that both areas contain glutamate-immunoreactive neurons.

Our results suggest that both dcSN and pEP fulfill the morphological criteria of being the anuran homologue of the mammalian STN. We conclude that an indirect basal ganglia pathway is present in anuran amphibians. Whether anurans possess one or two structures homologous to the STN remains to be determined.

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# Sensorimotor adaptation of the grasp component in prehension: Evidence for a continuous mechanism

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Little is known about the adaptive plasticity of prehensile movements. In the present study subjects saw objects (VisObj) of various orientations, and were instructed to seize them with their thumb and index finger. VisObj was presented in a mirror whereas the actual object to be grasped (HaptObj) was behind the mirror. The subject's grasping hand thus disappeared from view during the movement, just prior to contact with HaptObj. This assured only tactile feedback about the orientation of and the contact with HaptObj without any visual cues.

The baseline condition (BL) was such that HaptObj was identically oriented with respect to VisObj. Distortion conditions (DC) were created when HaptObj differed in orientation with respect to VisObj. This difference was a single 30° rotation in group A (n = 12). In group B (n=11), the rotation was done in two steps, first a 15° difference, then the full 30° difference relative to BL. Terminal grasp orientation (TGO) was determined as the orientation of the subject's thumb and index finger in the principal plane just prior to contact with HaptObj.

Both groups achieved about 80% of the adaptation in TGO during the first 20 movements of DC. This rapid adaptation was followed by a further increase in TGO until adaptation reached about 100%. This refinement phase was more gradual and spanned across the next 25 movements. In group B's second DC, a facilitation effect was observed. After achieving full adaptation to the 15° phase of DC, at the onset of the 30° DC, participants of this group continued to alter their responses beginning at their already accomplished level of TGO change. Our findings suggest that adaptation of TGO to a visual-haptic distortion was achieved by a continuously acting mechanism.

# Histochemical characterisation of sensory neurons in the trigeminal ganglion innervating the extraocular muscles of the rat

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The mammalian extraocular muscles are not only supplied by a motor innervation, but they also receive afferents from sensory neurons in the ipsilateral trigeminal ganglion (TG). In order to gain more knowledge about their properties the sensory neurons were histochemically characterised by combined tract-tracing and immunohistochemical methods in the rat. The used markers for the TG neurons were peripheral cholinacetyltransferase (pChAT), Calretinin (CR), Parvalbumin (PV), substance P (SP) and nitric oxide synthase (NOS). Injections of the non-toxic tract tracer choleratoxin subunit B (CTB) were placed centrally into the medial rectus muscle (MR). One injection into the conjunctiva served as a control. After transcardial perfusion with 4% paraformaldehyde the TGs and brains were cut at 15µm. The detection of the tracer and the markers was revealed by double-immunoflourescence.
After an eye muscle injection CTB labelled cells were found in a limited area within the TG, whereas after the conjunctival injection labelled neurons were distributed throughout the whole ophthalmic-maxillary part of the TG. The retrogradely labelled motoneurons in the oculomotor, trochlear and abducens nuclei served as an internal control for the judgement of the size of the uptake area. In addition each case revealed a very small number of retrogradely labelled small neurons in the mesencephalic trigeminal nucleus. Within the population of CTB-labelled neurons in the TG up to 60% contain CR, and up to 8% PV. Both populations consist of large cells (mean diameter 34,6 µm). SP and pChAT were found in smaller retrogradly labelled neurons (mean diameter 25µm), whereby the SP-positive neurons included up to 50%, the pChat-positive neurons up to 39%. Approximately 24% of the CTB-labelled neurons contained NOS involving large and small neurons.
In keeping with previous observations on the spinal ganglia, the large CR and PV-positive neurons in the TG may relay proprioceptive information, whereas the SP-labelled neurons are likely to relay nociceptive signals from the eye muscles and/or the conjunctiva. For the first time we could show a robust population of pChAT-positive neurons in the TG, that innervates the extraocular muscles and/or the conjunctiva. Because of their location in the TG these cholinergic neurons are considered as sensory neurons, but their function is unclear. NOS-positive neurons may participate in the processing of nociceptive information, but alternatively they may contribute to proprioception.

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# Impaired vestibular-neck interaction in cerebellar patients

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Coordinating sensory information from the vestibular and proprioceptive system is an essential aspect of motion in space. The cerebellum is believed to be part in this integrative process: electrophysiological data obtained from the rostral fastigial nucleus (FN) in rhesus monkeys indicate that the spatial response properties of vestibular neurons in FN are systematically modulated by proprioceptive information from neck receptors. This study examines whether vestibulo-spinal interaction is impaired in humans with cerebellar deficiency.

Vestibulo-proprioceptive interaction was investigated in patients with severe cerebellar disorders. Binaural, sinusoidal galvanic vestibular stimulation was applied, while static head-on-trunk position was systematically altered in the horizontal plane: 0° straight ahead; 30°, 45°, 60°, each left and right. In healthy controls, alteration of head position resulted in corresponding rotation in body sway direction, keeping it aligned with the interaural axis. A head-centered reference frame appeared to be transformed into a trunk-centered frame.
Patients with mild disorders showing no or minimal gait and stance ataxia (equal to or below 7 points in the Klockgether rating score for cerebellar ataxia), exhibited an alteration of body sway direction similar to controls. However, in cerebellar patients suffering from prominent gait and stance ataxia, with a Klockgether rating of 12 points or higher, these systematic changes in vestibularly evoked sway direction were greatly reduced or absent. Their reference frame appeared to remain head-centered.

These results suggest that impaired interaction between vestibular and proprioceptive inputs may be a fundamental component of cerebellar ataxia. Furthermore, the cerebellum appears to be essential for the transforming of spatial reference frames.

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# Functional cell groups of the oculomotor nucleus complex in the rat

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The oculomotor nucleus complex contains several functional cell groups including motoneurons of the singly- (SIF) and multiply-innervated muscle fibers (MIF) of the extraocular muscles and the parasympathetic preganglionic neurons for the pupil and the lens. In mammals the parasympathetic preganglionic neurons are considered to lie in and around the Edinger-Westphal nucleus (EW). A recent study has suggested that the boundaries of EW can be defined using urocortin (UCO) expression. The aim of the present study is to investigate the location of these functional cell groups by their histochemical characteristics in the oculomotor nucleus complex in rats.

In order to label SIF- and MIF-motoneurons in the oculomotor nucleus the extraocular eye muscles of rats were injected with the non-toxic cholera toxin subunit B, a retrograde tracer. Visualization of the motoneurons was combined with the detection of non-phosphorylated neurofilaments (NP-NF) and UCO by immunofluorescence. In addition we compared the location of UCO-positive and choline acetyltransferase (ChAT)-positive neurons, as well as the location of UCO-positive cells and Calretinin (CR) positive cells to see whether they are collocated within the oculomotor nucleus complex.

Our data revealed tracer-labelled NP-NF positive neurons within the oculomotor nucleus which correspond to SIF-motoneurons and at the medial border tracer-labelled NP-NF negative neurons representing MIF-motoneurons. None of the tracer-labelled SIF- and MIF-motoneurons were UCO-positive. UCO-positive cells were located as a separate group of neurons immediate above and rostral to the classical oculomotor nucleus, a location usually considered to be the Edinger-Westphal nucleus. The combination of ChAT- and URO-immunolabelling revealed that the Edinger-Westphal nucleus contains at least two subgroups: UCO-positive cells and ChAT-positiv parasympathetic preganglionic neurons, which do not overlap. Combined immunolabeling with CR and UCO revealed that CR-positive cells surround the UCO-positive cells, few being intermingled with UCO-positive cells, but no double-labelled neurons were found.

In contrast to the common assumption, the results imply that the UCO-positive cells are not the ChAT-positiv parasympathetic preganglionic neurons which mediate accommodation of the lens, as well as constriction of the pupil.

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# Modules of Locomotor Control in the Central Complex of the Fruit Fly - an Analysis of the *tay bridge* Mutant

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Several aspects of locomotor control have been ascribed to the central complex of the insect brain [1]. The *tay bridge*$^{1}$ (*tay*$^{1}$) mutant of *Drosophila* was isolated on the basis of a reduced walking speed and activity. In addition, *tay*$^{1}$ is defective in the compensation of rotatory stimuli during walking. This hypomorphic allele of *tay* causes a mid-sagittal constriction of the protocerebral bridge, a constituent of the central complex. Two additional lethal alleles of *tay* facilitated the identification of the *tay* gene which encodes for a novel protein of yet unknown function. Using different GAL4 driver lines [2] for a partial rescue strategy we are able to express the wild-type *tay* cDNA in various subsets of brain neurons of otherwise *tay*$^{1}$-defective flies and thereby associate the aberrant walking-speed control on approach of visual objects with the anatomical defect in the protocerebral bridge. In contrast, the compensation of rotatory stimuli during walking could be rescued by driving *tay* to three small subsets of neurons in the brain, among those a group connecting the fan-shaped body with the ellipsoid body of the central complex. Proper compensation is achieved without a restoration of the neuroanatomical integrity of the protocerebral bridge. These findings show for the first time a separation of the control of walking towards visual objects and the control of compensatory turning into different substructures of the central complex.

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Literature:
[1] Strauss R (2002) Curr Opin Neurobiol 12: 633-638.
[2] Brand AH, Perrimon N (1993) Development 118: 401-415.

# The Control of Gap Climbing in *Drosophila melanogaster*

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We are interested in understanding the neuronal circuits and principles of the adaptive initiation of motor programmes and the goal-oriented recruitment of groups of muscles within a given motor programme by the insect CNS. To address these questions, we use a paradigm, in which flight-disabled flies can cross gaps in their walkway. *Drosophila* lends itself for this analysis as there is a plethora of genetic tools at hand to manipulate the neuronal basis.

In our setup, a single fly with clipped wings can walk on a 45 mm long, 14 mm high and 4 mm wide block of polycarbonate. The block is interrupted by a cleft of initially 4 mm width which can be widened by sliding one part of the block along a dovetail. The block is surrounded by a water-filled moat to prevent the fly from escaping and a white cardboard cylinder to shield the fly from outside visual stimuli. Two synchronized 200 Hz digital cameras monitor the gap region from the side and from above.

The tendency to initiate a climbing attempt and the rate of success have been measured at gap widths between 1 mm and 6 mm. Wild-type flies can manage to cross gaps of up to 4.3 mm with a body length of 2.5 mm. The decision to engage in climbing behaviour is depended upon the size of the gap. The probability for the occurrence of climbing declines with growing gap size. At 6 mm, wild-type flies hardly show any climbing attempts. The estimation of the gap size is achieved visually most likely by evaluating parallax motion [1].

The question remained, whether the execution of climbing is under visual control as well. To this end the landing side of the gap has been moved backward to extend the gap during an ongoing trial. Would the fly continue to attempt crossing when a gap widens to an insurmountable width after the decision had been taken to climb the previously surmountable gap? Wild-type flies complete an ongoing bout of reaching movements by the front legs after the gap has been widened. However, new bouts were never initiated thereafter. In contrast, flies with ablated mushroom bodies initiated additional bouts.

In our ongoing screen for mutant lines with specific defects in distinct subroutines of climbing behaviour we found both, lines with up-regulated and down-regulated motivation to climb. The up-regulated line, which we named *sisyphus*, initiates climbing attempts at gaps of clearly insurmountable width, although it has a seemingly normal ability to judge distances in other paradigms. The finding confirms the notion of the modular structure of climbing control inferred earlier from three mutant lines with specific climbing defects [1]. A study in structural brain mutants revealed that the protocerebral bridge of the central complex is involved in the execution of climbing.

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Literature:
[1] Pick S, Strauss R (2005) Goal-driven behavioral adaptations in gap-climbing *Drosophila*. Curr Biol. 2005; 15(16):1473-8.

# Obstacle avoidance in human walking: Task-dependent flexor reflex facilitation

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The aim of this study was to investigate the influence of learning a precision locomotor task on spinal reflex modulation. Healthy subjects had to minimize foot clearance when repeatedly stepping over an obstacle. The subjects walked with reduced vision and were informed about the approaching obstacle and task performance by acoustic warning and feedback signals, respectively. The flexor reflex (FR) was randomly elicited by tibial nerve stimulation of the right leg (with high and low stimulus intensities) during normal and obstacle steps. In a "late warning signal" (LWS) group, the warning signal and release of the FR were applied simultaneously at mid stance (before swing over the approaching obstacle). In the "early warning signal" (EWS) group, the warning signal was provided already at heel strike, while the FR was released at mid stance. Foot clearance and electromyographic activity (EMG) of several right leg muscles were analysed. With repeated obstacle steps, improved performance was reflected in a decrease of foot clearance, number of obstacle hits and of EMG required to overcome the obstacle. Independent of stimulus intensity FR amplitude was enhanced only when the delay between the warning signal and release of FR was sufficient to allow a reflex facilitation. It is concluded that obstacle stepping is associated with a facilitation of FR pathways probably due to supraspinal drive. No correlation was found between improvement of performance and FR amplitude. Thus, motor learning and performance obviously are associated with a general and unspecific reflex facilitation connected with the awareness of an approaching obstacle.

# Decoding of hand grasping signals from the macaque parietal and premotor area

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Hand manipulations are crucial for human and non-human primate behavior. Recently, cells in the anterior intraparietal area (AIP) and the ventral premotor area F5 have been shown to encode planning signals for hand grasping movements. Here, we investigate the possibility to decode such grasping intentions from single neuron activity in AIP and F5 during a delayed grasping task, in which the presentation of a visual grasp target is separated from the planning and the movement execution phase.

Macaque monkeys were trained to grasp a single object (handle) with either a power grip or a precision grip (as instructed by a colored LED) and with the handle positioned in one of 5 different orientations. All trial conditions were presented randomly interleaved. Trials started with fixation of a red LED light in the dark and the placement of both hands on a resting position (baseline period). Then, the grasp target was briefly illuminated while the type of the required grasp (power or precision) was revealed to the animal by the color of a second LED (cue period). In the following planning period, the animal could plan, but not execute the movement, until the dimming of the fixation light gave the instruction to reach and grasp the target. Planning and movement epochs were in complete darkness except for a red LED light that the animal had to fixate (monitored by optical eye tracking).

Recordings from 116 task modulated neurons in AIP of one animal revealed that that 101 of 116 cells are grip type specific in at least one task epoch and 91 cells are significantly modulated by the target orientation. In area F5 of the same animal, 84 neurons were task-modulated in at least one task epoch. Of those, 60 cells were specific for the grip type and 56 cells were modulated by the target orientation. Furthermore, a simulated decoding using Bayesian classification showed that the intended movement (grip type and orientation, 10 conditions) can be predicted from the mean firing rate in 83 % of all simulated trials from cue, in 96 % from the planning, and in 96 % from the movement period using 116 AIP-neurons, whereas from 84 F5-neurons, the Bayesian classification predicted the intended movement in 61 % of the trials from the cue activity, in 70 % from the planning, and in 45 % from the movement.

These results provide further evidence that AIP and F5 represents the grip type and target orientation during memory-guided hand movements and that neural activity from AIP and F5 could be used for hand movement decoding in neural prosthetics.

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# Poster Topic

## T24: Motor systems III: Muscle physiology

**T24-1A** Calcium activation of the asynchronous flight muscle using FRET-based imaging in *Drosophila* expressing a Cameleon transgene
*HP. Bustami, A. Kabat and FO. Lehmann, Ulm*

**T24-2A** Efficiency and mechanical power output of the asynchronous flight muscle limit locomotor capacity in *Drosophila* expressing a flightin transgene
*FO. Lehmann, M. Mronz, B. Barton, G. Ayer, N. Heymann, DW. Maughan and JO. Vigoreaux, Ulm and Vermont (USA)*

# Calcium activation of the asynchronous flight muscle using FRET-based imaging in *Drosophila* expressing a Cameleon transgene

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Muscle mechanical power production in flying *Drosophila* is due to high frequency contraction of the asynchronous indirect flight musculature (A-IFM). In contrast to synchronous muscles that contract in response to their neural drive, IFM is stretch-activated during the flapping cycle due to cyclic length changes of the thoracic exoskeleton of approximately 2-3%. The steep slope of the calcium activation curve suggests that the IFM's intracellular calcium functions as a switch allowing the muscle to respond to stretch activation rather than directly controlling magnitude and length of the muscle twitch. In this scenario, however, it remains unclear how the animal controls muscle mechanical power output during maneuvering flight without further changes in the recruitment of motor units. We approached this problem using calcium imaging and electrophysiological techniques in intact behaving transgenic fruit flies. The animals express the transgene FRET-based $Ca^{2}$+-indicator molecule Cameleon that alters its fluorescence pattern depending on the concentration of intracellular calcium. The $Ca^{2}$+binding protein typically emits light at two different wavelengths (485 and 530 nm) that was recorded using a dual-emission fluorescence microscope and commercial software (Slide-book). Relative calcium concentration in the muscle fiber may be derived from changes in ratio between the light intensity of the two wavelengths. We employed this approach to map spatio-temporal activation pattern of the IFM when the animal altered both its neural drive to the IFM and mechanical power output during maneuvering flight in response to external stimuli. Previous data demonstrate that calcium concentration of the entire flight musculature increases linearly with increasing stimulus frequency of the neural drive and significantly varies within the physiological range of muscle spike frequencies found in the behaving animal. Thus, the results suggest that IFM power output during stretch-activation might be adjusted by changes in calcium concentration similar to the mechanism found in the synchronous flight muscles of other insects and birds.

# Efficiency and mechanical power output of the asynchronous flight muscle limit locomotor capacity in *Drosophila* expressing a flightin transgene

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Maximum mechanical power output of the asynchronous flight muscle is a key factor that limits aerial performance in flying insects. To assess the relationship between muscle power, efficiency and locomotor performance in behaving fruit flies *Drosophila*, we developed a biophysical experimental essay based on simultaneous measurements of flight force, wing motion, and the flux of respiratory gases. The primary goal of this research is to explore how mechanical power output constrains different classes of flight maneuvers in flying animals. Employing transgenic muscle mutants, we evaluate the consequences of changes in muscle protein composition on the interplay between the nervous system and two physiologically distinct classes of flight muscles. Recent experiments on transgenic *flightin* mutants demonstrate that alteration in molecular muscle structure changes the saccadic flight style in freely flying animals due to a reduced capability of the animals to produce aerodynamic forces. *Flightin* is a multiply phosphorylated, myosin binding protein found specifically in indirect flight muscles (IFM) of *Drosophila*. A null mutation in the flightin gene (fln0) compromises thick filament assembly and muscle integrity resulting in muscle degeneration and lost of flight ability. Using P-element mediated transformation with the full length flightin gene driven by the Actin88F promoter, we have achieved rescue of all fln0 related ultrastructural and functional defects of the IFM. Transgenic P[fln+] fln0 'rescued' flies have less thick filaments per myofbril than wild-type flies but have otherwise normal IFM. Transgenic P[fln+] fln+ 'tetraploid' flies have normal number of thick filaments. Flightin expression levels in both transgenic strains are similar to wild-type. In contrast, flightin expression levels are reduced in a myosin heavy chain tetraploid strain that produces excess myosin and excess thick filaments. Experiments in a flight simulator show that these small changes in molecular structure of the IFM produce small reductions in muscle mechanical power output but no significant changes in the chemo-mechanical conversion efficiency of the IFM.

# Poster Topic

## T25: Homeostasis

**T25-1B** Effects of oxygen tension on mitochondrial metabolism and single unit activity in organotypic hippocampal slice cultures
*C. Huchzermeyer, J. Otahal, K. Albus, HJ. Gabriel, R. Kovács, U. Heinemann and O. Kann, Berlin*

**T25-2B** Impaired homeostatic plasticity in migraine: a combined tDCS/rTMS study
*K. Boros, A. Antal, N. Lang, S. Arlt, Z. Chadaide and W. Paulus, Göttingen*

# Effects of oxygen tension on mitochondrial metabolism and single unit activity in organotypic hippocampal slice cultures

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Most of the energy that is required for maintenance of ionic gradients across neuronal membranes and for neurotransmission is provided by oxidative phosphorylation in mitochondria. Thus tissue oxygen tension is critical for mitochondrial ATP supply in neurons and neuronal function. We changed oxygen tension in hippocampal slice cultures by gassing the recording solution with either 95% oxygen (high $pO_2$ level) or 20% (low $pO_2$ levels) and explored the effects on mitochondrial metabolism and neuronal activity using NAD(P)H and FAD fluorescence measurements as well as electrophysiological techniques under submerged recording conditions. Absolute $pO_2$ values were determined using a Clark style microelectrode.

The switch from high (448 ± 25.5 mmHg $pO_2$; n=4) to low $pO_2$ (47 ± 10.2 mmHg $pO_2$; n=4) levels in slice cultures was associated with positive baseline shifts in NAD(P)H fluorescence, indicating enhanced $NAD(P)^+$ reduction. At low $pO_2$ levels, repetitive electrical stimulation (10 s, 20 Hz) of stratum pyramidale in hippocampal area CA3 led to biphasic NAD(P)H transients showing similar initial 'dip' components (-0.61 ± 0.1 $\%\Delta F/F_0$ versus -0.92 ± 0.1 $\%\Delta F/F_0$, p=0.15; n=18) but significantly larger 'overshoot' components (2.2 ± 0.4 $\%\Delta F/F_0$ versus 0.71 ± 0.1 $\%\Delta F/F_0$, p<0.01; n=18) as compared to NAD(P)H transients at high $pO_2$ levels. Interestingly, rise time (5.47 ± 0.48 s versus 7.55 ± 0.5 s, p=0.01; n=8) and decay time (8.2 ± 1.83 s versus 16.93 ± 1.69 s, p<0.01; n=8) of the initial 'dip' components were significantly faster at low $pO_2$ levels. Biphasic FAD transients showed similar effects. Moreover, spontaneous single unit activity and stimulus-evoked field potentials were enhanced at high $pO_2$ levels. The minimum value of oxygen tension after repetitive stimulation was 7 mmHg at low $pO_2$ levels and 293 mmHg at high $pO_2$ levels.

We conclude that tissue $pO_2$ and diffusion distances in slice preparations critically affect mitochondrial energy metabolism and thus neuronal activity, in particular under conditions of sustained neuronal stimulation.

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# Impaired homeostatic plasticity in migraine: a combined tDCS/rTMS study

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Migraine is a very common neurological disorder in which the central nervous system dysfunction has a pivotal role. Abnormal cortical excitability has been suggested to play an important role as a predisposing factor to spontaneous, cortical spreading depression (CSD), that has been suggested to represent the pathological basis of aura during migraine. Recently it has been shown that preconditioning of the primary motor and visual cortices by excitatory or inhibitory transcranial direct current stimulation (tDCS) can shape the direction of repetitive transcranial magnetic stimulation (rTMS) induced after-effects in the motor cortex of healthy subjects. The aim of the present study was to observe the dynamics of the basic interictal state of migraineurs with aura (MA) by further modulating the excitability level of the visual and motor cortex using tDCS and rTMS. The measurement of phosphene thresholds (PTs) was used to quantify the excitability level of the visual cortex and recording of motor -evoked potentials (MEPs) of the motor cortex. Facilitatory pre-conditioning with anodal tDCS resulted in an increase of PTs and decrease of MEPs by a subsequent period of 5 Hz rTMS when compared to baseline level in both groups, however, in the MA group the decrease of PTs and increase of MEPs after anodal stimulation was more pronounced than in healthy subjects. Conversely, inhibitory pre-conditioning with cathodal tDCS caused 5 Hz rTMS decreasing PTs and increasing MEPs to baseline in healthy subjects. In patients cathodal stimulation resulted in a decrease in PT that was followed by a more pronounced decrement after rTMS. The MEP changes were similar in migraineurs than in controls after cathodal preconditioning. In both groups no changes in visual cortex excitability were observed when 5 Hz rTMS was preceded by sham-tDCS. Our results show that changing the initial excitability level of the visual and motor cortices by a period of DC stimulation, reversed the conditioning effects of 5 Hz rTMS in healthy subjects, showing an existence of a homeostatic-like mechanism in order to stabilize cortical excitability within a physiologically useful range. However, this kind of plasticity is differentially impaired in the visual and motor cortices of migraineurs probably due to the inbalance of the inhibitory and excitatory circuits.

# Poster Topic

## T26: Neuroendocrine systems

**T26-1C** Targeted deletions of Mel1a and Mel1b melatonin receptors affect pCREB levels in lactotroph and pars intermedia cells of mice.
*P. Sheynzon and HW. Korf, Frankfurt/Main*

**T26-2C** Role of the thyroid hormone transporter MCT8 in the murine CNS
*M. Trajkovic, TJ. Visser, J. Mittag, S. Jungk, S. Horn, J. Lukas, K. Bauer and H. Heuer, Jena, Rotterdam (NL) and Hannover*

# Targeted deletions of Mel1a and Mel1b melatonin receptors affect pCREB levels in lactotroph and pars intermedia cells of mice.

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The pineal hormone melatonin acts on the target cells through two subtypes of membrane-bound, G-protein-coupled receptors: the MT1 (Mel1a) and MT2 (Mel1b) melatonin receptors. Through its receptors in the pars tuberalis (PT) of the pituitary, melatonin regulates prolactin secretion from the pars distalis of the hypophysis. We first analyzed the activity state of lactotroph cells in the pars distalis of melatonin-proficient C3H and melatonin-deficient C57BL mice at four different time points of a light/dark cycle. These analyses were performed by immunocytochemical demonstration of Ser133-phosphorylated pCREB in identified lactotroph cells and have revealed a subpopulation of lactotroph cells whose pCREB levels differed between the two mouse strains. We then attempted to identify the melatonin receptor type responsible for the regulation of lactotroph cells. For this purpose we analyzed the levels of Ser133-phosphorylated pCREB in immunocytochemically identified lactotroph cells of wild-type mice (MelAABB) and of mice bearing targeted deletions of Mel1a receptor (MelaaBB), the Mel1b receptor (MelAAbb) or of both receptor types (Melaabb) at five different time points of a light/dark cycle. Moreover, we have included the pars intermedia in our investigations in order to analyze whether pCREB levels are controlled by intact melatonin signal transduction cascades also in this part of hypophysis. In wild-type and MelAAbb mice the percentage of lactotroph cells with nuclear pCREB immunoreactions varied significantly over 24-h period, whereas in MelaaBB and Melaabb mice no significant differences were found between the five time points analyzed. pCREB levels in the pars intermedia did not show rhythmic variations in wild type or Melaabb animals but wild type mice had higher pCREB levels than Melaabb. Our results indicate that Mel1a and Mel1b melatonin receptors are involved in the control of the activity state of lactotroph and pars intermedia cells of mice. We currently examine, by using in situ hybridization, whether and how melatonin affects the expression of prolactin in the pars distalis.

# Role of the thyroid hormone transporter MCT8 in the murine CNS

Marija Trajkovic[1], Theo J. Visser[2], Jens Mittag[3], Sven Jungk[3], Sigrun Horn[1], Jan Lukas[1], Karl Bauer[3] and Heike Heuer[1]

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As a crucial factor for proper brain development, thyroid hormone is involved in the regulation of neuronal migration and differentiation, glia cell proliferation, myelination, and synaptogenesis. Consequently, absence of thyroid hormone during critical periods of brain development results in irreversible brain damage. In humans, thyroid hormone deficiency may even lead to the syndrome of cretinism, a disorder characterized by severe mental retardation, neurological deficits and hearing impairment.

A prerequisite for proper thyroid hormone actions in the brain is the presence of thyroid hormone transporters that mediate not only thyroid hormone passage at the blood- brain-barrier but also the cellular uptake of thyroid hormone in glial and neuronal cells, respectively. However, only a limited number of proteins putatively involved in TH transport processes have been identified so far. Among these candidates, the monocarboxylate transporter 8 (MCT8) was characterized as a very specific and active thyroid hormone transporter. Furthermore, MCT8 was found to be highly expressed in the murine CNS with a predominant neuronal localization pattern. Most importantly, patients were identified carrying mutations or deletions in the X-linked MCT8 gene. These patients suffer from a severe form of psychomotor retardation in combination with abnormal thyroid hormone serum levels. Though theses findings suggest a function of MCT8 as an important thyroid hormone transporter in the CNS the pathogenic mechanisms underlying this syndrome are not yet understood.

In order to elucidate the pathophysiological function of MCT8 we analysed mice in which the MCT8 gene was inactivated by a conventional knock out strategy. While MCT8 null mice exhibited the same abnormal serum thyroid hormone parameters as diagnosed in patients the animals did not develop any signs of ataxia, motor dysfunction or other neurological deficits. Furthermore, histological analysis of the cerebellum did not display any abnormalities in MCT8 deficient animals though its development is highly dependent on proper thyroid hormone supply. Even Purkinje cells devoid of MCT8 responded normally to thyroid hormone treatment in vitro indicating the existence of other thyroid hormone transporting proteins in these cells. In contrast, analysis of thyroid hormone regulated gene products revealed highly increased transcript levels of TRH in the paraventricular hypothalamic nucleus and decreased levels of neurogranin/RC3 in the striatum suggesting that in striatal and hypothalamic neurons of MCT8 deficient animals, intracellular thyroid hormone levels are decreased. We therefore conclude that MCT8 indeed plays a role in neuronal thyroid hormone uptake. However, depending on the cell-specific repertoire of other - yet unknown - thyroid hormone transporters absence of MCT8 can be compensated at least in mice.

# Poster Topic

## T27: Learning and memory I: LTP, LTD

**T27-1A** LONG-TERM DEPRESSION (LTD) IN BURST SPIKING (BS) AND REGULAR SPIKING (RS) NEURONS OF THE RAT SUBICULUM
*P. Fidzinski, O. Shor and J. Behr, Berlin*

**T27-2A** Nuclear Calcium signals during L-LTP induction do not predict the degree of synaptic potentiation
*FW. Johenning and K. Holthoff, Berlin and Munich*

**T27-3A** Slowness: An Objective for Spike Timing-Dependent Plasticity?
*H. Sprekeler, C. Michaelis and L. Wiskott, Berlin*

**T27-4A** Impairment of LTP after chronic mGluR5 antagonism is associated with decreased power of gamma (30-100 Hz) oscillations in the dentate gyrus of freely moving rats
*A. Bikbaev and D. Manahan-Vaughan, Bochum*

**T27-5A** Homeostatic plasticity in human motor cortex: a study combining transcranial direct current stimulation and paired associative stimulation
*MF. Kuo, A. Roth, AK. Fischer, W. Paulus and M. Nitsche, Göttingen*

**T27-6A** Up-regulation of vesicular GABA content and inhibitory synaptic efficacy during late-LTP
*V. Lopantsev, S. Kolbaev and A. Draguhn, Heidelberg*

**T27-1B** Role of the actin network during long-term potentiation and synaptic tagging in hippocampal CA1 neurons
*R. Binu, S. Sajikumar and JU. Frey, Magdeburg*

**T27-2B** Modulation of hippocampal CA1 synaptic plasticity by a complex holeboard learning task: implications for induction of LTP and LTD.
*D. Makhracheva-Stepochkina, JU. Frey and V. Korz, Magdeburg*

**T27-3B** Different role of protein kinase A during synaptic tagging in apical versus basal dendrites of hippocampal CA1 neurons
*S. Navakkode and S. Sajikumar, Magdeburg*

**T27-4B** Induction of hippocampal early- and late-LTP modulates monoamine transmitter level:
a microdialysis study
*F. Neugebauer, JU. Frey and V. Korz, Magdeburg*

**T27-5B** Different properties and requirements of synaptic tagging and cross-tagging in hippocampal CA1 neurons, compartmentalization and de-compartmentalization
*S. Sajikumar, S. Navakkode and JU. Frey, Magdeburg*

**T27-6B** A comparative study of long-term potentiation in the basal and apical dendrites of CA1 pyramidal neurons in hippocampal slices in vitro
*HK. Sreepathi, S. Sajikumar and JU. Frey, Magdeburg*

**T27-1C** BDNF dependent synaptic plasticity in organotypic hippocampal slice cultures.
*T. Brigadski and V. Lessmann, Mainz*

**T27-2C** The actin filament depolymerizing factor cofilin1 is a major player of activity induced actin cytoskeleton dynamics in dendritic spines
*MB. Rust, CB. Gurniak, H. Vara Rivera, M. Al Banchaabouchi, M. Giustetto, M. Sassoe-Pognetto and W. Witke, Monterotondo-Scalo (Roma) (I) and Turin (I)*

**T27-3C** Magnetic stimulation of one dimensional neural cultures *in-vitro*
*A. Rotem and E. Moses, Rehovot (Israel)*

**T27-4C** Presynaptic HCN channels modulate NMDA receptor-dependent synaptic plasticity in the immature rat medial perforant path
*T. Kirschstein, RA. Bender and H. Beck, Rostock, Hamburg and Bonn*

**T27-5C** Hyperpolarization-activated Cation Channels Impair DHPG-induced LTD in Rat Hippocampal CA1 Region
*T. Tokay, S. Krabbe, R. Köhling and T. Kirschstein, Rostock*

# LONG-TERM DEPRESSION (LTD) IN BURST SPIKING (BS) AND REGULAR SPIKING (RS) NEURONS OF THE RAT SUBICULUM

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The subiculum is the main output structure of the hippocampus and has been shown to play a pivotal role in learning and memory. On cellular level, learning processes are thought to occur by changes of synaptic strength. However, little is known about the precise mechanisms of synaptic plasticity in subicular neurons. In a recent study (Wozny et al., *in prep*) it was found that the form of LTP (pre- or postsynaptic) expressed in subicular neurons after tetanic stimulation correlated with the intrinsic properties of the cells (BS vs. RS). Here we report that also low frequency stimulation (LFS) has different effects on BS and RS neurons.

In acute brain slices of juvenile Wistar rats, EPSPs/EPSCs of single subicular neurons were recorded upon stimulation of CA1 efferents. For LTD induction, paired pulse stimulation of 15 min duration at 4 different frequencies (0.5-5 Hz) was tested. In 80% of BS cells, LTD was induced at all 4 frequencies, whereas RS cells responded with much larger variance, showing LTD in 40% and LTP in 60% of investigated cells. No difference in LTD strength between BS and RS cells was observed (BS: 67.7±7.9% of baseline and RS: 65.1±6.7%). In 80% of the cells showing LTD, analysis of paired pulse index and coefficient of variation indicated a presynaptic contribution to LTD expression. Inhibition of the NMDA-R (D-APV 100 μM) blocked LTD in both cells types without affecting the LTP component in RS cells. Interestingly, scopolamine (30 μM) increased the LTD ratio (vs. no LTD or LTP) in both cell types, indicating a modulatory effect of cholinergic input to subicular neurons.

This is the first study to describe differences in LTD expression between BS and RS cells. Taking under consideration the findings of Wozny et al., a possible function of the subiculum could be the control of hippocampal output by the input frequency of CA1 efferents. As BS cells project to the presubiculum and RS cells to the entorhinal cortex (Stewart M., Brain Res, 1997), a chosen input to the subiculum could specifically facilitate the information output from the hippocampal loop.

# Nuclear Calcium signals during L-LTP induction do not predict the degree of synaptic potentiation

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The magnitude and/or duration of nuclear Ca2+ transients has been shown to dose-dependently modulate gene-transcription upon neuronal activation. This is an attractive model for synapse-to-nucleus communication. In order to encode synaptic information, these nuclear Ca2+ transients have to be correlated with changes in synaptic strength rather than changes in gene expression patterns. In this study, we analysed nuclear Ca2+ signals during L-LTP induction. Using a combined approach of fEPSP recordings and two-photon imaging, these Ca2+ signals were correlated with different degrees of synaptic potentiation in CA1 hippocampal slices. To refine our analysis on the single cell level, we developed a new approach called single-cell-excitability-probing (SCEP) to assay the plasticity outcome of individual cells by optical means. The degrees of synaptic potentiation we observed could be categorized into transcription-independent, transcription-dependent and reduced transcription-dependent. There is no consistent dose-dependent relationship between these different degrees of synaptic potentiation and the magnitude, the decay time and the area under the curve of nuclear Ca2+ transients during L-LTP induction. This indicates that nuclear Ca2+-transients during induction are unsuited to grade the degree of plasticity in an analogue manner. We propose a role for nuclear Ca2+ as a digital on/off switch for activating transcription.

# Slowness: An Objective for Spike Timing-Dependent Plasticity?

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Slow feature analysis is an efficient algorithm for learning input-output functions that extract the most slowly varying features from a quickly varying input. It has been successfully applied to the unsupervised learning of translation-, rotation-, and other invariances in a model of the visual system, to the learning of complex cell receptive fields, and, combined with a sparseness objective, to the learning of place cells in a model of the hippocampus.

In order to arrive at a biologically more realistic implementation of this learning paradigm, we consider analytically how slow feature analysis could be realized with linear Poisson neurons. Surprisingly, we find that the appropriate learning rule reproduces the typical learning window of spike timing-dependent plasticity. The shape as well as the timescale are in good agreement with what has been measured experimentally. This offers a new computational interpretation of the peculiar learning window of physiological neurons.

# Impairment of LTP after chronic mGluR5 antagonism is associated with decreased power of gamma (30-100 Hz) oscillations in the dentate gyrus of freely moving rats

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Oscillatory activity in the gamma frequency range (30-100 Hz) is closely related to theta rhythm, and represents the activity of distributed neuronal ensembles that are organized within time windows set by theta cycles. Several lines of evidence show an important role of gamma oscillations in processes of learning, memory, and cognition.

Recently, it was shown that chronic antagonism of mGluR5 *in vivo* leads to significant impairment of both working and reference memory in rats (Manahan-Vaughan and Braunewell, 2005, Cer Cor 15: 1703; Naie and Manahan-Vaughan, 2004, 14:189). On the other hand, mGluRs are involved in generation and/or maintenance of various patterns of rhythmic activity, and their activation can induce high-frequency oscillations *in vivo* and *in vitro*. The present study addressed the role of mGluR5 in hippocampal LTP and the maintenance of gamma oscillations in the dentate gyrus of freely moving rats.

Adult male Wistar rats underwent stereotaxic implantation of a bipolar stimulating electrode into the medial perforant pathway and a recording electrode into the dentate gyrus. After a 10d recovery period, fEPSPs and intrahippocampal EEG (sampled at 0.5 KHz) were recorded. Either vehicle or the non-competitive mGluR5 antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP), were administered once daily into the lateral cerebral ventricle via an implanted cannula. To induce LTP, high-frequency tetanization (HFT, 200 Hz, 10 x15 pulses) was applied 30 min after third (final) injection. In order to estimate the effect of MPEP on gamma oscillations, 4-s long artefact-free EEG epochs, cut 1s after test-pulses, were analyzed off-line using FFT algorithm. The results of spectral analysis, as well as fEPSP values, were normalized and analyzed further using analysis of variance, separately for injection, tetanization, and drug factors.

We have found that chronic mGluR5 antagonism caused a significant impairment of both early- and late-LTP in the dentate gyrus, when compared to vehicle-treated controls. This impairment of LTP coincided in time with the memory impairments described in the abovementioned earlier studies and, therefore, may reflect the decreased ability to form memory traces.

On the network level, MPEP significantly suppressed gamma band activity. In MPEP-treated animals, we have found a significant decrease of the relative gamma power after HFT, compared to pre-tetanisation levels. In controls, a trend towards an increase of gamma power was evident after both HFT and injection. There was no effect of injection *per se* on gamma oscillations in MPEP-treated animals. In the period prior to HFT no drug effect was revealed. However, in post-tetanization period the MPEP effect was found to be highly significant, reflecting lower values of the relative gamma power in MPEP-treated animals compared to controls.

Our data demonstrate the pivotal role for mGluR5 in long-term potentiation and network activity in hippocampal circuits, and provide a link between plastic changes in synaptic transmission, oscillatory activity in gamma frequency range, and spatial learning in rats.

# Homeostatic plasticity in human motor cortex: a study combining transcranial direct current stimulation and paired associative stimulation

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Homeostatic mechanisms have been suggested to maintain the stability of cortical neural networks, which is crucial for the modulation of neuroplasticity. Accordingly in human experiments the history of general/background cortical excitability determines the direction of its subsequent modulation: an identical stimulation protocol can enhance or reduce excitability, in each case counteracting the previous level of excitability. Transcranial direct current stimulation (tDCS) induces neuroplasticity through membrane polarization under the electrode: anodal tDCS increases cortical excitability while cathodal tDCS decreases it. In contrast to this global excitability change, a synapse-specific plasticity can be induced by paired associative stimulation (PAS) which combines peripheral nerve stimulation with single-pulse transcranial magnetic stimulation (TMS) of the motor cortex. We studied the interactions of these two long-lasting plasticity-inducing techniques on 12 healthy subjects. When applied before PAS, anodal tDCS further increased the excitability-enhancing efficacy of the PAS protocol, while cathodal tDCS diminished the PAS-induced excitability enhancement and reversed it to inhibition. On the other hand, homeostatic neuroplasticity was revealed by the simultaneous application of both protocols. PAS elicited inhibitory plasticity when background excitability was increased by anodal tDCS, while the generally reduced background excitability caused by cathodal tDCS strengthened the facilitatory PAS-induced plasticity. We conclude that background network excitability influence associative plasticity. However, the relationship might be more complex than previously thought: Whereas enhanced or diminished background activity established before the induction of plasticity has an anti-homeostatic effect, the results accomplished by simultaneous modulation of both parameters are in accordance with homeostatic rules. They provide further insight on the possible neurophysiological mechanisms involving learning and memory formation.

# Up-regulation of vesicular GABA content and inhibitory synaptic efficacy during late-LTP

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Long-term potentiation (LTP), including its late-phase (L-LTP, lasting >4 hours) is a well established phenomenon of activity-dependent synaptic plasticity in the hippocampus. Repetitive activation of Schaffer collaterals leads to a long-lasting increase in synaptic glutamatergic efficacy in CA1 pyramidal cells. However, stability of hippocampal networks requires maintenance of excitatory-inhibitory balance. Therefore, we analysed GABA-mediated inhibition in CA1 pyramidal cells after induction of L-LTP in mouse hippocampal slices. Field-potential recordings confirmed the lasting potentiation of glutamatergic transmission in the CA1 area, measured as an increased slope of field excitatory postsynaptic potentials in the dendritic layer. Intracellularly recorded monosynaptic GABAA receptor-mediated IPSPs were enhanced during L-LTP. No changes in the reversal potential of IPSPs or paired-pulse evoked responses were detected. Control slices demonstrated frequency-dependent reduction in the amplitude of IPSPs, whereas IPSPs were more stable or even increased during the trains in potentiated slices. Our preliminary data also indicate that miniature IPSCs were enhanced during L-LTP. We hypothesized that the observed increase in GABAergic synaptic efficacy was caused by increased filling of presynaptic vesicles with GABA.

The mechanisms underlying long-LTP are dependent on protein synthesis. We therefore stained potentiated slices for the key enzymes of GABA synthesis (glutamate decarboxylase, GAD65 and GAD67) and for the vesicular inhibitory amino acid transporter VGAT (IAAT). Indeed, expression of all three proteins was increased in the potentiated region within CA1, indicating transcriptional up-regulation of the machinery for filling of GABAergic vesicles. Quantification of Western blots from potentiated versus naive slices confirmed the increase in GAD65, GAD67, and VGAT-content.

Our data indicate increased GABAergic inhibitory transmission in CA1 pyramidal cells during L-LTP. Local interneurons produce more GABA and increase vesicular GABA content, leading to enhanced IPSPs and more sustained inhibition upon activation at high frequency. This new mechanism of homeostatic plasticity contributes to the maintenance of excitation-inhibition balance in the hippocampal network.

# Role of the actin network during long-term potentiation and synaptic tagging in hippocampal CA1 neurons

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Long-term potentiation (LTP) and long-term depression (LTD) are models which are considered to be cellular mechanisms underlying learning and memory formation. Similar to distinct types of memory formation, LTP and LTD can be separated into a protein synthesis-independent phase (early -LTP/-LTD) and a protein synthesis-dependent late phase (late-LTP/-LTD). Both, late-LTP and late-LTD in the CA1 region of the rat hippocampal slice in vitro are characterized by the heterosynaptic requirements during induction as well as by processes of synaptic tagging, a phenomenon which describes how a weak, protein synthesis-independent early-LTP can be transformed into a late, protein synthesis-dependent form (Frey & Morris, 1997). Here, we present preliminary data investigating the role of the actin network during LTP and specifically, its role for synaptic tagging using actin polymerization inhibitors, such as latrunculin A and B. In agreement with previous work from others, our results suggest that the polymerization of the actin network is important for the maintenance of late-LTP. Here we show that in addition to maintaining late-LTP the polymerization of actin network is required for processes of synaptic tagging. The actin polymerization inhibitors prevented the transformation of early-LTP induced in a synaptic inputs S2 by late-LTP which was induced in an independent synaptic input S1 prior to eary-LTP in S2. Our preliminary results support the hypothesis that actin is part of the synaptic tag machinery which is directly involved in capturing plasticity-related proteins and thus, it is essential for the maintenance of LTP and and synaptic tagging.

# Modulation of hippocampal CA1 synaptic plasticity by a complex holeboard learning task: implications for induction of LTP and LTD.

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Long-term potentiation (LTP) and -depression (LTD) are considered as cellular models for learning and memory. We studied the impact of holeboard training on the maintenance of LTP in the rat CA1 hippocampal region. In 8-week-old rats, a recording electrode was stereotactically and chronically implanted into the pyramidal cell layer of the CA1 of the right hemisphere and a stimulation electrode into the contralateral CA1 region.

Behavioral manipulations started 15 min after tetanization. Two groups of animals received a spatial training on a fixed pattern of baited holes of either ten or fifteen trials over two days. The last trial was performed after tetanization. A pseudotrained control group received a ten- trial training with changing patterns of baited holes after each trial. Rats significantly improved their spatial performance with increasing numbers of trials, indicated by decreasing times to pick up all food pellets and by the decrease in reference memory errors. A learning-related impairment of induction of LTP in both the population-spike amplitude as well as the EPSP could be noted: in animals trained over fifteen trials no significant long-term potentiation but instead a lasting depression was observed. Intermediately trained animals showed a significantly reduced potentiation as compared to pseudotrained animals. These results are in contrast to earlier studies in the dentate gyrus of the hippocampus (Uzakov et al., 2005), pointing to region-specific different plastic processes during holeboard training. This difference may reflect the region-specific encoding of different memories during spatial training.

Reference: Uzakov S, Frey JU, Korz V (2005) Learn Mem 12, 165-171.

# Different role of protein kinase A during synaptic tagging in apical versus basal dendrites of hippocampal CA1 neurons

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Hippocampal long-term potentiation (LTP) is thought to serve as an elementary mechanism for the establishment of certain forms of memory in the mammalian brain. As is the case with behavioural memory, LTP in the CA1 region has distinct stages: a short-term early phase lasting 1-3 hours (early-LTP), which is independent of protein synthesis and a long lasting stage (late-LTP) which is dependent on protein synthesis. Synaptic input-specificity can be explained by the concept of synaptic tagging, in which newly synthesized plasticity-related proteins (PRPs) activated by heterosynaptic interactions bind to recently potentiated, glutamatergic 'tagged' synapses thus maintaining LTP and input-specificity. Studies related to mechanisms of synaptic tagging are mostly restricted to the apical CA1-dendritic compartment. However, a typical CA1 pyramidal neuron has both - an apical and a basilar compartment - which differ in morphology, biophysical properties, LTP-induction and -expression. It is widely known that inhibitors of cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA) block late-LTP in apical dendrites. We therefore studied the role of PKA in maintaining late-LTP in basal dendrites and also its specific role in setting of synaptic tags in both apical and basal dendritic compartments. We could show that, as in case with apical dendrites, PKA also plays a role in maintaining late-LTP in basal dendrites. However, in apical dendrites PKA activity is specifically required for the induction of the synthesis of PRPs. In contrast, in basal dendritic compartments the joint activity of PKA and PKMzeta is specifically involved in the tagging process rather than in the synthesis of PRPs.

# Induction of hippocampal early- and late-LTP modulates monoamine transmitter level: a microdialysis study

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The study aimed at combining microdialysis and electrophysiological recordings in freely behaving animals. 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. A microdialysis guide cannula was chronically implanted into the ipsilateral hippocampus so that after insertion of the probe the membrane (1 mm in length) was adjusted to the granular cell layer of the dentate gyrus. Long term potentiation (LTP) was induced by tetanization of the perforant path (early-LTP: 3 bursts of 15 pulses, 200 Hz, 10 s interburst interval; late-LTP: 20 bursts of 15 pulses, 200 Hz, 10 s interburst interval). A third group receiving only test pulses controled for diurnal variations of transmitters. The flow rate of the perfusing ringer solution was 1,5 µl/min and dialysates were sampled every 20 min at 1 h before tetanus and up to six hours after tetanus. Samples were analyzed by HPLC with electrochemical detection. The first results reveal a tetanus-specific modulation of different monoamines and their metabolites.

# Different properties and requirements of synaptic tagging and cross-tagging in hippocampal CA1 neurons, compartmentalization and de-compartmentalization

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The ability of a neuron to modify its synaptic efficacy in response to incoming information has been suggested to be the cellular basis for learning and memory. Long-term potentiation (LTP) and long-term depression (LTD) are prominent cellular models used to study these processes. How pre-existing or newly synthesized plasticity related proteins (PRPs) interact with specific, activated synapses expressing LTP/LTD, but not with inactive synapses, is fundamental to the synapse-specificity of LTP/LTD which is critical for systematic information processing and memory formation. The' synaptic tagging' hypothesis (Frey & Morris, 1997) has been put forward as a way to address this problem. According to this hypothesis the persistence of LTP is mediated by the intersection of two dissociable events. The first event involves the generation of a local 'synaptic tag' at specific synapses in association with the induction of LTP. The second involves the production and diffused distribution of PRPs that are captured and utilized only at those synapses possessing a tag. We have investigated some of the key-molecules involved in synaptic tagging during LTP and LTD and found that the induction of late-LTP in apical dendrites of CA1 sets a process-specific synaptic tag that is mediated by calcium-calmodulin-dependent-kinase II (CaMKII), whereas induction of late-LTD also sets process-specific tags however which is mediated by mitogen-activated kinases (MAPKs, i.e., MEK-dependent extracellular-signal regulated kinases: ERKs). In addition, synaptic tagging and cross-tagging is restricted (or compartmentalized) to distinct dendritic branches. These results suggest that instead of the formation of a memory trace within a single synapse, functional synaptic populations exist within a dendritic compartment. These synaptic populations with their related intracellular messenger machinery could act as integrative units within neural networks. We present data describing compartmentalization properties of synaptic tagging and cross-tagging and the requirements for de-compartmentalization under distinct circumstances.

References

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Sajikumar S, Frey JU. 2004 . Neurobiol Learn Mem. 82(1):12-25.

# A comparative study of long-term potentiation in the basal and apical dendrites of CA1 pyramidal neurons in hippocampal slices in vitro

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Hippocampal long-term potentiation (LTP) is considered to be a cellular model for studying the mechanisms of learning and memory. Although a typical hippocampal CA1 pyramidal neuron has both apical and basal dendritic compartments, relatively few studies have been conducted in basal dendrites. Both apical and a basilar compartments are distinctively innervated by different afferent inputs, such as arriving from hippocampal sub-regions, the entorhinal cortex, the amygdaloid complex, or the thalamus. Apical and basal dendrites also differ in morphology and biophysical properties. The information received from the different inputs is processed and integrated in the specific apical and basal dendrites of the CA1 pyramidal neuron to enable the encoding and storage of spatial, contextual, or relational information. We therefore studied here more thoroughly the properties of LTP in basal dendrites in the stratum oriens of CA1 neurons. We could show that the protocol for induction of various forms of LTP differs between the two dendritic compartments, i.e. the basal and apical dendrites. A strong tetanization protocol (STET, 100Hz, 100 pulses, 0.2 ms) used for induction of late-LTP produced a robust long-lasting LTP for 6 h in both inputs. A weak tetanization protocol (WTET, 100 Hz, 21 pulses, 0.2 ms) used to induce a protein synthesis-independent early-LTP in apical dendrites yielded a long-lasting LTP in basal dendrites. A protein synthesis-independent early-LTP lasting 2-3 h was obtained by determining an intermediate tetanization protocol consisting of 100Hz, 14 pulses and 0.2 ms. We also studied the pharmacological properties of LTP in basal vs. apical dendrites. We could show that similar to apical dendrites, basilar LTP was dependent on NMDA-receptor activation, protein synthesis and D1/D5-receptor activation. Moreover it was found to be dependent on cAMP-dependent protein kinase A (PKA). These general studies of LTP and its properties in basal dendrites not only allows us to study the properties like synaptic tagging and trans-compartmental tagging, but it also helps us to understand the information transfer between various inputs in hippocampal neurons.

# BDNF dependent synaptic plasticity in organotypic hippocampal slice cultures.

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The protein family of neurotrophins consists of nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], neurotrophin-3 [NT-3] and neurotrophin-4 [NT-4]. The neurotrophins are secreted neuronal proteins modulating survival, differentiation and synaptic plasticity of CNS neurons. They are all expressed at different developmental stages in hippocampal neurons and are considered to play an important role in activity-dependent synaptic maturation and plasticity in the hippocampus. Especially one neurotrophin, BDNF, has been shown to be a messenger in long-term potentiation [LTP] of hippocampal synaptic transmission, and high frequency stimulation of glutamatergic synapses triggers synaptic secretion of BDNF. However, anterograde and retrograde signalling of BDNF in hippocampal LTP are discussed controversially.
We now have devised an experimental setting to investigate, at the single cell level, the role of postsynaptic BDNF in synapse maturation and LTP: hippocampal slices from newborn (P3-P5) BDNF k.o. mice were maintained in Stoppini-type slice cultures. Using single cell electroporation in the loose patch configuration of the patch clamp technique, selected single postsynaptic CA1 pyramidal neurons were transfected with BDNF-GFP cDNA at 16 DIV. Green-fluorescent BDNF vesicles were observed in the somatodendritic compartment of these CA1 pyramidal neurons, starting 8 hrs post-transfection.
Long-term potentiation was established in the slice cultures by pairing presynaptic theta burst stimulation (6 bursts (interval 200 ms) consisting of 4 pulses at 100 Hz) with postsynaptic depolarization to -10 mV. After 30 min, evoked epsc amplitudes were increased to 175 ± 72% (mean ± sem) of non-potentiated controls (n>8 cells per group). This paradigm did not induce LTP in similarly cultured slices from homozygous and heteroygous BDNF k.o. mice, indicating establishment of BDNF-sensitive LTP under our conditions. However, re-addition of BDNF to postsynaptic neurons in these BDNF deficient slices lead to a partial recovery of LTP, selectively in the BDNF expressing neurons.
These results suggest a role of BDNF as a retrograde messenger in activity dependent plasticity at Schaffer collateral synapses.

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# The actin filament depolymerizing factor cofilin1 is a major player of activity induced actin cytoskeleton dynamics in dendritic spines

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Dendritic spines are dynamic postsynaptic structures that show high plasticity during development and in response to synaptic activity. The actin cytoskeleton is the basic structural component of dendritic spines and is regulated by a variety of actin binding proteins. These molecules are important for changes of denritic spine morphology and hence are thought to be implicated in memory acquisition.
Cofilin1 is an actin filament depolymerizing factor enriched in the head region of dendritic spines. We hypothesize that it is crucial for regulating spine morphology and proper brain function. To address this question we have generated a conditional mouse mutant in which the cofilin1 gene deletion is restricted to the forebrain and occurs only during postnatal development. Electron microscopy studies revealed striking differences in dendritic spine morphology and electrophysiologial characterization of the hippocampal CA1 region demonstrated defective synaptic function, including decreased long-term potentiation. Adult mutant mice exhibited severe deficits in learning and memory tasks such as the Morris Water Maze or the Contextual Fear Conditioning. Taken together our data demonstrate the importance of cofilin1 for dendritic spine morphology and brain function.

# Magnetic stimulation of one dimensional neural cultures *in-vitro*

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Magnetic stimulation of nerves is attracting increased attention recently, as it has been found to be useful in therapy of neural disorders in humans. In an effort to uncover the mechanisms of magnetic stimulation we apply magnetic stimulation on *ex-vivo* neuronal preparations. Preliminary work on sciatic nerves demonstrated the dependence of magnetic stimulation on neuronal morphology and in particular the importance of curvature of axonal bundles[1]. A more recent work demonstrates the first magnetically evoked activity in cultures. We shall show how magnetic pulses initiate neuronal activity in 1D patterned cultures of hippocampal neurons, and explore the effects of properties such as geometry of the system, axonal morphology, pharmacology and neuronal density on the threshold of magnetic stimulation. We shall also describe our findings regarding the effect of repetitive magnetic stimulation on neuronal activity.

1. Rotem, A. & Moses, E. IEEE Trans Biomed Eng. 53(3), 414-20 (2006).

# Presynaptic HCN channels modulate NMDA receptor-dependent synaptic plasticity in the immature rat medial perforant path

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels play a major role in determining the resting membrane potential and neuronal excitability. Recent evidence for presynaptic HCN channels at the immature medial perforant path axon terminal, however, raises the intriguing question whether they may be involved in the development of synaptic plasticity at these synapses. Here we investigated the effect of HCN channel blocker ZD7288 on the induction of N-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (LTP). Both stimulation and recording was performed in the middle (mML) and outer molecular layer (oML) of the dentate gyrus in slices prepared from immature (P10-15) versus adult (P50-80) male Sprague-Dawley rats. Although long-term application of the HCN channel blocker ZD7288 at low concentrations (10 µM) did not significantly depress field excitatory postsynaptic potentials (fEPSPs), we performed interleaved control experiments inducing LTP with the same stimulation protocol, but in presence of the NMDA receptor blocker D-AP5 (50 µM). Thus, we were able to extract the NMDA receptor-dependent component of LTP at these synapses. In the mML of immature rats, HCN channel blockade with ZD7288 significantly enhanced the NMDA receptor-dependent LTP from 120 ± 8% (fEPSP slope after 60 min compared to pre-tetanus values) to 165 ± 13% ($P < 0.05$). In the mML of adult rats, however, LTP was not altered by administration of ZD7288, but it was indistinguishable from LTP levels obtained in immature rats without HCN blockade by ZD7288. In the oML of immature rats, LTP was again unchanged by ZD7288 application. These results suggest that presynaptic HCN channels may play a crucial role in the developmental regulation of NMDA receptor-dependent synaptic plasticity at the medial perforant path-granule cell synapse.

# Hyperpolarization-activated Cation Channels Impair DHPG-induced LTD in Rat Hippocampal CA1 Region

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The hyperpolarization-activated cation current ($I_h$) is a non-inactivating depolarizing current which is activated by membrane hyperpolarization and regulated by cAMP. $I_h$ channels are widely expressed in hippocampal pyramidal cells and inhibitory interneurons, and play a fundamental role in determining the resting membrane potential and regulating the synaptic function. We investigated the involvement of I in the basal synaptic transmission and the induction of (*RS*)-3,5-dihydroxyphenylglycine-induced long-term depression (DHPG-LTD) at the hippocampal Schaffer collateral-CA1 synapses by using field excitatory postsynaptic potentials (fEPSP) recording techniques. Both stimulation and recording were performed in the CA1 stratum radiatum of hippocampal slices prepared from 2-3 month-old male Sprague-Dawley rats. Continuous application of the $I_h$ channel blocker ZD7288 (10 µM) caused a substantial increase of the CA1 fEPSP amplitude accompanied by a significant decrease in paired-pulse facilitation (PPF) using interstimulus intervals of 10-200 ms. As $I_h$ channels are abundantly expressed on inhibitory interneurons in the CA1 region, we asked whether the significant decrease in PPF might be due to an altered GABAergic inhibition via interneuronal $I_h$ channels. Application of the specific $GABA_A$ receptor antagonist gabazine strongly reduced PPF ratio, however, when it was coapplied with ZD7288, no further reduction of PPF values were observed. We next examined the effect of ZD7288 on DHPG-LTD. Following a brief application of DHPG (100 µM, 10 min), the CA1 fEPSPs were strongly depressed resulting in stable LTD after 60 min of wash-out (69 ± 4% of control). In marked contrast, administration of ZD7288 significantly increased the DHPG-LTD (41 ± 8% of control, $P < 0.05$), indicating an impairment of DHPG-LTD by $I_h$ channels. To identify the site of ZD7288 action on LTD enhancement, we calculated the PPF ratio (interstimulus interval 40 ms) following DHPG-LTD normalized to interleaved experiments without DHPG. At the end of the experiment, the control PPF ratio was 107 ± 1% compared to 136 ± 3% in bath solution containing ZD7288 ($P < 0.05$). Taken together, our results suggest a presynaptic modulation of $I_h$ channels on Schaffer collateral terminals rather than those on inhibitory interneurons in basal synaptic transmission. Furthermore, $I_h$ channels also seem to impair DHPG-induced LTD by a presumably presynaptic mechanism.

# Poster Topic

## T28: Learning and memory II: Cognitive learning and memory systems

**T28-1A** Dopamine differentially affects the input-output relationship of layer 5 pyramidal neurons in the prefrontal cortex
*K. Thurley, W. Senn and HR. Luescher, Bern (CH)*

**T28-2A** Learning dependent gene expression of NCAM 180 in the Hippocampus of laying hens kept in different housing conditions
*A. Grund, I. Meier, L. Phi van and S. Petow, Bielefeld, Hamburg and Celle*

**T28-3A** Hippocampus dependent one trial passive avoidance learning in chickens (Gallus gallus domesticus)
*ET. Krause, M. Naguib and S. Petow, Bielefeld and Celle*

**T28-4A** Pigeons and Pikachu: Failure to Learn New Category
*R. Adam, M. Manns and O. Güntürkün, Bochum*

**T28-5A** Behavioural and pharmacological characterization of rats selectively bred for deficient sensorimotor gating
*M. Dieckmann, S. Klein, M. Koch and K. Schwabe, Bremen and Hannover*

**T28-6A** Effects of high frequency tactile stimulation on sensory and motor performance in younger and older adults
*AF. Knop, C. Voelcker-Rehage, M. Babanin and B. Godde, Bremen*

**T28-7A** Processing "movies" in neural nets using memory-strings
*T. Kromer, Ertingen*

**T28-8A** Dissimilarity of firing rate patterns suggest population coding of visual objects in macaque prefrontal cortex
*MHJ. Munk, ES. Städtler, G. Pipa, LF. Muckli and R. Goebel, Frankfurt/Main and Maastricht (NL)*

**T28-9A** Assessment of working memory by repetitive application of the running wheel-based Motor Skill Sequence (MOSS)
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*A. Lex and W. Hauber, Stuttgart*

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*B. Lex and W. Hauber, Stuttgart*

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**T28-8C** The Role of the Posterior Parietal Cortex in the Representation of Continuous and Discrete Quantities
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*B. Munivrana, Zagreb (Croatia)*

# Dopamine differentially affects the input-output relationship of layer 5 pyramidal neurons in the prefrontal cortex

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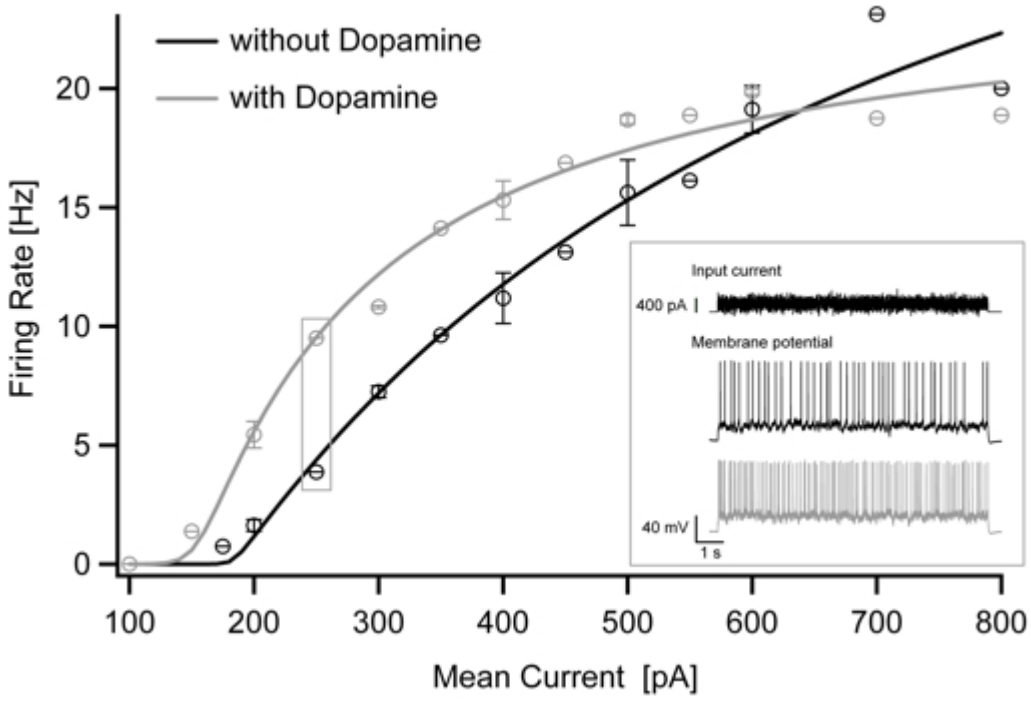
Prefrontal cortical activity plays a primary role in working memory; the mental ability to transiently store and manipulate information to guide forthcoming actions. Stored information can, for example, be the representation of a sensory stimulus or a memory item retrieved from long-term memory, or maybe a combination of both. Working memory is supposed to be represented by persistent neural activity in the prefrontal cortex (PFC), which outlasts the stimulus and enables a delayed response [Goldman-Rakic (1995) *Neuron* **14**]. Such persistent activity is hypothesized to be sustained by synaptic reverberation in an excitatory recurrent neural circuit [Wang (2001) *TINS* **24**], as it is composed of the pyramidal neurons in the PFC. In addition, *in vivo* animal experiments revealed that dopamine (DA) input to the PFC is critical for the regulation of working memory properties [see e.g. Seamans & Yang (2004) *Progress in Neurobiology* **74**].

To understand the actions of DA in the PFC at cellular level, we studied the influence of DA onto the activity of single layer 5 pyramidal neurons in the PFC *in vitro*. Using gaussian input currents to emulate realistic *in vivo* like input (see figure), we determined the input-output response function (f-I curve) of the pyramidal neurons in absence and presence of DA. The approach revealed excitability increase as well as decrease by DA depending on the amount of input current: The response and gain of the cell increases for small input currents after DA application; for large input, however, the response frequency decreases (see figure).

Inspired by our experimental results, we performed computer simulations in order to understand how the effect of DA onto single neuron activity translates into the behavior of the recurrent network of PFC pyramidal neurons. The experimentally determined f-I curves where fitted with an integrate-and-fire neuron model, which then was used to simulate a network of recurrently connected neurons. The simulations predict that under DA the emergence of persistent activity in the network is facilitated and becomes stabilized against distracting inputs compared to the DA free state.



# Learning dependent gene expression of NCAM 180 in the Hippocampus of laying hens kept in different housing conditions

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The mechanisms of synaptic plasticity can be investigated by learning-experiments with animals. Synaptic plasticity plays an important role in the processing of different environmental stimuli. The here used SDA (Step-down-avoidance-test) learning-test is a passive avoiding-test, whereby the learning-event can be scheduled exactly. The chickens have to learn that they will be punished with a mild footshock, when they step down from a platform. During the learning in the SDA test several brain areas, especially the hippocampus, are activated and can afterwards easily be analysed.In our case, the SDA test is used to examine gene expression of chicken that were kept under different housing conditions.The gene expression of the cell adhesion molecule NCAM180 was investigated. NCAM180 belongs to the best studied molecules and is highly conserved amoung different species. For NCAM180 it could be shown on protein level that it is involved during learning processes. Therefore RNA expression is expected to be deregulated up to six hours after the learning event. The RNA level of NCAM180 was verified by real time PCR experiments. We could show that the NCAM180 expression level differs between the housing conditions of the tested chicken. Within one housing group different NCAM180 RNA expression levels could be detected in the right and left hippocampus. These expression patterns correlate with the learning performance of these chickens.

# Hippocampus dependent one trial passive avoidance learning in chickens (Gallus gallus domesticus)

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Learning tests are common behavioural experiments, to measure the ability of animals to adapt their behaviour depending on previous experience. Especially in laboratory animals such as rats and mice, one trial learning test are commonly used. In many cases such tests are used to answer specific questions on the involvement of specific brain regions in the one trial learning. For rodents it was shown that the Step Down avoidance test (SDA) is an appropriate and hippocampus dependent one trial passive avoidance test. The SDA involves learning not to step down from a safe platform in order to avoid a mild foot shock. In our study we have shown that the SDA is also an appropriate one trial passive avoidance test for usage with birds, i.e. chickens (Gallus gallus domesticus). Moreover, using intrahippocampal injections (AP5 and Ibotenic Acid treatment), we show that learning this one trial passive avoidance task is hippocampus-dependent in chickens. These results enable to use the SDA to investigate further questions on learning, in the avian hippocampus and its underlying molecular processes as well as effects of housing systems on learning.

# Pigeons and Pikachu: Failure to Learn New Category

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Humans have concepts, meaning the ability to generalize within a class of stimuli and to discriminate between the classes. Many studies have shown that pigeons are capable of discriminating natural categories such as 'Human', 'Fish'. The learning was amazingly fast: pigeons need few sessions to establish the category 'Human'. Pigeons were shown to discriminate and generalize artificial stimuli like aerial photographs of 'Man-made objects' and polymorphous stimuli with defined dimensions and their features. However, it is unclear to what extent pigeons are really able to establish abstract concepts or even categories. To address this problem, we used a novel complex and artificial category, with no previously functional relevance for the birds to prevent embedded conceptual knowledge. We chose the category 'Pikachu', the main character in Pokémon, a kids fantasy world created by Nintendo©. Go stimuli were characterized by the presence of the character 'Pikachu'. Other Pokémon characters appeared in Go's and NoGo's. They appeared in various sizes and angles, and could be only partially shown. To prevent discrimination based on a single component, changes to 'non-Pikachu' characters were done, like coloring them in the yellow color of 'Pikachu'. Four pigeons were trained in a standard Go-NoGo task. In each session a mean of 20 Go's and 20 NoGo's were drawn randomly from a larger set. The traditional rho value was used to compare Go versus NoGo performances. 48±20 sessions were required until reaching the criterion of rho=.85 in three consecutive sessions. A transfer test examined the subjects' ability to generalize beyond the known examples. In each transfer session 12 new transfer stimuli were embedded in 40 old training stimuli. Two transfer procedures were conducted. Two pigeons had 5 transfer sessions with 64 new non reinforced, repeated transfer stimuli. The other birds were trained in six sessions with a total number of 68 reinforced and thus unrepeated transfer stimuli. The mean performances for the transfer stimuli were rho=.694±.15, .581±.17 for the two groups respectively, and hence below the criterion. The performances for the training stimuli were all above criterion: rho=.967±.05, .978±.01 for the two groups respectively. This indicated that categorization of 'Pikachu', as a novel artificial and complex 2D category was not achieved although the animals could memorize a larger number of highly complex stimuli. Past exposure as well as past functional relevance could play a role in previous categorical learning by pigeons. An open question is how crucial the language component is to novel concept formation and discrimination.

# Behavioural and pharmacological characterization of rats selectively bred for deficient sensorimotor gating

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Sensorimotor gating, measured as prepulse inhibition (PPI) of the startle reflex, is reduced in several psychotic disorders, such as schizophrenia, Tourette syndrome and attention deficit/hyperactivity disorder. To model these gating deficits, we selectively bred rats for either high or low PPI. For this purpose Wistar rats were tested for their PPI-level and the two females and males with the highest or lowest expression of PPI were selected as breeders. Offspring of these rats (F1-F5 generation) was tested for PPI as adults and rats with the highest or lowest PPI, respectively, were again selected for subsequent breeding. This breeding procedure resulted in two lines that significantly differed in their PPI-level (low group and high group).

Previous studies using a 4-arm baited 8-arm radial maze task implied that compromised function of the low group was not caused by impairment of learning or memory, but rather associated to the ability to switch between two different strategies during spatial learning. Therefore, we here tested this specific behavioural function. Rats were first trained in the radial maze for an egocentric (response) strategy by constantly baiting the arm next to the start arm. Thereafter, the rats were trained for an allocentric (place) strategy by baiting the same arm, independent from the start arm. In the test we switched between the two strategies. Although rats learned the two different strategies without difference, the low group was impaired in switching from a response to a place strategy. We subsequently confirmed previous results of undisturbed memory function by specifically testing this feature in a continuous delayed alternation T-maze task. Again, we found no differences between the high and the low group.

We tested for selective attention and social withdrawal in a behavioural paradigm based on novelty discrimination in a social context. An unfamiliar juvenile rat was presented to an adult rat for a period of 30min (P1). A second (novel) juvenile rat was then introduced at the end of P1 for a period of additional 5min (P2). The ability of the adult rat to discriminate between two juveniles, presented at the same time, was evaluated by measuring the ratio of the time spent with both juveniles during P2. No difference in selective attention was found between groups. However, during this task, the social behaviour of rats can be tested by evaluating the social interaction with the first juvenile rat in P1 measured as grooming, licking, and play behaviour. We found that rats of the low group were impaired for social interaction.

Together, our findings indicate that rats selectively bred for high or low PPI show concomitant features of psychiatric disorders such as compromised behavioural flexibility and social impairment. The social withdrawal found in the low group is particularly interesting, since so far only few animal models exist to test for negative symptoms of psychiatric disorders. Thus, rats selectively bred for high or low PPI may be useful to investigate the pathophysiological mechanisms underlying certain psychiatric disorders with special emphasis on negative symptoms.

# Effects of high frequency tactile stimulation on sensory and motor performance in younger and older adults

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Many activities of daily life such as eating and opening containers require the precise control of grasping forces. It is assumed that an important mechanism responsible for precise force control is tactile sensitivity (Cole, 1991; Gordon et al., 1991). However, also contradictory results exist (Cole et al. 1998) and the neural control mechanisms responsible for precise manipulation of grasping forces are largely unknown.

We examined the link between motor and tactile domains by applying a tactile training paradigm that has recently been introduced by Ragert et al (2005). They have shown that high frequency tactile stimulation (tHFS) profoundly improves tactile acuity.

We used a similar protocol of tHFS with a mean frequency of 20 Hz. Younger (18-28 years of age) and older (65-75 years of age) right handed participants were randomly assigned to an experimental and a control group. They performed a pre-test, 30 minutes of high frequency tactile stimulation on the tips of their left index finger and thumb, and a post-test. Control group received no stimulation. In pre- and post-test, participants performed two motor tasks (unimanual isometric precision grip task using a Mini Model force transducer and Purdue Pegboard test) and two tactile tasks (spatial discrimination of grating orientation and frequency discrimination in the flutter range) with their stimulated fingers.

Results indicated that tHFS significantly improved tactile discrimination of the left index finger, whereas motor performance as assessed with the pegboard task was impaired. These effects were found for both age groups with similar effect sizes even though absolute levels of performance in all tasks were lower for older as compared to younger adults.

Our results suggest that reorganization of the somatosensory cortex presumably induced by the applied protocol of passive tactile stimulation not only promotes sensory performance but also significantly affects fine motor control of the respective fingers. Moreover, our study reveals the remaining plastic capacity of the older brain.

*Cole KJ (1991) J Motor Behav 23:251-258*
*Cole KJ, Rotella DL, Harper JG (1998) Exp Brain Res 121: 263-269*
*Gordon AM, Forssberg H, Johansson RS, Westling GT (1991) Exp Brain Res 83:483-488*
*Ragert P, Kalisch T, Dinse HR (2005) SFN Abstract 173.6*

# Processing "movies" in neural nets using memory-strings

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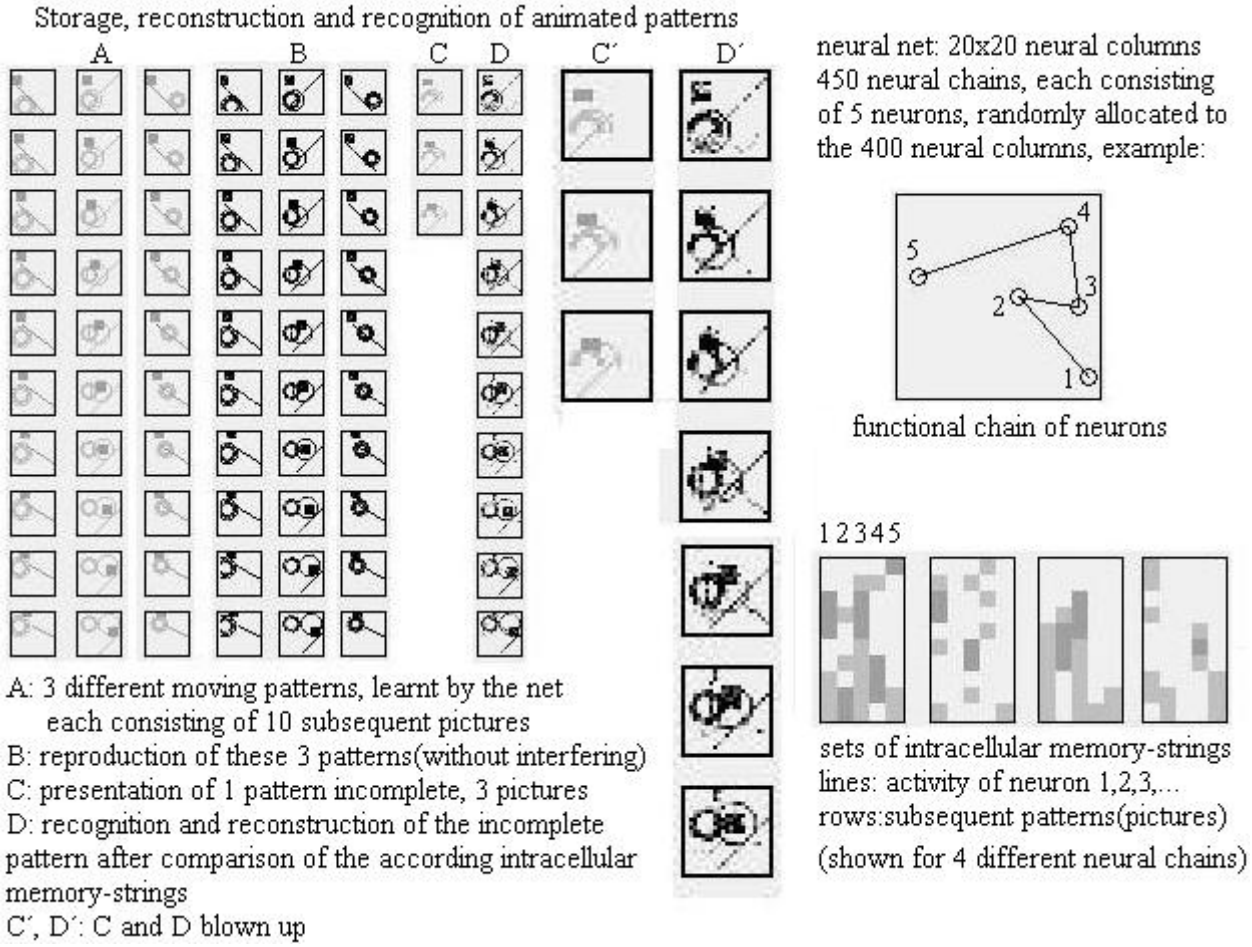
Introduction: It may be of interest to study models of neural nets, which are based on other paradigms of learning than synaptical changes. Neural nets using memory-strings to process sequences of pictures shall be shown.
Methods: 450 chains, each with 5 neurons, cover a net with 20x20 neural columns. Randomly allocated, 5-6 neurons of different neural chains belong to each column(fig.1). Any pattern presented to the net will cause specific activity in the 5 neurons of the chains. Neuron 1 will get by projection from neuron to neuron step by step this sequence of activity. The temporal sequence of incoming activity will be recorded by neuron 1 in form of a memory-string, representing the engram of long-term memory and may be used to reproduce the pattern by reversing this procedure(1,2). 10 subsequent simple pictures with moving geometrical patterns("movies") are presented to and learnt by the net. Then 3 incomplete pictures of one "movie" are presented. The according memory-strings are formed and compared to those strings, learnt earlier. Any storage neuron will choose the best fitting string to reactivate its neural chain.
Results: The net is able to store, compare, recognize and reproduce sequences of changing patterns of in principle unlimited length quite efficiently(fig.1).
Conclusion: In a hypothetical biological model(1,2), particularly nucleic acids as engrams of long term memory might encode temporal sequences of activity, (each base triplet representing different degrees of activity). The demonstrated efficacy should justify further efforts to develop those nets and to prove their biological relevance.

(1)T Kromer, "New Neural Nets",Lecture Notes in Comp. Sci., 2001, Vol. 2206, 772-781
(2)T Kromer, "Tomography in Fractal Neural Nets",Lecture Notes in Comp. Sci., 2001, Vol. 2206, 917-923



# Dissimilarity of firing rate patterns suggest population coding of visual objects in macaque prefrontal cortex

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The storage of visual information in short-term memory requires the temporary stabilization of neuronal representations of objects. The proposed neuronal mechanisms range from sustained firing of individual 'smart' neurons to highly distributed neuronal assemblies that could constitute by synchronizing reverberating activity. The large variety of described memory-related response patterns in prefrontal neurons supports the hypothesis that different neuronal populations are involved in the storage process. Here we investigated distributed patterns of cortical activity in lateral prefrontal cortex based on firing rate modulation. Although information coding by neuronal populations is an active field of research, there have been few direct demonstrations that information represented by groups of simultaneously active neurons exceeds the information that can be retrieved from multiple single sites, even if they are recorded simultaneously. We analyzed multi-unit activity from a 4x4 grid of microelectrodes in lateral prefrontal cortex of two macaques performing a visual short-term memory task. Significant modulations of activity for each site were detected when the firing rate exceeded a 1%-criterion of firing-variability during a pre-stimulus period. 78% of 158 sites showed task-related activity, 24% were influenced by the stimulus (ANOVA $p<0.01$) and 6% by the identity of the specific object (Scheffé $p<0.01$). Stimulus selectivity and specificity of distributed population activity were assessed by resampling of 100 bootstrapped binary activity patterns, computing the distribution of dissimilarity indices between original and scrambled sets of trials, and assessing differences at a significance level of 1% after correcting for multiple comparisons. Data from each recording session (n=12; mean number of trials: 597) showed significant results for both selectivity and specificity. For patterns involving seven sites, the best sliding window revealed 39% of pair wise comparisons of 20 objects as significant, while the best case involving 10 sites revealed 75%. When we systematically compared results for selectivity and specificity among single sites and patterns of multiple sites, we found a number of sessions without specificity at single sites, but clearly preserved information in activity patterns across sites. Although across all experiments, the percentage of significantly modulated single sites was correlated with the percentage of significant patterns during all task epochs, this correlation was strongest during the delay. Considering the relative contribution of multiple sites to a population code based on firing rates reveals that the population can provide more information about the stimulus than multiple single sites, even when they are recorded simultaneously. We conclude that comparatively small neuronal populations in prefrontal cortex may dispose of more information than predicted from single site analysis.

# Assessment of working memory by repetitive application of the running wheel-based Motor Skill Sequence (MOSS)

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## Background

Motor learning is an important compensatory mechanism in neurological diseases, such as stroke, multiple sclerosis or neurotrauma. Moreover, motor learning may become impaired during the progression of Alzheimer's disease. Several methods have been developed in rodent models to test motor and memory skills of various neurological disorders. However, assessing motor learning in mice still remains a challenging task.

## Aim

The running wheel-based motor skill sequence (MOSS), has successfully been used in displaying central motor skill deficits in the cuprizone mouse model of multiple sclerosis (Liebetanz and Merkler, 2006). These experiments suggest that MOSS may also determine motor learning capacity in the mouse. Subsequently, we tested the potential of a repetitive version of MOSS (rMOSS) in quantifying motor learning capacity of mice.

## Methods

MOSS analyses voluntary wheel-running behaviour on a sequence of two different types of running wheels: a simple running wheel (SRW) with regularly spaced crossbars and a complex wheel (CRW) with irregularly spaced crossbars The latter wheel design was chosen to make wheel running more difficult in such a way that improved running abilities would require motor skill learning on a supraspinal (i.e., cortical) level. After two weeks training on a SRW, 3 groups of mice were placed on a CRW for one week. After an initial drop, mice usually learn to increase maximum running speed (Vmax) over the next 5 days (learning phase). Mice were then placed on a SRW for 3, 7 or 14 days, after which they were tested on the CRW for a second time (recall). In a fourth group, this cycle was repeated continuously throughout the duration of the study.

## Results

All 3 groups (the time-points depict the animal groups) showed a similar learning curve, when placed on the CRW for the first time. In the recall phase, the 3 day interval group showed nearly identical performance compared to the first cycle (Vmax = 73.8%), while the 7 day group showed a moderate recall (Vmax = 57.1%). However, the interval prolongation to 14 days resulted in low memory recall (Vmax = 33.9%).

## Conclusion

This study showed that applying rMOSS is effective at quantifying motor learning capacity in mice. In normal C57/Bl6 mice, an interval of 7 days revealed optimum for long-term follow-ups of motor learning. Subsequent use of rMOSS will become invaluable, when assessing memory-altering effects in mouse models of various neurological diseases.

# Stimulus modality differentiates human and non-human timing and memory of rhythmic light and tone signals

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Humans and non-human primates engage in behaviors that critically depend on their ability to accurately time and remember the duration of sensory events. In order to perform accurate timing of signal durations, memory processes are of importance. They include working memory which keeps track of the timing process, retrieval of internal representations of temporal patterns learned by prior experience, and a mechanism which compares current working memory values to these representations. Several studies of human temporal coding revealed more accurate timing of auditory signals in a rhythmic series of auditory events, compared to signals presented in the visual modality. This modality effect indicates a reduced ability to make use of temporal information contained in visual stimuli. Several authors relate this effect to the phonological processing loop of human working memory, which is not only considered to be involved in processing of verbal information, but might also be involved in coding rhythmic sequences of sensory events. If this notion is correct, the phonological loop might prefer auditory rhythms and trigger a translation of visual rhythms into acoustic information. Variability in the beginning or ending of this translation should induce temporal uncertainty, and consequently reduce the accuracy of timing in the visual domain. Here we test these assumptions, by conducting joint behavioral timing experiments on linguistic human and non-linguistic non-human primates. Recent studies revealed remarkable similarities between primate vocal behavior and human speech. However, monkeys lack the requirements for meaningful speech processing, and their vocal development is more innate and hardwired than that of humans. If the modality effect of timing depends on language-shaped processes, monkeys are expected to show a minor auditory dominance and less visuo-acoustic translations. We trained human and non-human subjects to remember the duration of rhythmic signals presented simultaneously in the visual and auditory modality. Within this rhythmic series of events subjects had to indicate the presence of a target-signal of longer duration. Modality-specific processing capacities were measured by testing either visually, acoustically, or simultaneously in both modalities (crossmodal). Whereas humans showed a superior ability of timing tone targets, monkeys were more accurate when timing visual events. The ability of timing visual events did not differ between both species. In humans, crossmodal performance was comparable to their ability of timing auditory signals, whereas monkeys showed a crossmodal performance similar to their performance in timing visual events. These results suggest that ambient linguistic experience alters the brain's strategy and processing capacities of making use of temporal information contained in auditory stimuli. However, there is no evidence that a translation of visual events into acoustic information underlies modality effects of timing in a rhythm task in human and non-human primates.

# Perceiving learned action effects as investigated by fMRI

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**Introduction**

According to the ideomotor principle, performing a certain movement and perceiving its consequences leads to an integration of the corresponding motor and perceptual codes. Furthermore, as learned associations between actions and action effects are expected to be bidirectional, perceiving or imagining action effects should lead to a backward activation of the action itself. The current study investigated behavioral and neural correlates of perceiving learned action effects. Particularly, we sought to test three hypotheses: (A) Perceiving learned action effects facilitates the movement that previously caused the perceived action effect. (B) Perceiving learned action effects interferes with movements that are different from the one that previously caused the perceived effect. (C) Perceiving learned action effects activates brain areas that are involved in planning and performing the movement that previously caused the perceived effect.

**Methods**

The experimental procedure comprised two phases. During the acquisition phase, subjects performed a simple go/no-go task, during which they carried out free-choice button presses (left or right), while each button press produced a particular auditory effect (high or low pitch). In the subsequent test phase, subjects underwent fMRI while they were presented with circles which they had to classify according to their color by either a left or right button press. Before responding, subjects had to wait for an auditory start signal, consisting of one of the previous effect tones, or a third medium-pitch tone. With respect to the previous acquisition practice, tones could be either compatible, incompatible, or neutral (no effect tone) with the response to be given. Furthermore, we created no-go trials both with and without auditory start signal.

**Results & Discussion**

The fMRI data clearly indicated that perceiving action effect tones in the current study is different from perceiving neutral tones. Figures 1-3 depict and denote significant neural activations related to the perception of incompatible and compatible tones during go trials as well as, regarding no-go trials, activations related to the perception of tones associated with a left-hand response. Activations during go trials (e.g. temporo-parietal, inferior frontal, premotor, primary auditory) can be interpreted as reflecting effects of motor inhibition / facilitation by incompatible / compatible tones. Furthermore, activations during no-go trials (e.g. temporo-parietal, right premotor, primary auditory, cerebellum, hippocampus) may reflect response activation by tones associated with a left response. Tones associated with a right response (vs. neutral tones) during no-go trials, on the other hand, revealed no significant activations. The latter finding suggests a basic asymmetry in ideomotor learning that, however, needs further investigation.

**Conclusion**

The study provided evidence for the ideomotor principle and thereby raised the question whether ideomotor learning is influenced by the side of motor action and the agent's handedness.

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# Chaining Actions in a Sequence of Tasks

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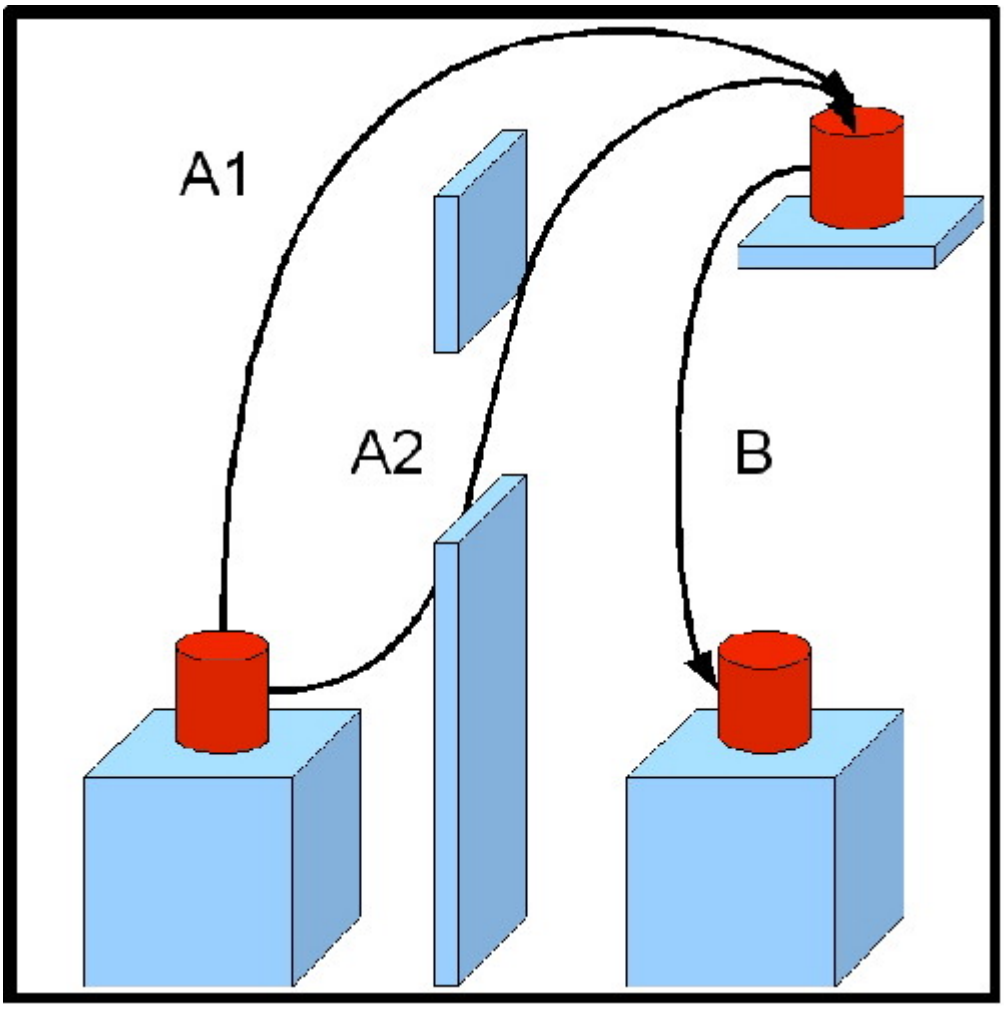
Humanoid robots have been able to learn and perform well defined tasks such as reaching, tracking or manipulating objects. The world that these robots interact with is highly structured. However, if a robot was to be designed to function in a real world environment, for instance a kitchen, like in the European PACO-PLUS-project, several issues like perception, learning and control have to be tackled. Such a robot will have to be highly adaptive in order to learn and adjust in an unstructured environment. One possible way to organize control is to introduce a dynamic, hierarchical set of actions that can adapt to new high-level tasks. In this work we examine, in how far humans rely on such a hierarchical structure, especially how actions are chained when solving a sequence of tasks.

For our experiments we use a manipulandum (PHANTOM Premium 3.0/6DOF) that serves as a haptic interface for a virtual environment. The manipulandum has a freely moving arm over which forces can be applied to the user in such a way that surfaces of 3D objects and their dynamical behaviour can be felt.

A test person is solving a simple task like the one sketched in the figure: The cylindrical object is picked up from the left side and dropped to the platform on the top right via either way *A1* or *A2*; from there it is then transported to the lower right box (*B*). In this context, we examine how the choice of action is affected by the difficulty of each alternative (i.e. gap size for path *A2*). Furthermore we inquire whether the choices of previous actions (*A*) influence the character of later actions (*B*).

These experiments are combined with a measurement of the test person's attention by tracking the eye movement during the task for to give further insight into the planning and execution of complex actions.



# Mammalian ependymin-related proteins (MERPs) in the mouse brain

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Ependymins are secretory goldfish brain glycoproteins synthesized by leptomeningeal fibroblasts. Except short regions homologous to N-CAM, laminin, fibronectin and tubulin, they exhibit no homology to any known protein. Piscine ependymins exist in N-glycosylation variants (in goldfish: 37, 31 and 29 kDa, respectively). They bind calcium and serve extracellular calcium homeostasis, supposed to be of crucial importance for the proteins´ participation in CNS plasticity during memory formation.
Recently, ependymin related proteins were found in invertebrates and amphibians. Genes of mammalian ependymin-related proteins (MERPs) were identified by reverse transcription differential display in mouse (mMERP1 and 2), monkey and in humans (huMERP1). Alignment studies revealed that MERPs and fish ependymins contain 4 conserved cysteines and possess a signal sequence, typical of secretory proteins. Mouse MERPs are of similar length to fish ependymins and, despite low amino acid sequence conservation, the hydropathy profiles are remarkably alike, suggesting similar protein conformation and function.
In earlier immunological studies on mice and rats we observed ependymin-like proteins in the hippocampal formation and in neurones obtained from the embryonic hippocampus. In the present study we isolated proteins from the mouse brain that are recognized by anti-fish ependymin antibodies and share glycosylation properties and calcium binding characteristics with fish ependymins. We examined their localization in the adult mouse brain and during postnatal development of the hippocampus immunohistochemically.
In the adult we found specific signals in ependymal cells outlining the III. and IV. ventricle, in cells of the chorioid plexus, around pyramidal neurons of the CA1, CA2 and CA3 hippocampal subfields, and around granule cells of the dentate gyrus. Staining at the granule cells was prominent at the axon initial segments.
The intensity of the ependymin-like immunoreactivity in the granular cell layer of the dentate gyrus increased during postnatal development. This observation is consistent with our data obtained from experiments using the reverse transcriptase-polymerase chain reaction to measure MERP1 and MERP2 mRNA expression.

# Analysis of Nex function in the murine forebrain

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Nex (neuronal helix-loop-helix protein) is an atonal-related bHLH transcription factor, which is expressed in postmitotic pyramidal neurons throughout CNS development and in adulthood. In adult mouse brain expression is maintained in hippocampus proper, amygdala, entorhinal cortex, mammillary bodies and subiculum, i.e. areas implicated in memory formation and retrieval. Recent cell culture studies have shown that Nex promotes neurite outgrowth and regeneration in PC12 cells and facilitates neuronal survival via upregulation of anti-apoptotic genes.

Our aim is to investigate the function of Nex in neuronal survival and plasticity. It has been shown that exposure of animals to an enriched environment enhances neurogenensis and synapse formation. We are currently investigating the role of Nex in these processes by means of microarray analysis of hippocampal neurons of null mutant and wildtype mice that have been exposed to an enriched environment. The virtual lack of obvious motor or perception abnormalities in Nex null mutants also allows us to perform behaviour and memory tests, which will include classical fear conditioning, spatial learning and memory consolidation paradigms.

Our preliminary data indicate that the survival of Nex null mutant hippocampal neurons is impaired in culture. The transcription changes of potential Nex target genes will be assessed in vitro in acute brain slices. In a complementary approach we have generated a transgenic mouse line, in which expression of Nex can be induced by Cre-recombinase. This line will be used to investigate the potential effect of Nex overexpression in neuronal progenitors and postnatal pyramidal neurons.

# Inducible ablation of NCAM gene in the adult mouse brain causes deficits in formation and retrieval of contextual memory in a repetitive auditory fear conditioning paradigm

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Mice constitutively deficient in the neural cell adhesion molecule (NCAM) exhibit impairment in contextual and tone fear memories (Stork et al., 2000, Eur J Neurosci, 12: 3291-3306; Senkov et al., 2006, J Neurosci, 26(42): 10888-10898). To rule out the possibility that this cognitive deficit is due to developmental abnormalities in these mice, we took an advantage of the tamoxifen-inducible Cre-ERT recombinase transgenic system (Weber et al., 2001, Eur J Neurosci, 14: 1777-1783). This allowed us to inducibly and acutely ablate NCAM expression in the adult brain and perform within-subject comparison of learning and memory before and after NCAM ablation. We found that NCAM-floxed mice expressing Cre-ERT under control of the PrP promoter showed normal levels of NCAM and normal contextual memory on the 1st and 7th days after the first fear conditioning. However, after injection of tamoxifen for 5 consecutive days these mice exhibited a strong reduction in expression of NCAM and in contextual memory tested on the 14th and 21st days after the first fear conditioning, suggesting that acute NCAM ablation weakens preformed memories or their retrieval. As controls, we used vehicle-injected NCAM-floxed Cre-ERT expressing mice, or NCAM-floxed mice not expressing Cre-ERT, which were injected either with tamoxifen or vehicle. No difference between control groups was found in fear conditioning. When NCAM-floxed Cre-ERT expressing mice were repetitively conditioned (using a context and tone distinct from those used in the first fear conditioning) after induced ablation of NCAM, they showed a robust deficit in formation of contextual memory on the 1st and 7th test days. Retrieval and formation of amygdala-dependent tone memory was, however, not disturbed after NCAM ablation. Thus, our data suggest that induced ablation of NCAM in the adult mouse brain impairs contextual but not tone memory.

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# Musical long-term memory and its relation to emotions and psychophysiology

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What influence do emotions have on long-term memory for music? Music can elicit strong emotions (Sloboda, 1991; Panksepp & Bernatzky, 2002) and can be remembered, possibly in connection with these emotions, even years later. However, until now, the episodic memory for highly emotional music compared to less emotional music has not yet been examined.

We investigated whether emotionalle rated music is more easily retrieved from episodic long-term memory than less emotionally rated music. Furthermore, we examined the influence of musical structure on memory.

Method
Twenty-four non-musicians participated. In the first session, all participants listened to 40 excerpts of film music as targets. Half of the participants (emotion group) indicated valence and arousal induced by the music on a five-point rating-scale according to the valence-arousal model of emotions by Russel (1980). The other half (control group) estimated the length of the musical excerpts (detection group). In the second session, all participants listened to 80 music pieces (40 target excerpts randomly mixed with 40 distractor pieces) and were asked both to perform a recognition task and to indicate the valence and arousal induced by the music. Musical features, previously evaluated by experts as possibly influencing recognition were considered. To determine the degree to which emotions were elicited in the participants, heart rate, breathing rate, skin conductance level, and skin conductance reaction were measured during the presentation of each musical excerpt.

Results
In both groups, musical excerpts rated with high arousal and extreme (positive or negative) valence were recognized significantly better than pieces rated with moderate or low arousal and moderate valence. The emotion group showed better recognition performance than the detection group but both groups answered in a similar way concerning the relationship between emotions and memory. Musical features did not have a direct influence on recognition performance.

Discussion
Music pieces rated as emotional are more easily retrieved from memory. Both valence and arousal seem to be important in episodic long-term memory for music. Evidently, strong emotions related to the musical experience facilitate memory formation and retrieval. Focusing on the emotional rating of the music (emotion group) caused a more elaborate processing of the stimuli which in turn enhanced memory performance as compared to the detection group (who only attended global non-musical features).

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# Cellular properties of CA1 pyramidal cells during 200 Hz oscillations in hippocampal slices

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Sharp-wave ripple (SPW-R) complexes occur during slow-wave sleep in the mammalian hippocampus. They travel throughout the hippocampus and recruit a minority of pyramidal cells. It is thought that these activated cell assemblies represent the replay of recently formed memory traces. Information processing of pyramidal cells in CA1 during ripples at ~200 Hz requires a fast and precise output signal from few pyramidal cells and thus depends on a high signal-to-noise ratio. Using our model of SPW-R complexes in mouse hippocampal slices we tackled the question of how this computational task is brought about by single CA1 pyramids. Cells were divided into two groups according to whether they displayed ripple-associated firing or not.

Spontaneous or evoked action potentials (APs) outside ripples emanated from a slow EPSP and were followed by a fast and medium AHP. In contrast, ripple-associated APs were preceded by a presumably GABAA-receptor mediated fast hyperpolarization and arose abruptly from baseline. These spikes were followed by an afterdepolarization. Some participating cells displayed a notch on the ascending phase of the spike. Strong hyperpolarization of the cells could not abolish spiking during ripples but sometimes revealed partial spikes. The peak of the partial spike matched the potential of the notch in full-blown APs.

These observations point to a possible mechanism of AP timing: Rapid inhibition followed by a fast excitatory transient is known to be a crucial determinant of precise spike firing. This effect is mediated by de-inactivation of sodium channels which leads to a lower firing threshold. Indeed we found a significant difference in threshold between both spike types. The existence of notches and partial spikes indicates a dendritic or axonal spike origin. Such ectopic spikes are the fastest known excitatory transient invading the soma.

Firing outside ripple episodes was usually sparse in both cell types. We were never successful in eliciting a phase-coupled AP by depolarisation of non-participating cells. Surprisingly, depolarisation of SPW-R-coupled neurons did not enhance their firing rate during SPW-R. This finding points towards a high signal-to-noise ratio separating non-participating from participating pyramidal cells and allowing for action potential firing phase-locked to certain ripple cycles. The underlying mechanism appears to involve strong synaptic inhibition of pyramidal cells going along with SPW-R.

Unexpectedly we found that the input resistance of cells that exhibit ripple-associated firing is significantly smaller than that of cells which remain silent. This suggests that suprathreshold activity does only occur when CA1 pyramidal cells receive coincident excitatory input from the CA3 area. This mechanism may be especially important when input resistance decreases even further during ripples due to massive inhibitory input.

Hippocampal pyramidal cells are traditionally regarded as imprecise signalling devices. Our experiments challenge this view and propose cellular mechanisms that enable CA1 pyramidal cells to fire within ~200 Hz network oscillations in a highly precise and defined manner.

# Specificity of information transfer during sharp wave-ripple complexes

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Sharp wave-ripple complexes (SPW-R) occur spontaneously during slow-wave sleep in the mammalian hippocampus. During each SPW-R, a small subgroup of principal cells takes part in the network oscillation which travels through the different hippocampal subfields from CA3 to CA1 before propagation into the neocortex. It is suggested that these signals represent engrams of previously acquired spatial information (episodic memory). However, it is still not fully understood whether the local ensembles within different hippocampal subfields are triggered by a rather unspecific input or whether each local SPW-R depends on specific, temporally and spatially precise input patterns from preceeding regions. This question is yet more important as the axonal gap junction hypothesis predicts high frequency oscillations in an isolated axonal network of pyramidal cells (Traub et al., Neuroscience 92, 407-426, 1999).

In the present study we investigated spontaneously occurring SPW-R in CA1 of acute mouse hippocampal slices and compared them to field potentials that were evoked by electrical stimulation of different input and output pathways of the CA1 region. To compare the resulting field potentials we performed time-frequency (TF) analysis based on continuous wavelet transform.

Antidromic stimulation of the CA1 axonal plexus showed an initial population spike followed by a positive field potential. TF analysis revealed a clearly different frequency behavior compared to spontaneously occurring SPW-R with a dominant slow component. The lack of a high frequency component could not be overcome by repetitive stimulation at ripple frequency. In addition, the removal of the alveus close (~100 µm) to the pyramidal cell layer left spontaneously occurring SPW-R in the pyramidal cell layer largely unaltered.

As SPW-R are normally generated in CA3a/b and travel to CA1 via the Schaffer collaterals, we evoked field potentials by weak stimulation of the Schaffer collaterals. The evoked signals are strongly reminiscent of spontaneously occurring SPW-R. However, the exact timing between low and high frequency components was different to native SPW-R. Also, at larger stimulation strengths, the high frequency component vanished. Thus, simultaneous activation of a small portion of CA1 inputs via the Schaffer collaterals triggers network oscillations within CA1 which are similar, but not identical to native SPW-R.

Stimulation in stratum pyramidale of CA3b revealed evoked field potential close to the stimulation electrode with some similarity to spontaneously occurring field potentials in this area. These events were followed by SPW-R in the CA1 region. In contrast to the events evoked by Schaffer collateral stimulation, SPW-R evoked by stimulation in CA3 showed the full frequency characteristics of native SPW-R in CA1.

These results show that high-frequency network oscillations at ~200 Hz can be generated by unspecific activation of local networks in CA1. However, the full pattern of native SPW-R in CA1 does depend on a highly specific, temporaly organized input from a subgroup of pyramidal cells in CA3, likely through excitatory synaptic activation of a subset of CA1 pyramidal neurons.

# Compensatory hyperactivations as markers of latent working memory dysfunctions in obsessive-compulsive disorder

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Prior studies indicated that OCD is associated with deficits in working memory (WM), but the findings are inconsistent. This inconsistency could be explained by the ability of some OCD patients to compensate for disturbances in WM. To test this hypothesis we investigated 11 selected OCD patients, who did not present any deficits in WM, and 11 matched healthy controls during performance of a spatial and two verbal WM tasks which had been previously proven to reliably activate different domain-specific networks of WM. In each of the WM tasks, OCD patients activated the same set of brain regions as did the healthy controls. However, statistical comparisons between groups revealed significantly enhanced brain activation in OCD patients both during the two verbal tasks (left inferior frontal junction area (IFJ), left frontal opercular cortex, middle third of the left inferior frontal gyrus, and left/right intraparietal sulcus) and during the spatial WM task (left IFJ). These data support the notion that WM is dysfunctional in OCD by providing evidence that the patients need greater activation in regions known to underlie WM to maintain a similar level of performance as healthy controls during performance of verbal and spatial working memory tasks. The observed hyperactivations may allow the patients to compensate for latent WM dysfunctions and may thus serve as markers of such dysfunctions.

# Sleep deprivation facilitates the ‚learning' of express saccades

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Introduction. Saccades are visually triggered gaze shifts. Several cortical processing steps have to take place before the motor command is transferred to the brainstem and the eye muscles. The frequency distribution of saccadic reaction times shows at least two peaks: a narrow peak of fast saccades (express saccades, 100-130 ms) and a broader peak with regular saccades (about 180 ms). For express saccades it is assumed that one processing step (the disengagement of attention) has already elapsed when the saccade target occurs. Furthermore, it is known that learning of express saccades builds up within days of training and then persists for a long time period. In this study we investigate how sleep and sleep deprivation effects the learning of express saccades.

Methods. Twenty healthy subjects performed visually triggered saccades in a gap-paradigm (test0). Then they performed a saccade training of about 1000 saccades. Thereafter, 10 subjects slept in a sleep laboratory environment, the other 10 subjects stayed awake. After 8 hours, the gap-paradigm was repeated in the morning (test1) and one day later (test2). After 4 weeks this procedure was repeated, but groups were switched (the sleep group stayed awake and vice versa).

Results. Just after one single training session sleep deprivation facilitated the learning process of express saccades. In contrast the sleep group showed hardly any effect. The learning effect persisted over the 4 week interval. Then the former sleep group had to stay awake and now showed the same learning effect for express saccades, while the former awake group now had to sleep and could not benefit from the second training session.

Conclusion. Learning of express saccades can be enhanced by sleep deprivation already after one training session. Starting from the hypothesis that express saccades occur in a state of disengaged attention, we suggest that sleep deprivation reduces focused attention and increases the probability to be in a disengaged attention status. Consequently, the occurrence of express saccades is somewhat increased. This facilitates the learning process, which then persists across the second night of normal sleep.

# Audiovisual category transfer - an electrophysiological study in rodents

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The formation of perceptual categories by abstraction from particular stimulus features is an elemental constituent of cognitive processes. Our previous work in the rodent (gerbil) auditory system has demonstrated that the formation of auditory categories is accompanied by the emergence of identifiable spatiotemporal activity patterns in auditory cortex (Ohl, Nature, 2001). Here we address the question of whether perceptual categories formed in one sensory modality (audition) can be transferred to another sensory modality (vision).

We trained Mongolian Gerbils (Meriones unguiculatus) first to discriminate two different presentation rates ("slow" and "fast") of tone bursts using a footshock-motivated Go/(No-Go) paradigm (shuttle box). Subsequently, the same animals were trained to associate the learned Go or No-Go behaviours with "slow" and "fast" presentation rates of a visual flash stimulus. In the latter training, the contingency between presentation rate and conditioned response was congruent for one experimental group (i.e. fast presentation rates had to be associated with the Go response in both sensory modalities, and slow presentation rates with the No-Go responses in both modalities) and incongruent in a second group. It is argued that under certain conditions a higher acquisition rate of the conditioned responses in the congruent group as compared to the incongruent group is indicative of a crossmodal transfer of the rate-response contingency learned in the auditory modality to the visual modality.

In order to illuminate potential physiological mechanisms underlying this crossmodal transfer we have recorded local field potential activity from 21 depth electrodes chronically implanted in auditory, visual and prefrontal cortex. Directional aspects of neuronal interactions between separate cortical areas were addressed using Granger causality analysis.

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# Anterior cingulate cortex and its role in spatial learning and behavioural extinction

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Numerous studies have implicated the role of the anterior cingulate cortex (ACC) in attention (Posner and Petersen, 1990), pain (Zhang, Zhang and Zhao, 2005), stimulus-reward association (Bussey, Everitt and Robbins, 1997) and temporal organization of behavior (Meunier, Jaffard, and Destrade, 1991) among other things. In a previous study (Noblejas and Poremba, thesis 2005) that explored the role of the ACC in a cross-maze task (Packard and McGaugh, 1996), we found that the ACC may be involved in determining which spatial strategy would be maintained. Male Long Evans rats were trained to retrieve food reward in a goal arm that remained constant throughout two weeks of training. The starting arm of the rats remained constant as well, except during probe trials where the arm was changed to that directly opposite of it on training trials. This allowed us to assess what spatial learning strategy, whether place or stimulus-response, the rats would use when the reference point is changed. Intact rats normally displayed a shift in spatial strategy upon extended training. However, rats with ACC lesions displayed a lack of shifting which was consistent with perseverative behavior attributed to lesions in areas that included the ACC (Dias and Aggleton, 2000).

Considering the potential role of the ACC in perseverative behavior that could prove to be maladaptive, we sought to explore the role of the ACC in extinction in either an appetitive or aversive conditioning in the current study. After training to lever press for stimulation in the ventral tegmental area (VTA), gerbils are trained to jump over a hurdle in a shuttle box in response to an auditory tone as the conditioned stimulus (CS). The response of treatment-predetermined groups is conditioned by either applying VTA stimulation upon jumping promptly or presenting footshock upon failure to respond to the CS in a timely manner. After eight sessions of training trials, gerbils are given lesions in the ACC prior to acquisition of extinction. We then investigate the impact of the lesion on the acquisition and retention of extinction in both experimental conditions.

# Sequential behaviour: effects of striatal dopamine depletions in rats performing a Serial Reaction Time Task

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Sequential learning, a type of procedural learning, has intensively been investigated in humans using so-called serial reaction time tasks (SRTT), in which subjects have to respond rapidly to simple visual stimuli by pressing a corresponding response key. Speed-up of the reaction times to sequentially presented stimuli is taken as an indicator of learning the sequence. Further studies have revealed the implication of the neurotransmitter dopamine and the basal ganglia and identified some cortico-striatal networks, however, the study of the underlying brain mechanisms stays limited in human research. Hence, we designed an analogous of the classical human test in rats, using food-reinforcement, to study the role of dopamine and striatal structures in such a task. Here, we present results from bilateral 6OHDA-lesions in the nucleus accumbens or the neostriatum, placed after the rats have learned the sequence, in well-trained animals. These results will be discussed considering the general effects on serial responding and the specific effects on sequential behaviour.

# Testing a songbird's auditory memory with a DNMTS procedure

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To evaluate a sequence of acoustic communication signals it is necessary to keep previous elements in memory. This applies to the analysis of speech by a human observer, but also to the analysis of a sequence of song elements by a songbird. Female starlings (Sturnus vulgaris), for example, base their mate choice decision on the diversity of elements that an individual male sings (Eens et al. 1991, Behaviour 116: 210-238). To assess song diversity a female needs to store information about the elements in short-term memory. Thus, starlings should be suitable models to investigate the role and mechanisms of auditory memory. In this study we applied a delayed non-matching-to-sample (DNMTS) paradigm to estimate the persistence of the auditory memory store.

The starlings were trained in a Go/NoGo procedure. In each trial of a session, a series of up to 6 identical sample stimuli and a final test stimulus were presented with random inter stimulus intervals (range 1-27 s). The auditory memory duration was measured as a function of the delay between the last sample and the test. The test stimulus was either the same as or was different from the sample stimulus. Test parameter was either the frequency of the tonal carrier (6 sinusoids in the range of 1100 to 3492 Hz, step size was 1/3 octave) or the amplitude modulation rate of broadband noise stimuli (range 20 to 113 Hz, step size was a Weber fraction of 1.41). Test stimuli became sample stimuli in the following trial. The 400-ms stimuli were presented with a level that randomly varied between 58 and 64 dB SPL (average 61 dB SPL). Memory performance was expressed as the proportion of correct responses in relation to the total number of pairs of sample and test stimulus that were presented with a specific delay. The calculated persistence of auditory memory in this bird species was rather long (up to 26 s) but showed distinct individual variation. Persistence did not differ substantially for the two stimulus types. Increasing the number of samples presented before the test stimulus improved the performance. Performance was also better for more salient differences between samples and test.

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# Reconstruction of network dynamics in the prefrontal cortex during a two-interval discrimination task

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We investigate the evolution of neural activities in the monkey prefrontal cortex during a two-interval-discrimination task. In this task, a subject must compare two stimuli, separated by a time delay of a few seconds, and make a binary, categorical decision based on that comparison. The task involves storing a short-term memory trace of the first stimulus and computing a decision. Electrophysiological recordings from neurons in the prefrontal cortex of macaque monkeys show that neural firing rates encode information about the first stimulus during the delay period (short-term memory). The firing rates of many persistently-active neurons vary slowly with time. The variety and prevalence of such temporal dynamics is remarkable. To analyze the evolution of neural activities on the population level, we here used phase-space embedding as well as dimensionality reduction methods. Pooling data from all cells (n=395), we find that the dimensionality of the network dynamics collapse to only four dimensions during the delay period of the task. Information about the memorized first stimulus evolves in two of these four dimensions and the corresponding phase space trajectories carry out a rotational movement. The remaining two dimensions capture purely temporal aspects of the network dynamics. The activity of individual neurons during the delay period is well preserved by this reduction to four dimensions and can be reconstructed by linear combinations of the four corresponding coordinates. The weights of these linear combinations are distributed randomly and sparsely. Accordingly, the diversity of neural activities in the prefrontal cortex is due to the randomness in the combination of a few fundamental components. Altogether, our analysis yields a complete phenomenological description of how the prefrontal cortex reorganizes its short-term memory representation during the delay period of the task in anticipation of the second stimulus.

# NMDA- but not D1- or D2-Receptors in the Orbitofrontal Cortex Are Involved in Reversal Learning

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The orbitofrontal cortex (OFC) has been suggested to be part of a circuitry through which information on the incentive value of stimuli influences the selection and execution of reward-directed behavioural responses. Further evidence implicates the OFC to adjust responding when previously learned stimulus-reward contingencies change. For instance, electrophysiological studies demonstrated an involvement of the OFC in reversal learning as neurons altered their encoding properties after changing the reinforcement contingencies[1]. Also, lesions to the OFC have been found to impair reversal learning[2].

The OFC receives glutamatergic inputs from numerous areas involved in processing the motivational significance of stimuli such as the basolateral amygdala. Glutamate signalling in the OFC is critical for reversal learning as an intra-OFC NMDA-receptor blockade inhibited behavioural adaption to changes of learned stimulus-outcome contingencies[3]. In addition, the OFC is projected upon by dopamine (DA) fibers from the midbrain. The prediction-error hypothesis of DA action states that DA signals are necessary for the brain to update the predictive significance of cues. Yet, little is known whether D1- or D2-receptor-mediated signals in the OFC are required to learn a reversal of the predictive significance of cues.

Here we examined the effects of an intra-OFC blockade of DA D1- and D2-receptors as well as glutamate receptors of the NMDA subtype on learning a reversal of previously acquired cue-reward magnitude contingencies. Rats were trained in a reaction time (RT) task, that demands a conditioned lever press in which discriminative stimuli signal in advance the upcoming reward magnitude (one or five pellets). After seven days of training, RTs were guided by cue-associated reward magnitudes, i.e. RT of responses with expected high reward magnitude were significantly shorter. Thereafter, cue–reward magnitude contingencies were reversed. Reversal learning was tested for 10 daily sessions with intra-OFC microinfusions being given on sessions 1-6. Subjects received pre-trial infusions of the selective D1-receptor antagonist SCH23390 (0.5µg per side), the D2-receptor antagonist eticlopride (1µg), the NMDA-receptor antagonist AP-5 (0.5µg) or vehicle.

Preliminary results confirmed our previous findings that an intra-OFC NMDA-receptor blockade inhibited discrimination reversal learning. By contrast, an intra-OFC D1- or D2- receptor blockade did not affect discrimination reversal learning[3].

Our data suggest that in a visual discrimination task as used here, NMDA-, but not D1- and D2-receptor-mediated signals in the OFC seem to be necessary to update the reward-predictive significance of cues and adapting behaviour accordingly.

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# Dopamine D1 but not D2 Receptors in the Anterior Cingulate Cortex Regulate Effort-Based Decision Making in Rats

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In order to make adaptive decisions, subjects have to analyze costs and benefits of the available response options. A number of studies indicate that the anterior cingulate cortex (ACC) is involved in these evaluative processes and might serve to encode whether or not an action is worth performing in view of the expected benefit and the cost of performing the action. For instance, after excitotoxic ACC lesions rats no longer selected the high cost - high reward option in a cost-benefit T-maze task, if having the choice between climbing a barrier to obtain a large reward in one arm or to run for a low reward into the other arm with no barrier present[1]. Recent studies indicate that mesocortical dopamine (DA) fibers projecting to the ACC are important in effort-based decision making as well. Like rats with excitotoxic lesions, rats with DA depletion of the ACC no longer chose effortful but high reward action in a T-maze cost-benefit task[2]. Prefrontal DA plays an essential role in numerous cognitive processes through actions on D1 and D2 receptors. However, the role of D1 and D2 receptors of the ACC in effort-based decision-making is yet unknown.

Here we examined the effects of a selective intra-ACC blockade of D1 and D2 receptors on response selection in the rat using a cost-benefit T-maze task which is sensitive to ACC dysfunction. In this task, subjects could either choose to climb a barrier (30 cm) to obtain a high reward (4 pellets) in one arm or a low reward (2 pellets) in the other arm without a barrier. Results show that unlike vehicle-treated rats, rats with intra-ACC blockade of D1 receptors exhibited a reduced preference for the high cost-high reward response option when having the choice to obtain a low reward with little effort. By contrast, in rats with an intra-ACC blockade of D2 receptors the preference for the high cost-high reward response option was not altered relative to vehicle-treated rats. These findings suggest that DA input to the ACC acting on D1 receptors is critical for effort-based decision making and regulates response selection based on the relative values associated with different actions.

Prefrontal DA not only plays a critical role in the form of decision making tested here, but also in working memory or behavioral flexibility[3]: Prefrontal D1 receptors mediate working memory, while both D1 and D2 receptors act in a cooperative manner to facilitate behavioral flexibility. Furthermore, prefrontal D1, D2 and D4 receptor contribute to a form of decision making that requires rats to direct instrumental responding to minimize conditioned punishment, while our results suggest that effort-related decision making in a T-maze task requires prefrontal D1, not D2, receptor activity. Therefore, mesocortical DA modulation of different types of cognitive and executive functions appears to be mediated by dissociable patterns of prefrontal DA receptor activity.

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# Dopamine acting on D1 and D2 receptors in the nucleus accumbens mediates Pavlovian-instrumental transfer

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Pavlovian stimuli can markedly elevate instrumental responding, a phenomenon known as Pavlovian-instrumental transfer (PIT). PIT is thought to reflect the motivational influences of appetitive Pavlovian stimuli over instrumental performance. The amygdala and nucleus accumbens (ACB) are known to be key structures in mediating PIT, but little is known about the neurochemical basis of PIT. Recent findings provide evidence for a dopaminergic involvement, as systemic administration of dopamine receptor antagonists eliminated[1], whereas intra-accumbens microinjection of the indirect dopamine receptor agonist amphetamine enhanced PIT[2]. Furthermore, the inactivation of the ventral tegmental area (VTA) also abolished PIT[3], suggesting that PIT may depend upon the regulation of the mesoaccumbens dopamine neurotransmission by projections from the amygdala to midbrain dopamine neurons. However, the involvement of dopamine D1 and D2 receptors within the ACB in mediating PIT is yet unknown. Therefore, we investigated the effects of intra-ACB microinfusions of the selective dopamine receptor antagonists SCH23390 (D1) and raclopride (D2) on PIT.

Thirty-five male Lister hooded rats were implanted bilaterally with guide cannulae aimed at the ACB. Following at least 5 days of recovery all animals were habituated to the operant chambers for one session. During 12 subsequent sessions of Pavlovian training an auditory stimulus (3 kHz tone with 80dB) was paired with reward delivery (i.e. food pellets). Then, animals received 9 instrumental training sessions, where responding on a lever was reinforced by food pellets. Finally PIT was assessed in a single test session, in which lever presses were recorded during presentation of the auditory stimulus and an interval where no stimulus was present. Subjects were assigned to five treatment groups and received either SCH23390 (0.5µg and 0.75µg), raclopride (0.5µg and 0.75µg) or saline prior to testing.

The motivating effects of Pavlovian stimuli on instrumental responding were clearly visible in the saline group. These animals made significantly more lever presses during presentation of the conditioned auditory stimulus than during the period where no stimulus was present, thus showing a robust PIT. In contrast, in animals treated with SCH23390 and raclopride PIT was dose dependently abolished. These findings suggest that dopamine in the ACB acting both on D1 and D2 receptors plays a major role in mediating the excitatory effect of Pavlovian stimuli on instrumental performance. PIT is thought to represent one possible mechanism of relapse to drug use in humans and animals[4], since Pavlovian stimuli associated with former drug abuse are capable of inducing reinstatement of drug taking behaviour. Hence, the finding that PIT can be blocked by ACB dopamine D1 and D2 receptor antagonism indicates a possible neurochemical target for relapse prevention medication.

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# The Role of Prefrontal Dopamine in Instrumental Learning

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Instrumental performance in rats is critically determined by their ability to encode both the incentive value of the outcomes as well as the causal relation between actions and their outcomes. Thus, post-training procedures that decreased the incentive value of the instrumental outcome ("outcome devaluation") modified the rats' choice towards actions that were rewarded by the non-devaluated outcome[1]. In addition, treatments that degrade the instrumental action-outcome contingency ("contingency degradation") have been found to attenuate the rate of performance of an action[2]. At the neural level, the medial prefrontal cortex (mPFC) is a key structure subserving instrumental learning. For instance, cell body lesions of the mPFC have been shown to render rats insensitive to changes in the instrumental contingency suggesting that this region is involved in encoding action-outcome relationships[3]. The mPFC receives a prominent dopamine (DA) input from the midbrain and reinforcement learning models suggest a critical role of DA in value and action learning[4, 5]. However, little is known about the role of prefrontal DA in instrumental learning. Here, we investigated whether a prefrontal DA depletion impairs the encoding of the incentive value of the outcomes as well as the causal relation between actions and their outcomes.

Before the onset of behavioural testing, rats received prefrontal 6-hydroxydopamine (6-OHDA) or sham lesions. In experiment 1 ("outcome devaluation"), rats were first trained to press two levers. One lever delivered food pellets, the other a sucrose solution. After training, one of these outcomes was devalued using a specific satiety procedure in which the animals gain access to one of the outcomes ad libitum 1h before a choice extinction test made on the two levers. In experiment 2 ("contingency degradation"), we tested whether the lesioned animals were still sensitive to a degradation of the instrumental contingency. Therefore a protocol was applied in which the outcome earned by pressing one of the two levers was now also given non-contingently with the same probability without a response. Thus the experienced probability for that outcome was the same, regardless if the animal performed that action or not. For the other lever the non-contingently given outcome was different from the one earned by performing the action, so that this action-outcome contingency was not degraded.

Our initial results revealed that in animals with prefrontal DA depletion the sensitivity to outcome devaluation and contingency degradation was moderately reduced. However these data are preliminary as a quantitative neurochemical analysis of prefrontal DA depletion is not yet available. These data tentatively point to the view that prefrontal DA is involved in instrumental learning.

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# Mapping 'Numerals' to Quantities in the Primate Prefrontal Cortex.

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Judging numerical quantities is an evolutionary old concept shared by humans and animals. Humans perfected this ability by assigning number symbols to quantities, a prerequisite for verbal counting. Children learn the usage of numerical symbols as mental tools while they develop language skills. One of the important steps during this learning process is the mapping of numerical quantities onto visual forms, which then become numerals, numerical symbols that enable humans to grasp a most exact, digital representation of quantity.
To investigate the neural underpinnings of this abstract mapping process, we trained two monkeys in a delayed match-to-sample task to associate the number of items in multiple-dot patterns with Arabic numerals. On any given trial, the sample stimulus was either a numeric 'symbol' (the Arabic numerals 1 to 4) or a set of one to four dots. Monkeys learned to associate numerosities with the respective numerals, e.g., two dots were associated with the numeral '2'. Two monkeys discriminated the stimuli on an 85% correct performance level for all numerosities and numerals. The performance for the numeral protocol was slightly better than for the dots (Chi-square test, $p < 0.001$).
We recorded 818 single-units in the ventrolateral prefrontal cortex in two monkeys. To determine the proportion of purely numerosity encoding cells we run a combined analysis of 2-factor ANOVAs and shuffle corrected normalized cross correlation. A fraction of 54 cells (54/628 or 9 %) were found to encode both formats during the sample period, irrespective of the notation. Even more neurons (149/628 or 24 %) showed this behavior during the delay. An ROC-analysis revealed that the encoding of numerals was more accurate during the sample period than the encoding of multiple-dot patterns (Wilcoxon signed rank text $p < 0.001$).
To our knowledge we show here for the first time on a single neuron level how the brain implements the mapping of visual forms ('numerals') to numerosity and how it may take advantage of symbolic numerical formats.

# The Role of the Posterior Parietal Cortex in the Representation of Continuous and Discrete Quantities

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Our intuitive need to link numbers (discrete quantity) to space (continuous quantity) and to make use of numbers to measure extent reflects the close similarities in the mental representations of continuous and discrete quantities. Behavioural studies in humans reveal interference between judgements of number and spatial magnitude. Functional imaging studies suggest that such judgments of quantity engage overlapping neural areas of the posterior parietal cortex. Neuropsychological investigations demonstrate, furthermore, selective impairment for both spatial and numerical interval estimates after lesions in the parietal lobe. The precise neuronal mechanisms underlying quantity representations, however, remain unclear.

We trained two monkeys (Macaca mulatta) to discriminate in a delayed match to sample protocol the numerosity of items in multiple-dot displays and the length of lines within the same working session. Subjects were able to discriminate numerosities from one to four as well as lines of four different lengths with comparable accuracy.

We then recorded the activity of 399 single units in the depth of the intraparietal sulcus while these monkeys performed the task. One hundred and three of the recorded neurons (26%) responded as a function of either the numerosity or the length of the line ($p < 0.01$ two-way ANOVA). Tuning was observed during stimulus presentation as well as during a memory delay when the monkeys had to maintain the quantity information in mind. Both in the sample and the delay period, neurons tended to respond selectively to either the line length or the number of dots, indicating segregated neuronal populations involved in the encoding of continuous and discrete magnitudes, but there was a third sub-population present, which responded selectively to both continuous and discrete magnitudes. These observations suggest that continuous and discrete quantities are both encoded in the same anatomical areas in the depth of the IPS, in partly overlapping neuronal populations.

# Reinforcement learning in an actor-critic spiking network model

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Computationally powerful reinforcement learning algorithms have been developed in the context of machine learning [1]. Experimental evidence [2] suggests that such algorithms are used by the mammalian brain [3]. However, it is still unclear how reinforcement algorithms capable of solving non-trivial tasks could be implemented in biological neuronal systems. A major obstacle to overcome is that neuronal systems operate in continuous time whereas common reinforcement learning algorithms are formulated in discrete time.

Here, we demonstrate how the arctor-critic architecture [4,5], an efficient and widely used realization of reinforcement learning, can be implemented by a suitably structured network of spiking integrate-and-fire neurons. The fundamental components of this architecture, the policy and value functions, are both represented by synaptic weights. The synaptic learning mechanisms are local plasticity rules motivated by biological findings.

We test the learning capability of the neuronal system on navigational tasks in one and two dimensional "grid-world" environments [1]. The network is able to accurately evaluate the quality of a state and adapt its policy accordingly, resulting in a learning curve similar in speed and stability to that of the corresponding discrete time computer algorithm (Fig. 1). This work shows that, in principle, reinforcement learning can be realized in a network of spiking neurons with plastic synapses.

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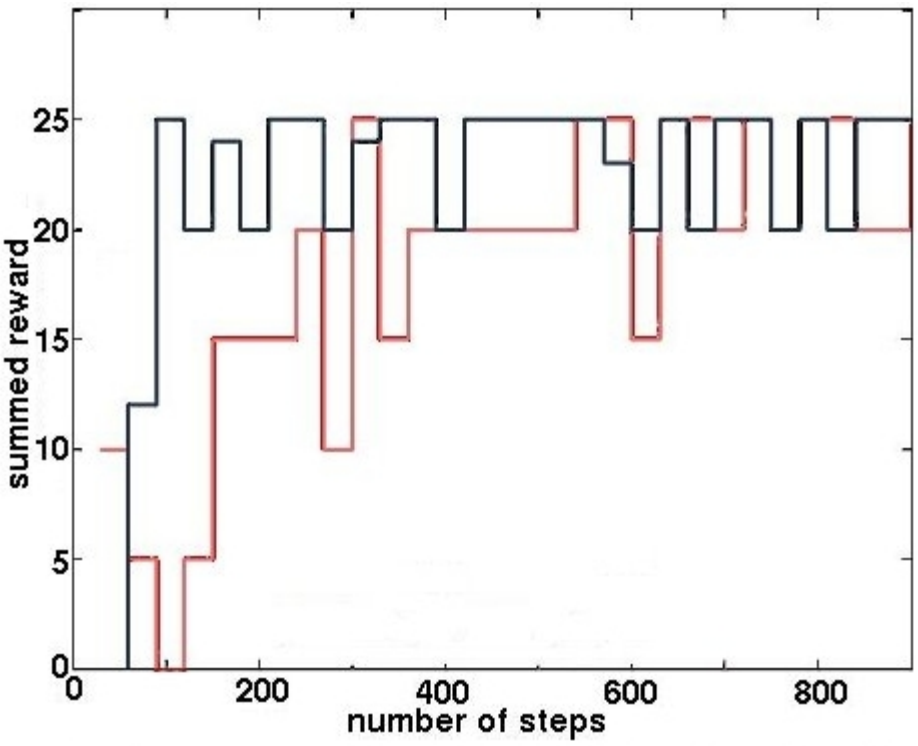


Performance on a one-dimensional grid-world task: continuous time neuronal network, red curve; discrete time computer algorithm, blue curve.

# Sources of human theta EEG activity during working memory

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When human subjects perform a working memory task, their EEG from frontal electrodes may show enhanced activity in the 4-9 Hz theta frequency band. The sources of this activity in humans are still being explored.
In our study, healthy subjects performed a modified Sternberg task. In the task, sets of consonants have to be retained in memory for 3 seconds. The setsize (4, 6, or 8 letters) determines the memory workload. During task performance we recorded EEG. We focused the analysis on the last 2 seconds of the retention interval. Among the subjects we selected 8 subjects who displayed high theta activity during high memory workload.
To analyze the workload-dependence of theta activity, we compared the EEG from setsize 8 to setsize 4. Most prominent was an enhanced theta activity around 5 Hz in middle frontal electrodes. To identify the cortical sources of this enhancement, we used sLORETA analysis. The EEG correlate of increased workload was generated in the dorsal part of the medial frontal gyrus.

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# DOES THE SHORT TERM MEMORY OR WORKING MEMORY HAS GOT INFLUENCE TO LANGUAGE(SPEECH)ACQUISITION IN THE CHILDREN WITH HEARING IMPAIRMENT ?

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The concept of working memory results from the concept of short term memory in opposite of long term memory.The short term memory is not the unique system, but rather it is short storeroom for information of limited capacity, where mental operation can be perfomed. It is measured in seconds and minutes. Working memory can receive information from sensoric memory, but also from long term memory and it can be the place where that information is processed.

Working memory has at least two components - storeroom and part for processing.

There is also a central executor which controls and regulates attention and is hierarchically superordinated systems: articulatory / phonological loop and visuo-spacial sketchpad.

This poster presents measures at short term memory obtained from children with impaired hearing ( with cochlear implants and with hearing aid).We investigated auditory and visual retention at word and number series, capacity for visual retention of pictures and their distribution on paper as well as capacity for retention of light stimulation order.

Obtained results for each participant were discussed in relation to his/her speech development.

# Poster Topic

## T29: Learning and memory III: Invertebrates

**T29-1A** Sleep in honeybees: Searching for the role of sleep in memory consolidation
*L. Bogusch, SA. Hussaini, M. Schubert, U. Greggers and R. Menzel, Berlin*

**T29-2A** Local blockades of neural activity reveal distinct roles of insect mushroom bodies during learning and memory transfer
*B. Grünewald, C. Bartsch, A. Blunk, J. Podufall, M. Giurfa and JM. Devaud, Berlin and Toulouse (F)*

**T29-3A** The home vector of desert ants does not extend into the third dimension
*G. Grah, R. Wehner and B. Ronacher, Berlin and Zürich (CH)*

**T29-4A** Context-dependent learning in honeybees *(Apis mellifera)*
*SA. Hussaini and R. Menzel, Berlin*

**T29-5A** NMDA glutamate receptor mediates long-term memory formation in the honeybee.
*G. Leboulle, R. Roessler and L. Muessig, Berlin*

**T29-6A** The influence of reward duration on reconsolidation and consolidation of extinction memory in PER conditioning in honeybees
*N. Stollhoff and D. Eisenhardt, Berlin*

**T29-7A** MONITORING NUCLEAR CALCIUM SIGANLS USING RECOMBINANT CALCIUM PROBES IN DROSOPHILA LARVAL BRAIN
*JM. Weislogel, J. Schlüter, C. Schuster and H. Bading, Heidelberg*

**T29-8A** Fast manipulation of cellular cAMP levels by transgenic
Photo Activated Cyclases (PACs) in *Drosophila*.
*F. Dohler, P. Cabrero, G. Nagel, U. Mueller, J. Dow and M. Schwaerzel, Saarbrücken, Glasgow (UK) and Würzburg*

**T29-1B** Chromatin remodeling and its contribution to long-term memory and neuronal development
*J. Hättig and U. Mueller, Saarbrücken*

**T29-2B** Sub-cellular anchoring of PKA as organizing principle in Drosophila associative memory processing
*A. Jaeckel, M. Schwaerzel and U. Mueller, Saarbrücken*

**T29-3B** The role of Phosphodiesterases in *Drosophila*
associative memory processing.
*E. Jost, JP. Day, U. Mueller, S. Davies and M. Schwaerzel, Saarbrücken, Glasgow (UK) and Saarbrücke*

**T29-4B** Linking satiation levels and learning: a function of the conserved cellular energy sensor AMP-activated protein kinase
*T. Laeger and U. Müller, Saarbrücken*

**T29-5B** IMPACT OF VIRAL INFECTION ON LEARNING, MEMORY AND THE IMMUNE SYSTEM OF HONEYBEE
*J. Iqbal and U. Mueller, Saarbreucken*

**T29-6B** Epigenetic control of gene expression in honeybee long-term memory
*D. Hatakeyama and U. Mueller, Saarbruecken*

**T29-7B** Mushroom bodies are necessary for non-elemental forms of learning in the honeybee.
*JM. Devaud, T. Papouin, B. Grünewald and M. Giurfa, Toulouse (F) and Berlin*

**T29-1C** Stress and learning in honeybees: effects of exposure to an alarm pheromone component on associative conditioning and memory.
*E. Urlacher, B. Francés, M. Giurfa and JM. Devaud, Toulouse (F)*

**T29-2C** Desert ants, *Cataglyphis fortis*, adjust their approach of a familiar goal with experience
*H. Wolf, Ulm*

**T29-3C** The Learning Mutant *dunce* is Impaired in Ethanol Tolerance
*M. Franz, A. Klebes, A. Saratsis and H. Scholz, Würzburg and Berlin*

**T29-4C** TOWARDS LOCALIZING A SYNAPSIN-DEPENDENT OLFACTORY MEMORY TRACE IN THE BRAIN OF *DROSOPHILA* LARVAE
*B. Michels, B. Gerber, H. Tanimoto and E. Buchner, Würzburg*

**T29-5C** Out of Sight, but not out of Mind - A Spatial Memory Trace in the Brain of *Drosophila melanogaster*
*K. Neuser, T. Peter and R. Strauss, Würzburg and Mainz*

**T29-6C** Rutabaga-independent learning and memory in *Drosophila*
*R. Wolf, W. Plendl, S. Yamaguchi and M. Heisenberg, Würzburg and New York (USA)*

**T29-7C** A role of the presynaptic protein SAP 47 in associative learning
*T. Saumweber, B. Michels, N. Funk, E. Buchner and B. Gerber, Wuerzburg*

# Sleep in honeybees: Searching for the role of sleep in memory consolidation

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Sleep, though much better characterized in mammals, has been also found in insects like the fruitfly (Drosophila melanogaster) and the honey bee (Apis mellifera). In mammals sleep is important for long-term memory consolidation. Since the honey bee provides us with an insect model for long-term memory consolidation, we studied sleep behavior in honeybees in the context of learning and memory consolidation. Two experimental approaches were applied, monitoring sleep in freely behaving bees, and laboratory bees exposed to olfactory conditioning. A natural form of learning is foraging. Total times of foraging flights per day of individually (electronically) marked bees were correlated with movements inside their hive. We found that the sleep behavior of forager bees differs from that of nurse bees. Foragers usually do not rest during the foraging hours but do so at night, quite often with prolonged resting times directly before and after the foraging period. Nurse bees, though more active at daytime than during the night, show also resting periods during the day and longer activity phases during the night. Furthermore, we found that the sleeping time of foragers is correlated to the number and length of their foraging trips. Since foraging is associated with learning these results suggest that sleep may indeed be important for the consolidation of new memories.

In the laboratory setting antennal movements in bees were used as an indicator of sleep. Harnessed bees were conditioned to odors, and their sleep behavior during the following night was monitored. Half of the animals were conditioned with an odor stimulus in a 5-trial classical conditioning protocol, and the other half were not conditioned and were used as control. Also, half of the bees were exposed to a normal light/dark 12h/12h light/dark cycle throughout 24h, and the other half were disturbed by continuous repetitions of 15'/15' light/dark cycles. We found that, similar to the foraging bees in the hive, harnessed bees also slept more during the night as compared to the day. Bees conditioned the previous day slept lesser than their unconditioned counterparts. Additionally, bees from 15'/15' light/dark cycle showed equally high learning scores than undisturbed bees.

* The authors contributed equally

# Local blockades of neural activity reveal distinct roles of insect mushroom bodies during learning and memory transfer

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Local blockade of spiking activity is a successful method to localize brain areas involved in learning behavior or memory formation. We injected the local anesthetic procaine into the mushroom bodies (MB) within the brain of the honeybee, Apis mellifera. Using the classical conditioning of the proboscis extension reflex we studied the role of the MB for reversal learning and memory transfers between the brain hemispheres.

1. Physiological effects of local anesthetics. Using patch clamp techniques we showed that 2% procaine reversibly blocks action potentials in cultured adult mushroom body Kenyon cells. Procaine acts on voltage-gated $Na^+$ and $K^+$ currents (complete block of $I_{Na}$ at 10mM). The effects are dose-dependent between 1-10mM and readily reversible within a few minutes of wash. Lidocaine (1-10mM) also blocks voltage-sensitive $Na^+$ and $K^+$ currents. However, it inhibited currents through $K^+$ channels more efficiently than $Na^+$ currents. Procaine was, therefore, chosen for behavior experiments.

2. Reversal learning: bees learn to reverse their responses to two odorants, one rewarded (A+) and one unrewarded (B-), if their contingencies are reversed (A- B+). We blocked MB neural activity by injecting procaine (0.5nl, 20%) into both MB alpha lobes before the reversal training and analyzed the behavioral consequences. Saline injections served as a control. Procaine injections impaired reversal learning. This was not due to impaired retention of the initial learning (A+ B-) immediately prior to reversal learning. Procaine injections neither blocked learning, because differential learning tasks involving novel odorants (C+ D -) were intact under procaine. In addition, procaine injections did not impair extinction (A- B-). This indicates that the MBs play an important role not only during memory recall, but also during complex learning tasks like reversal learning (see also poster by Devaud et al).

3. Memory transfer: bees were trained to learn an odour on only one side. During the test animals showed a conditioned response on both sides, indicating a transfer of the learned information to the untrained brain hemisphere. We injected procaine unilaterally into the alpha lobe of either the ipsilateral or the contralateral MB before acquisition. Although not yet completed, our experiments indicate that unilateral blockade of the MB during training left the transfer of learned information intact. This would imply that either the information transfer occurs at other brain areas (e.g., the antennal lobes) or at a different time point (e.g., during memory retrieval). We are currently testing these hypotheses.

Our experiments show that local anesthetic block insect neuronal spike activity and local brain injections of procaine may be used to map learning tasks onto specific regions of the insect brain. We conclude that intact mushroom body activity is required for the acquisition of reversal learning, but not for simple differential learning tasks.

# The home vector of desert ants does not extend into the third dimension

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Desert ants (*Cataglyphis fortis*) assess the position of their nest entrance with a path integration mechanism that uses the walking direction, and the distance walked with a particular bearing. Accurate homing is possible even in three-dimensional (3-D) environments. One possible explanation is that the vector orientation is fully functional in all three dimensions. In this case, a vertical deviation from the intended course should prompt an ant to try to compensate for it.

We tested this hypothesis by training ants to visit a feeder in a horizontal channel. Homebound ants were placed in a test channel that included a descending ramp (1.5 m / 70 deg) shortly after the release point. This descent connected with a horizontal channel, which led to an ascending ramp (test ramp, 2 m / 70 deg) at nest distance. In a control experiment, the test channel did not include a descent, but consisted only of a horizontal channel and a test ramp at its end. We analysed the length of the first ascent on the test ramp, which should be indicative of a remaining vertical component of the ants' home vector. If the home vector extends to the third dimension, we expect that animals from the critical test should show higher ascents than their peers from the control. Median ascent lengths were not different (U-test, $p = 0.41$) between critical test (median: 50 cm, $N = 37$) and control (median: 65 cm, $N = 39$). Thus, the initial descent that was imposed on animals in the critical test did not result in a greater tendency to ascend.

Although these data speak against a 3-D vector, earlier experiments showed that desert ants climb on ramps more often and higher if their current itinerary includes an ascent. One explanation is that ascents are stored as additional information of the current vector. Alternatively, desert ants could learn to accept ascents and descents in general, i.e. independently from the current vector. We tested the second hypothesis in an experiment where ants first learned to walk a route to a feeder that included an ascent, but later tested their acceptance of a ramp in a training situation that was exclusively 2-D.

We trained one group of ants along a path that included a ramp (critical test). A control group was trained in a horizontal channel. On the next day, both groups were trained in a flat channel, pointing in the opposite direction. Homebound ants were placed at the top of a descending ramp (1.5 m / 70 deg), which led into a horizontal channel. Whether ants accepted the descent was assessed by looking at the position of their first U-turn. We took a first turn on the ramp as indication that ants rejected this descent. After ramp training on the previous day, only 19 % of ants turned around on the ramp, as opposed to 63 % in controls (Fisher's exact test, $p < 0.01$). Ramp-trained animals that were tested again two days later still showed this high acceptance of a ramp that was not congruent with the current outbound training.

We conclude that ascents can be learnt as particular aspects of a journey. However, the home vector of desert ants does not extend to the third (vertical) dimension.

# Context-dependent learning in honeybees *(Apis mellifera)*

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Context-dependent learning involves the extraction of relevant information (stimulus) from potentially confounding and changing environmental conditions (context). It requires an animal not only to perceive particular stimuli, but also to evaluate and associate the environmental conditions surrounding such stimuli. Previously, free flying honeybees were shown to associate visual or odor stimuli selectively in two different context situations. It is also known that bees relate different flight routes to different landmarks and to learn to respond to particular odors at particular times of the day. In our experiments we used harnessed honeybees exposed to classical conditioning of the proboscis extension response (PER). We used 'odors' as conditioned stimuli, and 'temperature' and 'colors' as context stimuli. (Context stimuli will be indicated here in capital letters). In the first experiment, bees were conditioned differentially by 12 trials to two odors A and B. In the presence of HEAT, odor A was combined with sugar reward (A+) and odor B was not rewarded (B-), and in the presence of COLD, odor B was combined with sugar reward (B+) and odor A was not rewarded (A-). In the second experiment more complex context stimuli were used by adding colored light to the temperature contexts, YELLOW appeared together with HEAT, and BLUE together with COLD. The training regime was A+B- in the presence of HEAT and YELLOW, and B+A- in the presence of COLD and BLUE. Odors and contexts were used in all combinations to exclude odor and context specific effects. We found that in presence of only temperature context, bees could discriminate odors A and B in the HEAT and COLD contexts, but only partially. With the additional contexts YELLOW and BLUE, however, bees learned the context specific task of odor discrimination much more quickly and more precisely. This shows that additional contextual cues make learning of a particular task easier.

# NMDA glutamate receptor mediates long-term memory formation in the honeybee.

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Glutamate is an important excitatory neurotransmitter in the mammalian central nervous system (CNS) through its specific receptors (NMDA, AMPA, kainate and metabotropic glutamate receptors). The ionotropic NMDA receptor is intensively studied in relation to its participation in neural plasticity and memory processes. In insects, glutamate is the neurotransmitter at the neuromuscular junction and glutamate immunoreactivity was found in the CNS. The expression of several glutamate receptors, related to mammalian homologues, was also described.

In a previous study, we characterised the NR1 subunit homologue of the NMDA glutamate receptor of the honeybee Apis mellifera (Zannat et al. Neurosci. Lett. 398:274-9, 2006). This subunit is common to all NMDA receptors, and is necessary for the assembly of functional receptors. An essential role of the NMDA receptor was recently demonstrated in olfactory learning and memory in Drosophila (Xia et al. Curr. Biol. 15:603-15, 2005). In the honeybee, pharmacological evidence indicated the participation of glutamatergic neurotransmission in olfactory memory (Locatelli et al. J. Neurosc. 25:11614-8, 2005). However the role played by the NMDA receptor in memory formation remains to be demonstrated.

In this work, we provide evidence that several isoforms of the NR1 subunit are expressed in the honeybee brain. We also show that the induction of RNA interference by injection of dsRNA molecules specific for this subunit reduces its expression and impairs the formation of olfactory long-term memory in appetitive conditioning.

# The influence of reward duration on reconsolidation and consolidation of extinction memory in PER conditioning in honeybees

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In classical conditioning an animal learns that a previous neutral stimulus (conditioned stimulus, CS) acts as a predictor for the appearance of a biologically significant stimulus (unconditioned stimulus, US). After an association has formed animals display the conditioned response (CR) in anticipation of the US. Depending on the number of retrieval trials retrieval of that memory can induce two different processes: consolidation of a CS-noUS association and reconsolidation of the CS-US association.

A correlation has been reported between the stability of the retrieved memory and the control of retrieval induced behavior by that memory (Eisenberg et al., 2003, Science, 301:1102-4) indicating that the outcome of the competition between excitatory CS-US and inhibitory CS-noUS associations depends upon the intensity of the original training.. This means that if the original training is highly robust and/or the number of extinction trials is too small, the 'inhibitory' trace may not gain control of behavior and, therefore, will become insensitive to consolidation blockers. In order to evaluate these predictions in the context of appetitive learning, we manipulated the original training of animals that were subsequently exposed to retrieval. To do so, we varied the length of US presentation, which might be directly related to the associative strength.

Associative strength is thought to be reflected during acquisition by its effects on the animal's CRs. However, in the present study US length, showed no effect on the animals CRs during acquisition. Moreover, when bees trained with different US duration were exposed to CS presentations 24 h later no differences in their CRs were observed. However, a consolidation blocker applied prior to the presentation of CS-only trials revealed that CS-US associations underlying CRs of in these groups were probably different.

# MONITORING NUCLEAR CALCIUM SIGANLS USING RECOMBINANT CALCIUM PROBES IN DROSOPHILA LARVAL BRAIN

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The generation of calcium signals following synaptic activity is a fundamental property of neurons that controls many neuronal processes including cell survival, learning and memory. In cultured neurons, increases in nuclear calcium concentrations are known to be critical for the activation of gene expression mediated by the transcription factor CREB (1). CREB has been implicated in transcription-dependent plasticity (late phases of LTP).

Confirming the importance of nuclear calcium signaling in the intact brain is impeded by the restrictions and complexities of in vivo experimentation. However, using genetically encoded $Ca^{2+}$ indicators in the Drosophila central nervous system provides an excellent system to explore the role $Ca^{2+}$ in an intact behaving animal. Since we are particularly interested in $Ca^{2+}$ signaling of the nucleus, we are engineering genetically encoded nuclear $Ca^{2+}$ indicators.

To visualize nuclear calcium signals in Drosophila, we have engineered a transgenic fly based on the GCaMP 1.3 (2), which increases its fluorescence when binding $Ca^{2+}$. We made several different GCaMP constructs with nuclear localization signals (NLS) and used the Gal4/UAS-system to direct the expression of the indicators to cells of interest (3). Because the larval neuro-muscular junction has been a simple and useful system to study plasticity, we first expressed these indicators in muscle and motorneurons of third-instar larvae. In both cell types, the $Ca^{2+}$ indicator localized to the nucleus. In addition, we have tested both the standard GCaMP (4), and the NLS-GCaMP for detecting calcium transients in the larval brain in response to KCl-induced membrane depolarization.

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# Fast manipulation of cellular cAMP levels by transgenic Photo Activated Cyclases (PACs) in *Drosophila*.

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The flagellate *Euglena gracilis* contains a photo activated adenyly cyclase (PAC) consisting of the flavoproteins PACαand PACβ (Iseki et al., 2002). Upon heterologous expression the activity of PACs is strongly and reversibly enhanced by blue light, providing a powerful tool for light-induced manipulation of cellular cAMP levels in animal cells. When PAC expression is targeted to the *Drosophila* brain illumination results in fast and reversible behavioural changes of freely moving flies (Schröder-Lang et al., 2006).
In order to characterize the optimal illumination conditions of this novel tool in *Drosophila* we have systematically investigated the effects on behaviour in freely moving animals, as well as on physiology of isolated tissue. We addressed questions about (1) effects of light intensity and duration of illumination, (2) effects of repeated or pulsed illuminations, (3) effectiveness of different subunits and genetically optimized derivatives.

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# Chromatin remodeling and its contribution to long-term memory and neuronal development

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Transcriptional processes are triggered by strong associative training that is required for long-term memory formation. However, since these learning-triggered processes are transient, it remains unclear how learning-induced transcriptional changes are maintained for days, weeks, or even a lifetime. New findings support the idea that remodeling of the chromatin structure by modifications of histones and DNA, that together with the underlying biochemical machinery are termed epigenetic mechanisms, play a roll in learning and memory formation. Depolarization of neurons change the DNA-binding affinity of MeCP2 (methyl-cytosine binding protein 2) that is a component of a chromatin-remodeling complex, and this results in an enhanced transcription. A mutation in MeCP2 causes a severe neuronal developmental disorder called Rett-Syndrom. In mice the knock-out of MeCP2 leads to a learning defect while a mild over-expression enhances the learning ability.

However, it is yet impossible in mice to unequivocally separate developmental MeCP2 function from that observed in learning. Tools available in Drosophila and honeybee enable a high spatial and temporal manipulation and monitoring of enzyme function and prompted us to address the role of chromatin function in learning in these insects.

Morphological analysis revealed a first hint for a role of chromatin in neuronal development as shown in mice. Drosophila mutants with defects in proteins homologue to components of mammalian chromatin remodeling complex (MeCP2-Sin3A-HDAC) show a specific change in the volume of the mushroom bodies. This mushroom body specific effect provides now the basis for a detailed analysis of the role of chromatin structure in neuronal development.

By using a series of antibodies against defined acetylation sites of histones, we established techniques that allow the determination and localization of changes in the histone acetylation pattern. Immunohistological studies in honeybee brain reveal first evidence for a highly interesting result: the acetylation pattern differs between brain areas involved in distinct aspects of learning. Since the acteylation pattern of histones is closely correlated to chromatin function, these findings point to differences between distinct brain areas with this respect. While these results provide first evidence for a function of chromatin in the insect brain the ongoing analysis will show whether chromatin structure serves as a transcriptional memory that maintains learning-induced transcriptional changes.

# Sub-cellular anchoring of PKA as organizing principle in Drosophila associative memory processing

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The cAMP-PKA pathway plays a pivotal role in associative memory processing. Depending on the strength and time-course of stimulation during the training period, different molecular processes become activated leading to distinct forms of associative memories. This long-known and ubiquitous finding raises questions about the molecular mechanisms that differentially distribute signalling of the cAMP-PKA pathway. Scaffolding proteins of the A-Kinase-Anchoring-Protein (AKAPs) family have recently been discovered to localize PKA on the sub-cellular level. By that, AKAPs provide functionally distinct pools of PKA and thus add specificity to this pathway. Using transgenic techniques in *Drosophila* we recently could show that consolidation of olfactory associative memory requires a pool of AKAP anchored PKA. Since impairing catalytic function of PKA affects learning and memory formation our results support the idea that different pools of PKA contribute to distinct aspects of behavioural plasticity in vivo. For the further characterization of the underlying organizing processes we extend our analysis by variation of the training procedure.

# The role of Phosphodiesterases in *Drosophila* associative memory processing.

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The second messengers cAMP and cGMP play pivotal roles in associative memory processing and multiple cyclases and phosphodiesterases (PDEs) are involved in their formation and degradation, respectively. The PDEs can be grouped according to their substrate selectivity; enzymes that specifically degrade only a single type of cyclic monophosphates (cAMP or cGMP, respectively), as well as PDEs with mixed specificity do exist. Here, we explored *Drosophila´s* sophisticated genetic techniques to interfere with various PDEs, thus manipulating cAMP and/or cGMP levels, and analysed the effects on associative olfactory memory processing.

First, we determined which PDEs are involved in memory processing. To that end we expressed each knockdown constructs throughout the whole brain and analysed associative behaviour. In the second step, we restricted construct expression to various neuropils involved in memory processing to determine the necessary sites of PDEs activity. Finally, we will apply temporal control over transgene expression to distinguish between a developmental or acute function of the PDEs.

# Linking satiation levels and learning: a function of the conserved cellular energy sensor AMP-activated protein kinase

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The level of satiation is one of the parameters critically involved in learning and memory formation. Especially in appetitive learning paradigms the satiation level is strongly correlated with memory formation: only hungry animals with low satiation levels during training reliably form a long-term memory. Although the impact of satiation level on learning is well known, the underlying molecular processes are unclear. The AMP-activated protein kinase (AMPK) is an evolutionary highly conserved sensor of cellular energy and a 'metabolic master switch'. By monitoring cellular AMP-levels, the AMPK controls metabolic processes and supports the restoration of energy depletion by reducing anabolic pathways and elevating ATP generating catabolic pathways. We addressed the question whether the cellular energy sensor AMPK is a component of the satiation-dependent processes interfering with learning and memory formation. By using AICAr (5-Aminoimidazole-4-carboxamide-1-b-riboside), which mimics AMP and activates AMPK, it is possible to imitate a "low cellular ATP level" in satiated animals. This treatment leads to an enhanced memory performance after training of satiated animals and thus points to a link between AMPK-regulated mechanisms and processes underlying learning and memory formation. By photorelease of caged ADP, that is immediately degraded to AMP, we started to localize the brain area responsible for AMPK action. First experiments using habituation of the appetitive proboscis response show, that AMPK function can be attributed to distinct brain areas. These finding supports the idea that AMPK, the conserved sensor of cellular energy level, contributes to the link between satiation level and learning.

# IMPACT OF VIRAL INFECTION ON LEARNING, MEMORY AND THE IMMUNE SYSTEM OF HONEYBEE

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Honeybees (Apis mellifera L.) face a major threat to different infections that results in a drastic impairment in their performance up to the loss of whole colonies. Infections by bacteria, fungi, and protozoa, together with parasites (varroa) and their combinations are considered as potential reasons. Recently, viral infections are added to this list. Viral infections usually show no apparent symptoms in honeybees and it is unclear whether they affect the immune system or facilitate infections by other sources. Moreover, it is feasible that viral infections directly impair behaviours like foraging, navigation or social behaviour, by interfering with neuronal function. To address these questions, we established techniques to monitor viral infections and study their effect on behaviour and the immune system of honeybees. We use RT-PCR protocols that enable to identify viral infections of single bees and to monitor parameters like the time course of infection. The observation that artificially infected honeybees show a change in their sucrose responsiveness provides first evidence for effects on sensory capabilities. To determine effects of viral infections on the immune system we are presently investigating the implication of the highly conserved NF-kB/Rel-protein, which participates in triggering the immune response in mammals and insects. This combined analysis aims for a better understanding of the link between viral infections and its action on the immune system, brain function and behaviour.

# Epigenetic control of gene expression in honeybee long-term memory

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In eukaryotes, methylation of cytosines of genomic DNA is an epigenetic modification responsible for both chromosomal stability and functional activity of genomic loci. In addition, the transcriptional activity at distinct loci are regulated via histone modifications that change chromatin structure. Thus, DNA de-methylation and chromatin remodeling, mainly by histone acetylation, are essential processes for active gene expression. Although these epigenetic regulations of gene expression, considered as a "transcription memory", are actively studied in development and cancer research, there are only few reports on possible functions of DNA methylation and chromatin remodeling in processes underlying neuronal plasticity and learning. Our study aims to address the role of DNA methylation as a transcription memory in associative learning in honeybees, which enables the analysis across levels from behaviour to genomic sequence.

As a prerequisite for our studies we established techniques to identify DNA methylation in genomic DNA of honeybee brain. Using antibodies against 5-methyl-cytosine, we developed an ELISA-based procedure to quantify total methylation levels in whole genomic DNA. Similar as reported for *Drosophila*, the total DNA-methylation levels in honeybee brain is considerable lower as compared to mammalian brain. However, the established quantification of the level of total methylation allows to monitor the effect of drugs, known to change methylation in mammals. This provides us with the basic parameters to address questions how changes in total DNA methylation levels interfere with learning and memory formation.

Since we aim to study the link between DNA methylation and learning at the levels of genes, we started to identify potential genes regulated by learning-induced processes that contribute to memory formation. Here we focus on genes regulated by the transcription factor CREB (cAMP/$Ca^{2+}$-responsive element binding protein), that is a conserved key player in memory formation throughout species. The search for CRE motives, the binding-sites for CREB, uncovered a series of genes known to play important functions in processes underlying learning. In order to identify the DNA methylation pattern, we started to select primers for genes with CRE motives, including CREB itself, nitric oxide synthase, ubiquitin C-terminal hydrolase, calpain, metabotropic glutamate receptor, acetylcholine receptor, dopamine receptor, octopamine receptor and serotonin receptor. Monitoring learning-induced changes in the DNA-methylation pattern in the promotor regions of these genes will provide a first hint for a contribution of chromatin structure in memory formation.

# Mushroom bodies are necessary for non-elemental forms of learning in the honeybee.

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Honeybees learn to associate stimuli conisting of either pure odorants or their mixtures with a sugar reward during a pavlovian learning protocol (proboscis extension reflex conditioning). Although the neural pathways and memory dynamics associated with the formation of simple associations (e.g. odour A and sugar: A+) have been well studied, less is known about the brain processes underlying non-elemental forms of learning (Giurfa, 2003, Curr. Opin. Neurobiol. 13, 1-10). Non-elemental learning cannot be solved on the basis of simple links between stimuli (e.g. odour A - sugar) because non-elemental protocols raise ambiguity at the level of elemental stimuli. For instance, discriminations that oppose single odours (A, B) to their mixture (AB) imply treating the mixture as being, in principle, different from the simple sum of their elements. This situation corresponds to positive (A-, B-, AB+) or negative (A+, B+, AB-) patterning.

The mushroom bodies (MBs) are essentially involved in the formation and consolidation of a stable olfactory memory, and in memory retrieval. In addition, previous experiments on bees partially devoid of MBs after a hydroxyurea treatment suggested that they might be required for the resolution of non-elemental forms of learning (Malun et al., 2002, J.Neurobiol. 53, 343-363; Komischke et al., 2003, Behav. Brain Res. 145, 135-143). However, variations in the extent of ablations and potential developmental compensations were severe obstacles to further address this question. In this study, we have used a different approach to block transiently and selectively neural activity in the MBs: we injected procaine (a local anaesthetics) in the MBs (see also poster by Grünewald et al.) and tested the performances of animals during learning and memory retention.

Procaine (0.5nl, 20% in saline) was injected into the alpha lobes of each of the two MBs. Saline injections served as a control. Injections were performed 15 minutes prior to training. Four groups of bees were trained according to the following protocols: 1) positive patterning (A+, B+, AB-, 5 blocks of trials); 2) negative patterning (A-, B-, AB+, 6 trials) and their respective control conditions, using differential conditioning: 3) A+, B+, CD-) and 4) A-, B-, CD+ (where C and D are odours different from A and B). We show that interfering with MB function impairs the acquisition of non-elemental rules (groups 1 and 2), while the ability to learn elemental associations remains intact (groups 3 and 4). Thus, we show that elemental and non-elemental processing are not subtended by the same neural circuits and that local procaine injections are a useful tool to map the neural circuits required for learning in this species. Supported by the DAAD / MAE.

# Stress and learning in honeybees: effects of exposure to an alarm pheromone component on associative conditioning and memory.

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Stress is known to influence behaviour of many animals, and has been shown in particular to affect learning and memory in Mammals. Although adaptative responses to stress appear to be widespread in the animal kingdom, less is known about their importance and mechanisms in non mammalian species. We are interested in the relationships between stress and mnesic process in a somehow more simple organism, the honeybee (Apis mellifera). This species is an extremely valuable model for the study of learning and memory processes, as well as their neural and biochemical bases. Honeybees can be conditioned in a pavlovian protocol to associate an odorant with a sugar reward. The neural bases of this form of associative learning are well known, but its modulation through environmental factors is less well understood. Alarm pheromones, in particular isopentylacetate (IPA), the main component of the sting alarm pheromone, are considered as potential stressors because they are capable of activating an endogenous opioid system. It is therefore possible to combine olfactory conditioning and IPA exposure to understand how stress affects learning and memory in honeybees.

Based on previously published procedures, we exposed bees to IPA during 30 min and then trained them to associate an odour with sugar during 3 trials. Retention tests were performed 1 h later. The performance of these bees was compared to that of control bees exposed to solvent alone. We show that exposure to IPA leads to a dose-dependent decrease of learning performance. However, memory is not impaired. Indeed, odour-specificity of the memory at 1 h tends to be improved after exposure to low IPA doses. This last result is reminiscent of the positive effects of mild stress on learning in rodents. These results suggest a role for the opioid system in stress-induced modulation of mnesic processes. Injections of an opioid antagonist (naloxone) are envisaged to verify this hypothesis.

# Desert ants, *Cataglyphis fortis*, adjust their approach of a familiar goal with experience

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When visiting a familiar feeding site, desert ants steer some distance downwind of the feeder (Wolf & Wehner (2005) J Exp Biol 208: 4223), instead of attempting a direct approach that might miss the food source on the upwind side. In the downwind area, the ants are able to pick up the odour plume emanating from the food and follow it safely to the goal. On average, this strategy saves considerable walking distance and foraging time. The additional path necessitated by the downwind strategy is only about 0.75 to 2m, depending on nest-feeder distance. Missing the food on the upwind side, by contrast, affords search trajectories that are more than three times longer than the normal downwind approach, on average, and may be up to 30m long.

The distance steered downwind of the food is shortened over time, and it is also adjusted after changes in feeder location. Shortening of approach path and downwind distance, as well as a notable straightening of the approach trajectory, occur during the initial 4 to 5 visits of a novel food source. The approach trajectory stays relatively constant thereafter. On average, downwind distance and length of the approach path both decrease to about 50% of their initial values.

Desert ants further exhibit unexpected short-term flexibility in their approach trajectory, primarily with regard to ambient wind direction. Wind direction fluctuates slightly during the day, and notable changes in wind speed and direction occur over night. With regard to the minor diurnal fluctuations, experienced individuals are able to decide upon their departure from the nest which direction to choose towards the feeder, depending on momentary wind direction. With regard to the major changes in wind condition that occur over night, the animals adjust their approach en route to the altered wind direction during their first foraging trip in the morning. This is true for larger nest-feeder distances, while in the nest vicinity the approach of a feeder appears to be dominated by knowledge of the nest surrounds.

In summary, Cataglyphis ants exhibit unexpected and rather subtle adjustments of their approach of a familiar feeding site. These adjustments depend on experience and exhibit notable individual variability. They apparently serve to minimise foraging effort in terms of path length and time investment, and a concomitant reduction in exposure to heat and predators.

# The Learning Mutant *dunce* is Impaired in Ethanol Tolerance

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To understand the neuronal and molecular genetic basis underlying ethanol tolerance, we have established *Drosophila melanogaster* as a model system. Ethanol induced behaviour is in part learned and might be mediated by changes of cAMP levels (Lê and Mayer, 1996; Diamond et. al., 2002). However this has not been shown *in vivo*. We are interested to investigate whether learning and memory and ethanol tolerance has a common neuronal basis. Therefore we wanted to identify and analyze genes that are involved in both processes and determine whether they function in similar neurons to mediate the behavioural changes. We have previously isolated the *hangover* mutant that develops reduced ethanol tolerance. The *hangover* gene encodes a nuclear zinc finger protein. Here we provide evidence that the Hangover protein might modify RNA. Therefore we identified putative target transcripts of Hangover. One candidate gene is *dunce*. The gene *dunce* encodes a phosphodiesterase which regulates cAMP levels and in Drosophila *dunce* mutants show impaired learning and memory defects (Benzer et. al., 1976). We tested known *dunce* mutants for ethanol tolerance. The $dunce^{1}$ mutant shows a reduced ethanol tolerance phenotype. However the precise nature of the mutation causing the phenotype is unknown. The *dunce* gene consists of 14 transcripts which can be combined into four groups due to their function (Qiu and Davis, 1993). One transcript group has been implicated in initial learning. Therefore we made a transcript specific mutant $dunce^{\Delta 143}$ and tested this mutant for ethanol tolerance. Interestingly $dunce^{\Delta 143}$ mutants develop reduced ethanol tolerance. We are currently investigating whether this mutant is also impaired in learning. Taken together the phenotypic similarities between *hangover* and *dunce* mutants and the identification of *dunce* as a putative target of Hangover, we suggest that both proteins might act in the same pathway to promote ethanol tolerance.

# TOWARDS LOCALIZING A SYNAPSIN-DEPENDENT OLFACTORY MEMORY TRACE IN THE BRAIN OF *DROSOPHILA* LARVAE

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Synapsins are presynaptic phosphoproteins regulating the balance between the reserve pool and the readily-releasable pool of synaptic vesicles (Hilfiker, Phil. Trans. Royal Soc. (B), 1999). As regulation of transmitter release is a prerequisite for synaptic plasticity, we use the fruit fly *Drosophila* to ask whether Synapsin has a role in behavioural plasticity as well. We tackle this question for associative olfactory learning in larval *Drosophila* by using the protein-null mutant $syn^{97\ CS}$ (Godenschwege, EJN, 2004). We find that olfactory associative learning in $syn^{97\ CS}$ larvae is reduced to appr. 50 % of wild-type; responsiveness to the to-be-associated stimuli and all required motor faculties, however, are normal (Michels, L&M, 2005).
This learning phenotype now is the basis for localizing the cellular site(s) of Synapsin-dependent olfactory memory in the larval brain. Therefore, we have used the GAL4 binary transcription activation system to determine in which part(s) of the brain Synapsin expression is sufficient and/ or necessary for memory formation. Concerning sufficiency, both almost pan-neural (elav-GAL4) and mushroom body restricted expression (mb247-GAL4 as well as dunce-GAL4), fully rescue the memory defect of the $syn^{97\ CS}$ mutant. The expression pattern of Synapsin in these transgenic animals is confirmed by anti-Synapsin immunohistochemistry. Therefore, the mushroom bodies are a sufficient site for Synapsin-dependent memory. Concerning necessity, two different approaches, with either local suppression of GAL4 by GAL80 or by RNAi are currently used to test whether mushroom-body specific knock-down of Synapsin impairs learning.

# Out of Sight, but not out of Mind - A Spatial Memory Trace in the Brain of *Drosophila melanogaster*

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*Drosophila* posseses several distinct memory traces, which can be localized to different neuropils of the central brain: aversive and appetitive olfactory short term memory to the mushroom bodies (for review see Gerber et al. 2004), visual pattern memory to the fan-shaped body (Liu et. al. 2006) and tentatively pattern memory to the median bundle and/or the antennal lobe (Zars et al. 2000). Here, we assign for the first time a spatial memory for the position of visual objects in walking flies to the ellipsoid body of the central complex.

While flies approach a visual object in our paradigm, it becomes invisible. In this situation wild-type flies are able to pursue their chosen course for several seconds (Strauss and Pichler 1998). "After-fixation", as it has been termed, could be achieved just by maintaining a straight course (the fly is already on target) or by recalling a memory for the previous position. In order to discern between such a mere control of straight walking and the involvement of a short-term memory, we systematically lured the flies out of their straight path after the object disappeared by presenting an alternative object on the side of the fly. As soon as the fly walked up to the alternative object, it disappeared as well within less than a second. We then evaluated, whether the fly turned toward or away from the firstly presented visual object.

We found that wild-type flies resume a direct course toward the first seen object in (87 ± 6) % ($p<0.05$; t-test) of all cases. The outcome strongly implies the existence of a spatial working memory, where at least for a short time the position of the visual object is stored and can be retrieved. Whenever the neurotoxin tetanus toxin was conditionally expressed in various subsets of ellipsoid-body ring neurons via the GAL80 system (McGuire et al. 2004), flies took a random decision. The mushroom bodies, known centres for olfactory learning, could be excluded as a necessary neuropil for our particular spatial memory trace. Studies on the fan-shaped body are underway.

In summary, up to know exclusively the expression of tetanus toxin in the ellipsoid-body ring neurons prompted random orientation behavior, which speaks for the localization of a spatial working memory in the ellipsoid body.

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# Rutabaga-independent learning and memory in *Drosophila*

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The Rutabaga protein, a type 1 adenylyl cyclase that is regulated by $Ca2^{+}$/Calmodulin and G-protein and is considered a putative convergence site of the unconditioned and conditioned stimulus in olfactory associative learning[1-4]. The *rutabaga*(*rut*) gene is also required for visual pattern memory in the standard learning experiment in the flight simulator[5,6].

Interestingly, we found several alternative learning paradigms, in which the *rut* gene seems not to be required, as *rut* mutant flies do not show any learning impairment.

- In a purely operant learning experiment at the torque meter (yaw torque learning)[7], *rut*-mutant flies learn as normal as WT.
- No impairment of learning performance can be observed in *rut* in the "novelty choice[8]" experiment in the flight simulator.
- Utilizing the "novelty choice" learning paradigm for a colour discrimination task in which a single fly is made to control the appearance of two different colours by shifting a lever with its legs left or right, we find the learning performance of *rut* mutant flies not to be different from WT.

In these new paradigms the learning seems not to consist of an association between a conditioned and unconditioned stimulus. The data indicate that alternative biochemical/molecular mechanisms may underlie these more "incidental" types of learning/memory.

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# A role of the presynaptic protein SAP 47 in associative learning

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The SAP47 protein was discovered in an antibody screen for presynaptic proteins (1). It is member to a novel, phylogenetically conserved, gene family with unknown function. We here take a first step to understand the function of SAP47.

We use a deletion mutant in the *SAP47* gene (2) (*SAP47*$^{156}$); these animals suffer a 2.1 kb deletion in the regulatory region and the first exon of the gene. The mutant strain has been outcrossed to wildtype for nine generations to achieve equal genetic background in both strains. After verifying the lack of SAP47 in immunocytochemistry, these strains are tested in an associative learning task in larval fruit flies (3), during which the animals have to associate an odour with a food reward. We find that *SAP47*$^{156}$ mutants show reduced learning when compared to wildtype. Sensory ability with respect to detection of the to-be-associated stimuli and motor performance, however, are normal in the mutants.

Next, we will test whether transgenic expression of *SAP47*-directed RNAi constructs (2) has similar effects, and if so, where in the brain the expression of such RNAi constructs is sufficient to destroy learning ability.

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# Poster Topic

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**T30-1C** Structural Organization and Coupling of Cultured Brain Slices to Multi-Transistor-Array
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*E. Ofek and H. Pratt, Yokneam (Israel) and Haifa (Israel)*

**T30-6C** Imaging cellular network dynamics in three dimensions using fast 3D laser scanning

*W. Göbel, BM. Kampa and F. Helmchen, Zurich (CH)*

# Standardized Drosophila thoracic ganglia morphology

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Functional neuroanatomy requires an adequate analysis of the shapes of different parts of the nervous system. To development a quantitative toolkit to analyse functional neuroanatomy, standardized digital morphology atlases are currently being developed for a variety of brains. Great success has been made for specific parts of small insect brains. For Drosophila and honey bee brains standard average brains are now available on the web and can be used as a reference to register individual neurons, gene and protein expression patterns to develop a comprehensive framework to understand the construction principles of brains. However, no such standardized atlas exists for the thoracic ganglia, despite the fact that these contain the deeply analyzed insect motor pattern generators and most of the insect individually identified neurons. Combining knowledge on thoracic motor circuitry and identified neurons with a standardized brain morphology in a genetic model system will accelerate our understanding of functional neural network anatomy. Therefore, this study aims to generate a digital three-dimensional standard atlas of the drosophila thoracic-abdominal nervous system.
Neuropiles of female wild-type *Drosophila melanogaster* are labeled flourescently using a monoclonal antibody (Nc82) that marks active zones. Tracts are detected by labeling neuronal projections with anti-tubulin. Whole-mount preparations are imaged by confocal microscopy and data sets are labeled manually in all optical sections with AMIRA's segmentation tools. Multiple preparations are transformed on one reference label to correct for global and local shape differences and to compute one average label. The average label is now supplemented with multiple morphological details. Projections of legs, wings and halteres are visualized by backfilling and become registered into the standard. Furthermore, neuropile structure is functionally subdivided by expressing synaptic markers under the control of transmitter-specific drivers. And finally groups of individually identified neurons are visualized by selective GFP expression and become registered into the atlas. This atlas will then serve as a quantitative reference to address the neuroanatomical construction principles on the neuropile, network and single neuron levels by genetic manipulation experiments.

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# Impact of inhibitory mechanisms on cortical representation of adjacent fingers in human primary somatosensory cortex

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**Objective**

Thalamocortical projections originating in the somatosensory relay nuclei form excitatory synapses on pyramidal and spiny stellate cells as well as on inhibitory interneurons of primary somatosensory cortex (S-I) thereby mediating feedforward excitation and feedforward inhibition, respectively. Using fMRI, we have shown previously, that electrical finger stimulation closely below threshold for conscious perception ("subliminal stimulation") is associated with a focal BOLD signal decrease in contralateral S-I. We proposed that local inhibitory interneurons in S-I are preferentially activated during subliminal stimulation as strongly suggested by the functional properties of inhibitory interneurons, thus leading to a net reduction of neuronal baseline activity. Accordingly, intermingled subliminal stimulation leads to a significant reduction of contralateral S-I activation in response to supraliminal stimulation on the same finger (Blankenburg et al., 2003). Beyond this presumed correlate of cortical in-field inhibition, here, we investigated the impact of interneuron activity on cortical representation of adjacent fingers to assess lateral inhibition.

**Methods**

fMRI was performed on a 1.5 T scanner on 11 healthy subjects. Electrical current pulses were delivered via bipolar ring electrodes placed on the index (d2) and middle finger (d3) of the left hand. Sensory thresholds were determined. The paradigm consisted of two stimulation conditions: (1) stimulation of d3 at 4 Hz with an intensity markedly above sensory threshold, and, (2) stimulation of d3 (identical to condition 1) and concomitant stimulation of d2 closely below sensory threshold at 8 Hz, preceding the supraliminal pulse by 30 ms. Stimulation conditions (25 repetitions each) were presented in a pseudo-randomized order in blocks alternating with rest (duration 20 s). Standard imaging data analysis was performed using SPM2. T-maps of the random effects analysis were thresholded at $p<0.001$ uncorrected for multiple comparisons due to our a priori hypothesis.

In a psychophysical experiment, detectability of stimuli was testet with and without concomitant subliminal stimulation of the neighbouring finger.

**Results & Discussion**

For the group, condition (1) led to a focal BOLD signal increase within the hand area of contralateral S-I. Condition (2) was associated with a BOLD signal increase with a similar activation maximum. For the T-contrast "condition 1>condition 2" a significant signal difference was specified within S-I, but not for the inverse contrast.

Stimulus detection rate significantly decreased during additional subliminal stimulation of the neighbouring finger.

**Conclusions**

Cortical representations of adjacent fingers exhibit considerable overlap (Krause et al. 2001), and simultaneous electrical stimulation of adjacent fingers is associated with a suppressive interaction effect. Here, the S-I response to single finger stimulation is affected by concomitant subliminal stimulation of the adjacent finger. The spatial decrease of the cortical response to finger stimulation might reflect local interneuron activity serving lateral inhibition.

# Decision-making in eye-hand coordination: an event-related fMRI study.

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Deciding in favor of one out of several similar possible targets is a complicated process. Many factors are involved, making it difficult to control for. In our study, we nevertheless adressed this topic looking at simplified target selection in eye-hand coordination. Goal of this study was to identify areas in the human brain which are involved in the neuronal underpinnings of selecting one out of two physically equal targets.

We designed a task in which target selection was carried out using a combination of different effectors: eyes, eyes and index finger, and index finger alone while fixating a central target. Targets were presented either simultaneously or sequentially with variable intervals equally spaced on both sides of a fixation spot. Subjects had to indicate their choices with the appropriate effectors.
This kind of task allowed us to separate decisional from sensory and motor activation: trials containing every kind of effector or temporal sequence of target appearance could be grouped post hoc by decision for left or right target.
Recording of functional volumes was carried out using an event-related design. During imaging, target selection with the index finger as well as eye position were recorded.

We show the functional anatomy of deciding in favor of one out of two possible targets.

# Second-harmonic microscopy of microtubules in acute hippocampal brain slices

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Microtubules are a core component of the neuronal cytoskeleton. They not only serve structural functions but also play a crucial role in transport of proteins, in particular along the axon. Microtubules constitute highly ordered aggregates of tubulin heterodimers. As a consequence, they give rise to a second harmonic signal when excited with a pulsed Ti:Sa-laser. We have used a two-photon laser scanning microscope to elicit second harmonic signals from freshly prepared, unstained hippocampal brain slices, as well as cortical cell cultures, with an excitation wavelength of 970 nm and a resultant second harmonic wavelength of 485 nm.

In hippocampal slices, SH signals were observed particularly in axon initial segments, the mossy fibers, in apical dendrites and around blood vessels. The application of nocodazole (10 µM), a microtubule destabilizing agent, led to a pronounced decrease in the SH signals arising from neurons. Thus, generation of these signals requires ordered tubulin heterodimers and is a measure for the integrity of microtubules. We next examined the dynamics of changes in the SH signal arising from microtubules. These experiments revealed that the reductions of SH signals in axons initiate at the soma, thereafter propagating along the axons. This phenomenon reflects microtubular polarity, and allows us to determine an upper limit for the tubulin turnover rate in native neurons under physiological conditions. These techniques also allowed us to examine microtubular integrity and dynamics in hippocampal neurons of a chronic epilepsy model.

In conclusion, second-harmonic microscopy constitutes a promising tool to examine microtubule dynamics in living, unstained neuronal tissue.

# Quantitative aspects of the relationship between smooth pursuit eye movements and cortical activity as measured by fMRI

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Introduction

We investigated the quantitative relationship between smooth pursuit eye movements and cortical activations shown by blood oxygenation level dependent (BOLD) changes in the brain using functional magnetic resonance imaging (fMRI).

Methods

In the experiment we tested three stimulus frequencies of horizontal, sinusoidal target motion (0.09, 0.16 and 0.33 Hz, amplitude 10°). For control of the eye movement performance we used the Freiburg infrared eye tracking system for parallel eye data acquisition in the MR scanner. MR-imaging was performed in a 3T Siemens scanner. Eight healthy subjects participated in this study.

Results

Cortical activation was observed in the well-known smooth pursuit system: frontal and supplementary eye fields, cingulate gyrus, posterior parietal cortex, precuneus and V5/V5A region, depending on the frequency of the task.

The relationship between frequency and BOLD activation showed a V-shaped form with maximum activation at the highest frequency (0.33Hz) and lowest activation at the middle frequency (0.16Hz).

Eye movement performance remained nearly constant across tested frequencies with respect to smooth pursuit velocity gain and the occurrence of small correction saccades.

Conclusions

Our results demonstrate that the activation of the cortical smooth pursuit network varies with pursuit frequency (and peak velocity). Furthermore, there appears to be a pursuit frequency which is physiologically optimal and thus leads to less BOLD activation. This hypothesis needs further investigation as we only tested three different frequencies.

# Neural processing of food stimuli in eating disorders: a fMRI study

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**Introduction** Eating disorders affect 1-4% of young women and are commonly associated with a chronic course and social impairment. Somatic symptoms (e.g. underweight) and specific attitudes (e.g. drive for thinness, fear of fatness) are hypothesized to be secondary to alterations of cerebral neural circuits. In previous studies using functional brain imaging, abnormal frontal lobe responses to disease-related stimuli (images of food) in patients with eating disorders were found (Uher et al., 2001; Uher et al., 2004). Higher food-related activity occurred in the anterior cingulate and the medial frontal cortices in the whole group of eating disorders. So far there have been some experiments on anorexia nervosa but very few investigations in bulimia nervosa using functional brain imaging. Data from previous studies suggest that there are activation patterns which are common to the whole group of eating disorders while others are specific for bulimia nervosa or anorexia nervosa (Uher at al., 2004). The aim of this study was to confirm the findings from Uher et al. using a slightly different paradigm.

**Methods** We used fMRI to investigate brain responses to food images in women with eating disorders and healthy female comparison subjects. 20 female patients with eating disorders (10 with bulimia nervosa, 10 with anorexia nervosa) were matched for age, handedness and education. 3 hours after the last meal we presented food images to the participants of the study while brain activity was recorded. We were using colour photographs depicting savory and sweet foods presented on plates. These pictures were matched for colour and complexity with photographs of non-food items on neutral background. 10 food pictures in a 30 seconds block (on condition) were followed by 10 nonfood pictures (off condition). This sequence was repeated five times during the 10-minutes experiment. Data was acquired on a 3-T magnetic resonance system. After scanning participants rated the food images for pleasantness, disgust and fear.

**Results** In a preliminary analysis we found activation patterns in the expected regions as medial prefrontal and anterior cingulate cortices in anorectic and bulimic patients. Thus, we succeded in replication of data shown in first studies of bulimic patients before.

# The Human Corpus Callosum: Diffusion Tensor MRI of Regional Microstructure

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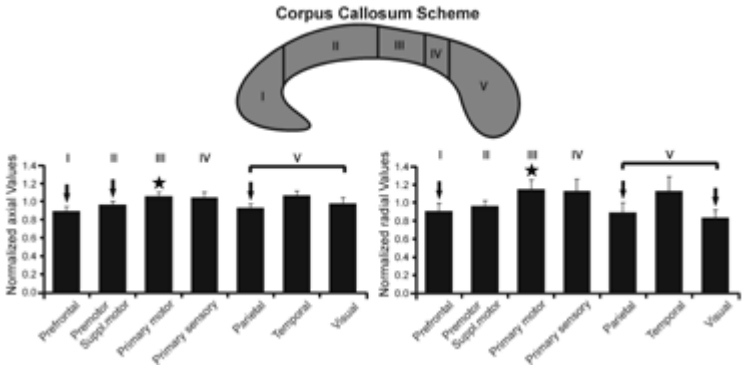
The corpus callosum (CC) serves as the major connection between the two brain hemispheres with more than 300 million fibers. Microscopy techniques (Aboitz et al. 1992, Brain Res 598:143) identified fibers with a relatively small diameter to be concentrated in the anterior and posterior third of the CC, whereas thick fibers were concentrated in the midbody and posterior splenium. The lack of morphologically discernable structures makes it difficult to perform comparative analyses of the CC topography using conventional MRI. In a recent work, we reconstructed comprehensive sets of callosal fiber bundles projecting to cortical hemispheres by tractography based on diffusion tensor MRI. These data were used to establish a new geometrical scheme of transcallosal connectivity (Hofer & Frahm 2006, Neuroimage 32:989). We could distinguish between fibers associated with prefrontal (see fig., region I), premotor (II), primary motor (III), primary sensory (IV), parietal, temporal and occipital cortical regions (V). Furthermore, in maps of the fractional anisotropy, we observed consistent differences in diffusion anisotropy across the CC in all subjects. The most pronounced anisotropy was found in anterior and posterior regions and the lowest values in middle regions. To further detail the regional microstructure of the CC, we evaluated the axial and radial water diffusivities, which characterize the magnitude of water diffusion along the longitudinal and perpendicular axis of the diffusion tensor ellipsoid representing an elongated fiber bundle.

MRI studies of healthy human subjects (n=8) were conducted at 2.9 T (Siemens Trio, Erlangen) using an 8-channel phased-array head coil. Diffusion tensor MRI was based on b values of 0 and 1000 s/mm$^2$ using 24 independent gradient orientations. Acquisitions were performed at 2 mm$^3$ resolution using diffusion-weighted STEAM MRI sequences and 5/8 partial Fourier encoding in combination with a projection onto convex subjects (POCS) reconstruction algorithm (Rieseberg et al. 2005, MRM 54:486).

In comparison with other regions of the CC, both the axial and radial diffusivities are significantly higher in the middle region (region III, see fig.) assigned to the motor regions (asterisks; arrows: significant differences to other regions). Significant differences were also observed between other regions (not shown). This is in general agreement with histological data showing different fiber diameters in particular regions of the CC. Hence, regions with less densely packed fibers with thick axon membranes, thick myelin sheaths, and large diameters (like the callosal motor fibers) lead to both high axial and radial diffusivities. In contrast, less densely packed and lightly myelinated thin fibers, as found in regions I, II, and V result in lower values. We therefore assume a correlation between the degree of axial and radial diffusivity in the CC and the microstructure of fiber bundles.



# Dopamine Transporter genotype alters N-Acetylaspartate in left putamen

Harald Scherk[1, 2], Martin Backens[3], Susanne Kraft[1], Claudia Kemmer[1, 2], Wolfgang Reith[3], Jobst Meyer[4], Peter Falkai[2] and Oliver Gruber[1, 2]

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Introduction: The neurotransmitter dopamine plays an important role in regulation of basic brain functions including motor behaviour, motivation and working memory. Several lines of evidence suggest that prefrontal dopamine has specific function in attention control and working memory mediated mainly through the D1 receptors. Dorsal striatal dopamine in the caudate and putamen is essential for execution of learned motor programs. We hypothesized that dopaminergic activity and neuronal viability in distinct brain regions is modulated by the gene of dopamine transporter DAT1 that mediates the dopamine re-uptake in the presynaptic neuron. In the DAT1 gene a 40-bp variable number of tandem repeats (VNTR) polymorphism was identified. The VNTR is repeated between 3-13 times, with greatest frequency in the 9- and 10-repeat forms in most human populations. Proton magnetic resonance spectroscopy (1H-MRS) is an imaging method which allows in vivo determination of several brain metabolites such as N-acetyl-aspartate (NAA), choline-containing compounds (Cho), creatine + phosphocreatine (Cre) and inositol + myo-inositol (Ins). The aim of this study was to determine whether the DAT1 VNTR alters the metabolic ratios NAA/Cho, NAA/Cre, Cho/Cre and Ins/Cre in the left prefrontal cortex, anterior cingulate cortex and putamen in healthy individuals and patients with bipolar disorder or obsessive-compulsive disorder.

Subjects: Thirty euthymic patients with bipolar I disorder, 17 patients with obsessive-compulsive disorder and 16 healthy control subjects participated in the study.
Imaging: Single-volume proton magnetic resonance spectroscopy was performed on a 1.5-Tesla Siemens Magnetom Sonata using a spin-echo sequence with water-suppression and 128 scan averages (TE = 30, TR = 1500). Regions of interest were determined in left dorsolateral prefrontal cortex, left anterior cingulate cortex and left putamen.

Results: The individuals with the homozygote DAT1 10-repeat genotype presented significantly higher ratios of NAA/Cho and NAA/Cre in the left putamen compared to the group of individuals with the homozygote 9-repeat or heterozygote 9/10-repeat genotype. No associations between DAT1 VNTR polymorphism and the other metabolic ratios in putamen or all metabolic ratios in the left prefrontal cortex or left anterior cingulate gyrus were observed.

Conclusion: The VNTR polymorphism of the DAT1-gene modulates NAA-ratios in putamen independent of psychiatric diagnosis status. These results suggest an association of DAT1 polymorphism and dopaminergic activity in putamen. Further studies are needed to enlighten the functional basis of this association.

# Disturbed cortico-amygdalar functional connectivity as pathophysiological correlate of working memory deficits in bipolar affective disorder

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Background: Patients suffering from bipolar affective disorder exhibit deficits in working memory functions. In a recent fMRI study we observed an abnormal hyperactivity of the right amygdala in bipolar patients during articulatory rehearsal in verbal working memory. In the present study we investigated the dynamic neurofunctional interactions between the right amygdala and the brain systems that underlie verbal working memory in both bipolar patients and healthy controls.
Methods: 18 euthymic bipolar patients and 18 healthy controls performed a modified version of the Sternberg item-recognition (working memory) task. We used the psycho-physiological interaction (PPI) approach in order to assess functional connectivity between the right amygdala and the brain regions involved in verbal working memory.
Results: In healthy subjects, we found significant inhibitory functional interactions between the amygdala and multiple brain regions involved in verbal working memory. By contrast, in bipolar patients these functional interactions with cortical areas were significantly diminished (absent) in the right hemisphere.
Conclusions: Together with our previous finding of amygdala hyperactivity in bipolar patients during verbal rehearsal, the present results suggest that a disturbed right-hemispheric "cognitive-emotional" interaction between the amygdala and cortical brain regions supporting working memory may be responsible for amygdala hyperactivation and verbal working memory deficits in bipolar patients.

# The initial emotional responses to pleasant and unpleasant music: An fMRI study.

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The present study used short pleasant and unpleasant music excerpts and their manipulated counterparts to determine neural correlates of emotion processing with functional magnetic imaging (fMRI). It contributes to the investigation of the timecourse of emotion, focusing on the initial response to acoustic stimuli with positive and negative valence. Furthermore, it disentangles the neural correlates of the perception of 1.) A corruption of a vertical organization of music (spectrum), and 2.) A corruption of a horizontal organization of music (forward/backward). A correlation of BOLD signal with decreasing valence revealed an activation of the right amygdala, the premotor cortex bilaterally, the right motor cortex, the paracentral lobe bilaterally, and the right superior parietal lobe. The activation of the amygdala in the response to increasing unpleasantness of the music replicates a previous finding (Koelsch et al., 2005), but now revealing that the amygdala is already activated in the initial response to unpleasantly manipulated music. A correlation of BOLD signal with increasing valence revealed activations in the frontomedial orbitofrontal cortex bilaterally, the right BA44, the left SMA, the left globus pallidus, the left putamen, the STG bilaterally, and the right cerebellum. The orbitofrontal cortex has previously been shown to be involved in emotion processing during music listening; the present data provide the first evidence for such an involvement with functional MRI. Further interesting findings are that the gyrus angularis and the gyrus supramarginalis, two regions that are involved in semantic processing, are activated by the perception of the corruption of a horizontal dimension of music (forward/backward).

# Suitability of Manganese as a Tracer for Functional Imaging in the Brain - A Study on the Inferior Colliculus and the Auditory Cortex of the Gerbil

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Manganese can be used as a contrast agent in magnetic resonance imaging. The paramagnetic tracer enhances the signal intensity in T1-weighted images. The divalent cation $Mn^{2+}$ is an attractive tracer for functional neuroimaging since neurons take up the ion in an activity-dependent manner. Quite recently (Yu et al. 2005), manganese-enhanced magnetic resonance imaging (MEMRI) has been used for high resolution imaging of neuronal activity in the inferior colliculus of mice. However, due to the poor blood-brain barrier permeability of $Mn^{2+}$ long stimulation times (24h-48h) are necessary to achieve a sufficiently high signal intensity in MEMRI after systemic application of the tracer. These long stimulation times could result in a redistribution of $Mn^{2+}$ in the brain due to either passive diffusion or active axonal transport.

We here compared the manganese distribution in the brains of acoustically stimulated Mongolian gerbils at early (2h) and late times (24h) after subcutaneous $Mn^{2+}$-injections in order to analyse the effects of redistribution. Especially for the early times, where $Mn^{2+}$ is difficult to detect in the brain with MEMRI, we made use of a sensitive histochemical technique, manganese autometallography, for manganese detection in the brain. We focused on the Inferior Colliculus (IC) and the Auditory Cortex (AC) of gerbils (Meriones unguiculatus) stimulated with alternating pure tones of 1 and 8 kHz for short-term (2h) and long-term (24h) periods.

Classical anatomical staining methods (Golgi-, Nissl-, PV-Fiber-stainings) served as a further control of the histochemical $Mn^{2+}$-findings to verify to which extent the $Mn^{2+}$-distribution reflects stimulus-induced activation patterns or simple anatomical differences.

With short-term stimulation we found, in the auditory cortex, evidence for manganese-uptake in tonotopically ordered bands indicating that the Mn-distribution reflects neuronal activity. Upon long-term stimulation we were unable to find such stimulus-related bands in the AC. In the Inferior Colliculus we could not find tonotopically ordered bands in either short-term or long-term stimulated animals. Instead, a prominent band of high Mn-uptake was present in the IC of all animals irrespective of the stimulus used.

Our results indicate that the $Mn^{2+}$-distribution in the brain is affected by a number of factors other than neuronal activity. Especially in long-term stimulation studies the manganese distribution may not necessarily reflect patterns of neuronal activity and the results of such studies have to be interpreted with caution.

# Single-cell resolution mapping of neuronal activity in rat Slow-Wave Sleep: A thallium-uptake study

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Until now there has been no method that could provide a sufficient spatial and temporal resolution to investigate distinct sleep-phases in rodents on the level of singular neurons. Here we used a modified version of the recently introduced Thallium-Autometallography, a method that utilizes the tight coupling of neural activity to potassium reuptake, to map the metabolic state of SWS in the rat.
Results from human- and animal studies indicate that sleep, and to a large part what is termed Non-REM or Slow-Wave Sleep (SWS), is essential for the acquisition of new declarative memory and procedural skills. Sleep is also of special interest for understanding neuronal correlates of waking consciousness by comparing it with states where consciousness is either altered or absent, as it is in dreaming and deep sleep. Although extensive research was carried out, especially in recent years, there remains a host of unanswered questions about these topics.
Our data suggest that, different than one would intuitively expect, despite the global decrease in metabolism, activity patterns in cerebral cortex remain highly complex with an activity-bias for the deeper layers (V/VI) and a significant diminution of activity in the upper layers. We also report the occurence of distinct metabolic patterns on the level of neuronal ensembles in the deeper layers and relative activity changes in certain cortical and subcortical fields.

# Structural Organization and Coupling of Cultured Brain Slices to Multi-Transistor-Array

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Recordings of neuronal activity with high spatiotemporal resolution over a large area of brain tissue give insight into correlations in space and time which are the key to understanding of complex nervous tissue on the level of its network. Therefore much activity is dedicated to the coupling of neuronal networks to arrays of electrodes or transistors.

Well-investigated planar systems for this purpose are organotypic hippocampal brain slice cultures. These organotypic cultures were coupled to a high-density two-dimensional Multi-Transitor-Array (MTA) based on an extended complementary metal oxide semiconductor (CMOS) technology [1]. Time-resolved maps of electrical field potentials at a spatial resolution of 7.8 µm on an area of 1 $mm^2$ results from recording with this MTA. This is an improvement to the spatial resolution of conventional multi-electrode array technology, which is rather low or restricted to small areas.

In this study we analyse the structure of the slices cultured on the MTA by means of two photon Laser-Scanning Microscopy. The intrinsic three-dimensional information achieved by this nonlinear optical technique makes it possible to reconstruct the detailed cellular structure. With this information it is possible to correlate the three dimensional structure to the two dimensional time resolved map of electric field potentials. A crucial step towards understanding of the linkage between three dimensional field-potential-map and recording with planar structures is done.

The mechanism of the coupling of complex tissue to planar structures is fundamental for every use of neuronal prosthetics. On the one hand the measurements give an insight into the detailed mechanism of the coupling of the slices to the chip. On the other hand it is now possible to study the changes in field potential over short distances with knowledge of the cellular structure of the slices. With this studies we aim for an improvement of the known theoretical models.

References:

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"High-Resolution Multitransistor Array Recording of Electrical Field Potentials in Cultured Brain Slices" J. Neurophysiol. 96: 1638-1645

# Long-range calcium waves in the brain of adult mice

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Recently, we reported the development of an optic fiber-based method for the fluorometric detection of calcium signals in vivo in behaving animals (Adelsberger et al., Nature Neuroscience, 2005). This single-point detector system records intracellular calcium changes occuring in groups of synchronously active neurons. Such calcium waves appear spontaneously or in response to sensory stimuli. In order to determine the action range of the cortical calcium waves, we now introduce a new detection system that uses two independent optical fibers for measurements in distinct brain regions. The new double-fiber system consists of separate optical pathways, but a common laser for the delivery of the excitation light and an integrated data recording module. We used our optic fiber-based recording device in combination with two-photon imaging to estimate the number of cells that contribute to individual calcium waves in vivo. For this purpose, we evoked calcium waves by locally applying glutamate in a pulse-like manner to the cells in the visual cortex of anesthetized mice. First, we performed recordings with the optical fiber-based system. Then we used the same stimulation protocol in combination with high-resolution two-photon imaging to detect all cells that were activated by the stimulus. From these recordings we estimate that the optical fiber-detected calcium waves are the result of the simultaneous activity of several hundred up to about 2000 neurons. We next investigated the spontaneous wave activity of the primary auditory cortex of adult mice. First, we studied mice that were minimally anesthetized (0.6 - 1% isoflurane) and detected recurrently occurring calcium waves of large amplitudes. Their frequency was about 0.4-0.5 Hz. In view of earlier work using electrical and calcium recordings in other cortical areas, we suggest that the spontaneous calcium waves represent neuronal activation that is associated with the up-down state of cortical activity. An important, controversially discussed question is whether this activity requires anesthesia. This question can be easily addressed under our recording conditions. We found that the activity persists in awake mice. Interestingly, the activity pattern changed from nearly regularly occurring waves to wave bursts that were interrupted by silent or plateau states. Finally, we used the double fiber system to identify brain structures that displayed calcium waves that were correlated to those measured in the auditory cortex. First we tested various cortical regions and found tightly correlated calcium waves in other primary sensory regions (visual and barrel cortex) as well as in secondary cortical areas (frontal and entorhinal cortex). As expected from earlier electrical recordings done by others, time-locked calcium waves were always observed in the thalamus. Interestingly, large amplitude correlated calcium waves occurred invariably also in the hippocampus. Taken together, our results demonstrate the presence of long-range correlated calcium waves throughout the cortex but also in remote subcortical areas.

# Age Related Cognitive Decline Correlates with Regional Brain Changes Measured by Q-space MRI

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Aging is a complex heterogeneous process accompanied by a cognitive decline. Although defined as a natural condition, many pathological neurodegenerative processes are involved in aging, manifested by reduced memory, executive function, motor abilities and processing speed.

The main hypothesis of this research is that age related cognitive decline is a complex, not specific and multi-regional process without repeated pattern across subjects.

In the present research we used Q space imaging (QSI), which is a non invasive, highly sensitive MRI method for detection of brain changes. QSI was performed in brains of 50 subjects, age 25-82y. Subjects also underwent a series of neuropsychological tests outside the scanner (including memory performance, executive function, motor abilities, visual-spatial and verbal function). The subject's test performance as well as subject's age served as covariate correlation inputs for voxel based morphometry (VBM) of MRI scans (see figure 1). Region of interest (ROI) analysis was performed based on the VBM results and the correlation coefficient was extracted for each test in each ROI.

The results showed that age is highly correlated with QSI indices in all ROIs, while the correlation of cognitive performance with QSI indices in each ROI was task specific. For example, in the right thalamus, the correlation coefficient with memory was r=0.46, while in other tasks (motor, visual spatial and verbal tasks) a correlation coefficient smaller then 0.35 (r<0.35) was found. An exception was the stroop interference task, which showed a high correlation in all ROIs, implying that it is a non specific multi-regional task (see table 1).

In conclusion, although brain changes with age are multi-regional, the cognitive abilities of subjects show region specific brain changes, which allow a more focused observation of the heterogeneous aging process.

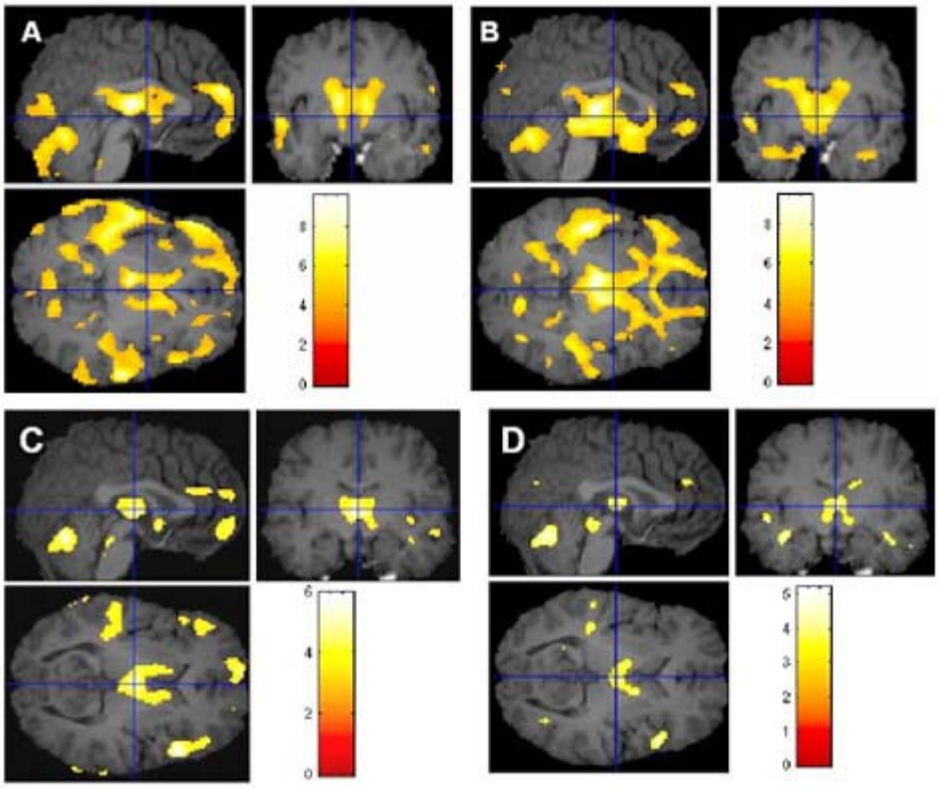


**Figure 1.** Signigicant correlation between (A) displacement (B) probability with age, ($p<0.0001$) and Correlation between displacement (C) probability (D) with accuracy in the non verbal memory task. ($p<0.001$). The colored areas indicate significant orrelation in the displacement and probability map.

# New Methods for the P300 Visual Speller

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The Visual Speller is a Brain-computer interface (BCI) based on ERP's. In the classical paradigm one trial consists of successive highlightings of one or more symbol(s) on a visual grid presented to the subject. The stimulus events in which the symbol of interest was highlighted will result in an enhanced ERP. The more symbols are highlighted simultaneously the faster the speller gets. A stimulus code that uses few events per trial is called dense. The tradeoff with dense codes is that the signal to noise ratio gets worse with increasing stimulus frequency: the P300 signal is reported to be strongest when the target symbol frequency is lowest. The stimulus code in which only one symbol per stimulus event is presented, is a maximally sparse code.
It has been proposed that high bitrates of information transfer in a visual speller can best be achieved with sparse stimulus codes. However sparse codes have long trial durations. In order to improve the information transfer rate, we tried to use denser stimulus codes that present fewer stimulus events per trial. To investigate the effect of stimulus type on classification accuracy and the interdependence of stimulus code and type, we explored new stimulus types including ones exploiting recent findings in neuropsychology, such as change blindness and isoluminant color motion. We show that, using appropriate stimuli, denser codes, and hence fewer stimulus events, yield sufficient classification accuracy to achieve competitive bitrates.

# The combined effects of cue validity and verbal distracters in a modified auditory cued attention (Posner) task - an ERP functional imaging study.

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Objective: To study the interaction between cognitive (cue validity) and emotional (subjectively significant verbal stimuli) effects on brain activity. Subjective significance effect in the invalid condition is of particular interest because of possible emotional impact of the invalid condition ("being lied to").
Methods: Event related potentials (ERPs) were recorded while 12 right-handed subjects performed an auditory tonal cued attention task with subjectively significant verbal distracters presented at different points between cues and targets. During the task, cues (in most cases valid) provided information on the appropriate response to the subsequent target stimulus. Verbal distracters were first names whose subjective significance was assessed after the experiment with a questionnaire. ERPs were averaged according to cue validity and the subjective significance of the distracters. Intracranial sources of the scalp activity were estimated from the ERPs by LORETA (Low Resolution Electromagnetic Tomographic Analysis). Interactions between cue validity and distracters' subjective significance were assessed.
Results: Cue validity effects included enhanced cortical activation to invalidly cued targets. Enhanced activation to invalidly cued targets was located mostly in the general location of the left cuneus. The effect of subjective significance in the valid condition has already been reported by us, with early activation (P1, 40 msec) in the right insula and only at later latencies (N1, 100 msec) in the left cingulate gyrus. In this study, in response to invalidly cued targets administered after subjectively significant distracters, enhanced early activation, as early as P1 (40 msec), was noted in the approximate locations of the auditory cortex and cingulate gyrus as well as the right parahippocampal gyrus. Effects of subjectively significant distracters were also evident for the late ERP components (P350 and P670). Enhanced activation was found in the right prefrontal cortex augmenting the cue validity effect.
Conclusions: Brain activity was found to be modulated by preceding subjectively significant stimuli, irrespective of their exact timing. Early ERP components (P1) to targets were affected by the subjective significance of the preceding distracters, in the invalid condition.
Significance: The results indicate an effect of subjectively significant distracters on subsequent brain activity with an interaction between cognitive and emotional processes.

# Imaging cellular network dynamics in three dimensions using fast 3D laser scanning

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Multi-cell bolus loading of calcium indicators in combination with 2-photon microscopy enables in vivo measurement of the distinct calcium dynamics in neuronal and astroglial cell populations. So far, however, population measurements with sufficient temporal resolution to resolve neural activity (>=10 Hz) have been restricted to a rather small number of cells (up to 40) within a single 2D image plane. Methods to image network activity in three dimensions with comparably high temporal resolution are lacking.

Here we present a novel 3D-linescan technology for 2-photon microscopy that permits fast fluorescence measurements from several hundred cells distributed in 3D-space. Using a piezoelectric focusing element the microscope objective is vibrated in a sinusoidal manner at 10 Hz along the axial direction (up to 400 µm amplitude). Standard galvanometric mirrors are used for xy-scanning with their movements matched to the axial vibration so that a closed scanline is generated in 3D space, which is continuously repeated. The scan pattern can be tuned to hit the vast majority (95%) of cell bodies contained within a volume of about 250 µm side length. In addition, the 3D scan trajectory can be user-defined to target pre-selected cells or structures such as blood vessels.

We applied this new method to 3D imaging of calcium dynamics in neurons and astrocytes in superficial layers of rat somatosensory cortex. Cells were bulk-loaded with the calcium indicator Oregon Green BAPTA-1 AM and astrocytes were counterstained with the red-fluorescent dye sulforhodamine 101 (SR101). 3D linescan measurements were stable over time (for at least 10 min) and simultaneous calcium measurements could be achieved from several hundred cells with 10 Hz temporal resolution. Thus, the spatiotemporal pattern of local network activity could be resolved in the superficial layers of a cortical column. Using counterstaining techniques (e.g. SR101 for astrocytes) different cellular subtypes can be identified, revealing the 3D activity pattern in specific sub-networks. In conclusion, the 3D-linescan method for the first time permits fast and comprehensive in vivo measurements of cortical microcircuit activity. We expect this method to enable fundamental insights in the function of local neuronal and glial networks.

# Poster Topic

## T31: Limbic System, Motivation, Emotion

**T31-1A** A 'depressive-like' state induced by a wasp's sting into cockroach brain
*R. Gal and F. Libersat, Beer-Sheva (Israel)*

**T31-2A** Dissociating the neural correlates of different types of biological significance
*EK. Schlüter and O. Gruber, Göttingen and Homburg*

**T31-3A** A model of temporal lobe epilepsy: Intrahippocampal kainate injection and its related behavioural phenotype in mice
*I. Gröticke, K. Hoffmann and W. Löscher, Hannover*

**T31-4A** How are subjective feelings and physiological responses related? The synchronicity of emotion components changing over time in response to music.
*O. Grewe, F. Nagel, R. Kopiez and E. Altenmüller, Hannover*

**T31-1B** Appetitive and aversive reinforcement and their interaction during auditory learning.
*AI. Micheal, W. Wetzel, H. Scheich and F. Ohl, Magdeburg*

**T31-2B** ULTRASONIC VOCALIZATIONS IN RATS: EFFECTS OF EXPERIENCE AND CONTEXT ON 50-kHz CALLS.
*M. Wöhr, B. Houx, RKW. Schwarting and B. Spruijt, Marburg and Utrecht (NL)*

**T31-3B** DIFFERENTIAL BEHAVIORAL PROFILE INDUCED BY THE INJECTION OF CHLORAZEPATE DIPOTASSIUM IN BRAIN AREAS THAT PROJECT TO THE NUCLEUS ACCUMBENS SEPTI
*PA. GARGIULO, L. LLANO, M. FRAILE, M. OLGUÍN, P. MELONARI, F. CAIF and A. LANDA, Mendoza (Argentina)*

**T31-4B** DIFFERENTIAL STRESS RESPONSE IN SPRAGUE DAWLEY (SD), WISTAR (W) AND OFA hr/hr (O) FEMALE RATS. EFFECTS OF DIAZEPAM.
*AS. GONZALEZ, EL. RODRIGUEZ ECHANDIA, S. VALDEZ and G. JAHN, MENDOZA (Argentina)*

**T31-1C** Does emotional state affect human performance in spatial cognition tasks?
*G. Hardiess, S. Saydam and HA. Mallot, Tübingen*

**T31-2C** Does the Modulation of the Insular Activity Affect Our Emotional Involvement?
A Real-Time fMRI Study during Emotional Pictures Processing
*A. Caria, R. Sitaram, R. Veit, A. Kuebler, M. Lotze and N. Birbaumer, Tuebingen*

**T31-3C** Differential regulation of synaptic vesicle proteins in serotonin transporter deficient mice - with and without acute stress exposure
*SL. Nietzer, AG. Schmitt, M. Maier, G. Ortega, C. Kriegebaum, L. Gutknecht, EM. Bogusch, P. Riederer, J. Deckert and KP. Lesch, Würzburg*

# A 'depressive-like' state induced by a wasp's sting into cockroach brain

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Insects are not just 'automatons', but rather undergo complex decision-making processes to select for appropriate behavioral responses. Yet, the insects' narrow behavioral repertoire combined with their relatively simple neuronal organization make them useful models for neuroethological studies. Hence, the question arises whether insects can be used as realistic models in behavioral neuroscience for complex mammalian behaviors and disorders.

Cockroaches stung by the parasitoid wasp A. compressa become dramatically hypokinetic, although not paralyzed. The wasp's neurotoxic venom cocktail is injected directly into the cockroach head ganglia and, as a result, the cockroach fails to initiate spontaneous locomotion and escape responses to tactile stimuli. The venom's effect persists for a few days, during which the stung cockroach appears to have lost the motivation to initiate movement, as if in a helpless- or depressive-like state. To explore this possibility, we compared stung to control cockroaches in two behavioral paradigms traditionally employed to study learned helplessness and depression as cognitive disorders in mammals. First, by applying electric shocks of measurable amplitude, we show that the number of escape failures to the shock is significantly higher in stung compared with control cockroaches. However, markedly higher shock amplitudes evoke an escape response, demonstrating that the stung cockroaches are physiologically capable of producing such responses. This elevated threshold for locomotion cannot be solely explained by an unspecific anesthesia since the startle (nociceptive) response to the shock is modulated to a lesser extent. Second, by submitting cockroaches to the forced swimming test, we show that swimming duration is significantly reduced in stung cockroaches compared with controls. Yet, during swimming episodes, specific features of locomotion such as inter-leg coordination, stepping frequency range and correlation between stance duration, discharge rate of leg muscles, and cycle frequency are unaffected in stung cockroaches. However, for a given stepping frequency, the discharge rate of leg muscles is decreased in stung cockroaches, suggesting a reduction in excitability of motor centers.

These results suggest that the venom cocktail of A. compressa manipulates central mechanisms involved in modulating the "motivational" state of its insect prey to impact on the initiation of movements. Furthermore, this behavioral manipulation can be analyzed and quantified using established paradigms in mammalian research.

# Dissociating the neural correlates of different types of biological significance

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Objective:
Infrequent events that require a behavioral adjustment have been shown to activate the posterior OFC (Schlueter & Gruber, 2005). The posterior OFC has also been reported to be involved in context-dependent reward processing (Elliott et al. 2000; Gottfried et al. 2003) and processing of salient rewards (Elliott et al. 2003). The observed OFC activation might therefore underlie a specialized mechanism that does not emerge exclusively in the context of reward, but whenever a salient behaviorally relevant event occurs. This mechanism would allow for the rapid adjustment of behavior towards or away from salient events and grants behavioral flexibility.

Methods:
In the present study, we systematically varied the exact nature of behavioral relevance which allowed us to directly compare neural mechanisms involved in reward processing with neural responses to unrewarded but rare events that required a rapid adaptation of motor-behavior.
10 subjects underwent fMRI in a 3-Tesla-Scanner while performing a cued task-switching experiment including different types of behaviorally relevant events. Two different objects were presented that could appear in four different colors. The objects and two of the colors were always mapped to a left or right manual response and appeared in both tasks. The remaining two colors were exclusively presented in the shape task and formed our "critical infrequent events", that could either be:
(1) response-irrelevant
(2) response-irrelevant and rewarded
(3) response-relevant
(4) response-relevant and rewarded.
Half of the remaining trials were also associated with a reward (reward was no infrequent event in itself). Cues provided both task and reward information. To prevent subjects from counting the rewarded trials, their mean performance in reward-trials was further considered for a ranking-list of all participants. The top-three players won ¬50. This competitive setting created the incentive for optimization of performance in reward trials. Preprocessing and analyses of the imaging-data were done using SPM2.

Results & Discussion:
The three contrasts including different types of behavioral relevance revealed a significant activation in the right posterior OFC. In addition, left medial OFC represented rewarded events, while right anterior OFC was solely activated by response-relevant infrequent events requiring a behavioral adjustment (Fig. 1). According to Kringelbach & Rolls (2004) different aspects of reward processing are located in distinctive OFC-subregions. Considering our results, right anterior OFC grants behavioral flexibility, while left medial OFC may code the positive hedonic value of rewarded events. Finally, the posterior OFC may indeed be considered as a candidate region for processing of salient behaviorally relevant events in general.


Figure 1: Subtraction contrasts showing the effect of different types of salient „behavioral relevance" displayed on a rendering of the orbitofrontal cortex.

# A model of temporal lobe epilepsy: Intrahippocampal kainate injection and its related behavioural phenotype in mice

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Epilepsy patients frequently develop psychiatric and cognitive comorbidities, but suitable mouse models modelling this phenomenon still do not exist.Constituting a model of temporal lobe epilepsy, the unilateral injection of kainic acid (KA) intra-hippocampally into the CA1 region of mice causes a non-convulsive status epilepticus (SE) as well as subsequent long-term electroencephalographic and pathohistological changes, whereas little is known about the related behavioural phenotype.

We stereotaxically injected KA into the right dorsal hippocampus of NMRI mice and implanted a bipolar electrode in the same place. Sham mice received intra-hippocampal saline instead. Furthermore, a naïve control group was included in the study. The first EEG and video recording was made immediately after surgery and lasted until the next morning.

Following KA administration, mice slowly developed SE lasting for several hours, whereas sham-injected mice did not show any EEG alterations. In the following weeks, EEG recordings once a week revealed the occurrence of spike-wave discharges in KA-treated mice. In some animals, generalized motor seizures could be observed during handling procedures. KA- and sham-injected mice as well as control mice passed a behavioural test battery consisting of several test domains testing for general health, motor deficits, exploratory behaviour, anxiety-related behaviour and spatial learning abilities. None of these tests revealed any behavioural changes in KA-injected mice compared to sham-injected or control mice except for two subtests of the Morris water maze test. In these tests, KA-treated mice revealed deficits in remembering the previously well learned platform position during retention test of the Morris water maze procedure, and the cued version seemed to prove an intact visus but difficulties to integrate new visual information.

Compared to previous experiments with the pilocarpine model of temporal lobe epilepsy in mice, our study indicates that intra-hippocampal injection of KA produces a much more discrete epilepsy with almost no behavioural alterations in a battery of test systems. The most likely explanation for this striking difference between models is the severe neurodegeneration produced by pilocarpine vs. the more subtle morphological alterations seen in the intra-hippocampal kainate model.

# How are subjective feelings and physiological responses related? The synchronicity of emotion components changing over time in response to music.

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Emotions are expected to show a subjective feeling as well as a physiological reaction component [1]. However, the exact relations between feelings and physiology, especially an eventual synchronization in time, are still unclear. Music is an ideal stimulus to study synchronization effects in emotion components since it develops over time. Additionally, music is known to frequently elicit affective peak experiences like goose bumps or shivers down the spine. These "chills" [2, 3] can be used as a paradigm for strong emotional reactions to music combining subjective and measurable bodily reactions.
The 95 participants (mean age: 39, SD: 16, 50 females, 45 males) listened to five movements from W.A.Mozarts Requiem KV 626 (Lacrimosa, Confutatis, Tuba mirum, Rex tremendae, Dies irae). A subgroup of 54 participants was highly familiar to the stimulus, being members of non professional choirs who performed the Requiem in public concerts (test group). The other 41 participants did not to know the Requiem (control group).
The participants were asked to report the intensity of their perceived feelings continuously on a scale from -10 to 10 while they were listening to the pieces. Additionally they pressed a mouse button whenever they perceived a chill. The self reported data was synchronized with continuous measurements of skin conductance response (SCR), heart rate (HR) and breathing rate (BR), as well as a loudness analysis of the musical piece.

Intensity ratings, reported chills and SCR are positively correlated to loudness in the Lacrimosa, whereas correlations of the same parameter are weak and even negative in the Confutatis. Additionally, the beginning of all pieces results in an increase in SCR and decrease in HR. The test group rated intensity significantly higher and reported more chills in response to all pieces but the Tuba mirum.

A detailed analysis of reported feeling intensity, chills and physiological responses suggests the conclusion that chills depend on cognitive evaluation of music, which is based on familiarity and liking of the stimulus. Physiological reactions seem to be influenced by mere physical stimulation (loudness), orientation or startle responses (e.g. beginning of a piece, entry of a voice) and higher cognitive reactions (e.g. feelings, individual associations).

This work was supported by the DFG (grant no. AL 269-6) and the Center for Systemic Neurosciences Hannover

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# Appetitive and aversive reinforcement and their interaction during auditory learning.

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In conditioning experiments, changes in behaviour can be elicited by either appetitive or aversive reinforcement. However, it is not clear how both types of reinforcement interact during classical and instrumental conditioning. Our learning paradigm employs the combination of both types of reinforcements in the same training session. Here, we study their interaction during auditory learning in the shuttle box using foot shock as aversive reinforcement and electrical stimulation of the ventral tegmental area as appetitive reinforcement. We are trying to address the links between the affective states (either avoiding the aversive stimuli or approaching the appetitive stimuli) using continuous and partial reinforcement procedures.
Combination of both reinforcements potentiated speed of acquisition and asymptotic level of performance as compared to using a single type of reinforcement only. Experiments in which one of the two reinforcers was omitted after animals had previously been trained to the combination of both reinforcements indicate that appetitive and aversive reinforcements act differently: Aversive reinforcers appeared to be more effective for acquisition while appetitive reinforcers were more effective for maintaining the learned responses. In all groups, we also studied extinction. The extinction was slower in the group trained with combination of both types of reinforcements compared to the groups trained with just one type.
In summary, we established an experimental learning paradigm that allowed us to study the appetitive reinforcement ("the carrot") and aversive reinforcement ("the stick") in the same training session.

Acknowledgements:
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# ULTRASONIC VOCALIZATIONS IN RATS: EFFECTS OF EXPERIENCE AND CONTEXT ON 50-kHz CALLS.

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Rats can emit calls in different ultrasonic ranges. 50-kHz vocalizations of adult rats are often emitted when animals experience or expect reward, like palatable food. Therefore, it has been suggested that such calls may reflect a positive affective state. However, two aspects of 50-kHz call production put the reliability of 50-kHz calls as indicators of positive affective states into question. Firstly, they are also emitted in novel environments with scents of other rats and even by victims of aggression. Secondly, huge inter-individual variability in call production can be observed. The studies presented here were conducted to determine factors which may influence calling.

The main aim of Experiment A was to explore the effects of experience and contextual cues on calling. Furthermore, it was asked whether inter-individual differences are stable over days, meaning that they reflect a trait. The calls of two groups of 12 male rats were recorded during the first 15 minutes in an empty cage with scents of another rat on 3 consecutive days. Unlike the naïve group, the rats of the trained group first had a 30-min appetitive T-maze learning task before being recorded. The results show that call emission in the range of 50 kHz can be affected strongly by prior experience. Trained rats emitted only few calls, whereas naïve rats emitted a surprisingly high number of 50-kHz calls, which was stable over days. Even more surprisingly, calling was rather stable irrespective of the fact whether they were tested in a clean cage without rat scents or in a cage with rat scents on the second day. Three weeks later, vocalizations and behavior were recorded during an open field test and elevated plus maze test. During both test 50-kHz calls were detected, however, at much lower levels. Interestingly, it was shown that 50-KHz calling is dependent on subject-dependent factors, since individual differences in call production were highly stable over days and related to individual differences in open field behavior.

In Experiment B, the amount of ultrasonic calling was not only determined for the rat which was isolated in a novel empty cage, but also for the rat which stayed in the home cage. Experiment B was conducted, since one of the possible explanations for unexpected results in Experiment A is that the rats call for their cage mate. It was predicted that the rat that remains alone in the home cage will also vocalize when the cage mate is removed. Indeed, it was found that the rats that remained alone in the home cage also call at 50 kHz after separation. They vocalized even more than the rats in the novel cage.

In total, it can be concluded that 50-kHz calls should be interpreted with care, since they can occur in contexts that are not necessarily pleasurable to rats, and since they are affected by prior experience and individual differences. Possibly, 50-kHz calls serve for communicative purposes.

# DIFFERENTIAL BEHAVIORAL PROFILE INDUCED BY THE INJECTION OF CHLORAZEPATE DIPOTASSIUM IN BRAIN AREAS THAT PROJECT TO THE NUCLEUS ACCUMBENS SEPTI

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The medial pre-frontal cortex (mPFC), amygdala (Amy) and ventral hippocampus (Hip) project to the Nucleus Accumbens Septi (NAS) through glutamatergic pathways, and these projections have been implicated in goal directed behaviors, emotional driving and contextual cues, respectively, and in the glutamatergic transmission within Acc modulates anxiety. We compared here behavioral changes induced in the plus maze by the injection of chlorazepate dipotassium within origin nucleus of Acc afferences. Rats bilaterally implanted by stereotaxic surgery were divided in three groups (mPFC, Amy, Hip) and injected with saline (1 µl each side, n=15-18), or chlorazepate dipotassium (1 or 2 µg in 1 µl, n=15-18). The mPFC group showed a decrease in time per entry ($p<0.05$ for both doses) when compared to its control group. The Amy group present an increased in time spent in the open arm ($p<0.01$, 2 µg), open arm entries and open/closed arm quotient ($p<0.05$), 2 µg), and extreme arrivals ($p<0.05$ for both doses). The Hip group showed high values in the time spent in the open arm in saline controls, that were decreased by the lower but not the higher dose ($p<0.05$), like open arm entries ($p<0.05$). The open/closed arm quotient , starting also with high control values, were decreased by both drug doses ($p<0.05$). We conclude that GABAergic blockade within these different areas leads to behavioral stereotyped entries to the open arm (mPFC), an anxiolytic-like profile (Amy) and an interference with hippocampal modulation of anxiety and environment recognition (Hip).

# DIFFERENTIAL STRESS RESPONSE IN SPRAGUE DAWLEY (SD), WISTAR (W) AND OFA hr/hr (O) FEMALE RATS. EFFECTS OF DIAZEPAM.

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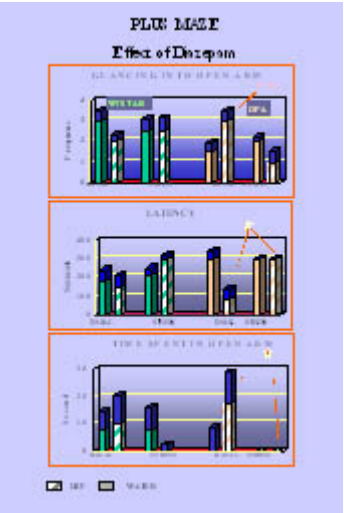
OFA hr/hr (O) rats are spontaneously hairless and hipoprolactinemic, with severe lactation deficit. Though prolactin (PRL) levels are low, this hormone exhibits a higher increase after ether stress than other strains. Sensitivity to stress depends upon genetic and environmental factors. In the present work we studied the behavioural stress response of female rats of three strains (O, Sprague Dawley (SD) and Wistar (W)) on oestrus day using two tests: a) 1 min. exposure to a white noise, recording motor and exploratory activity before, during and after stressor application. b) Restraint stress followed by plus-maze, to evaluate the ansiogenic component. In the last test only W and O were observed. Diazepam (3mg/kg/day) was administered in the drinking water to randomly chosen animals, three days prior to plus-maze testing. Basal animals were given tap water. c) In a separate experiment hypothalamic tyrosine hydroxilase (TH) expression and brain Dopamine (DA) and DOPAC were determined on lactating dams. Results: a) W and SD showed similar activity score and O was hyperactive. Freezing response was exaggerated in W, while O showed the lowest score. W and SD pre-stress head-dipping scores fell to near 0 after stress; O exhibited a low score all the time. Pre-stress scores of novel object exploration were similar in the three groups, but the stress-induced impairment of exploration was lower in the O group. b) In the closed arm, immobility basal scores were higher in O, but restraint stress lowered immobility. Basal and post-stress rearing were lower in O. Performance in the open arm was similar in W and O. Diazepam improved the plus-maze performance of basal O animals, but was unable to attenuate the effects of restraint stress. c)TH expression and DA and DOPAC levels were increased in MBH from pregnant and lactating O rats compared with the other strains. Conclusions: In O basal hole exploration is almost blocked, while novel object exploration is similar to the other strains. Stress response was lower in the O group, while W rats showed the highest sensitivity to the noise stress. The opposite effects in immobility scores of O in each testing condition may be due to motivational contextual cues. No differences in ansiogenic components were found. Results concerning TH, DA and DOPAC are consistent with previous works in other physiological situations. Dopaminergic systems are involved in most of the behavioral features observed in the present tests. It is also known that PRL interacts with GABA system. Low circulating PRL and hyperfunctioning DA systems could then be the basis of the differences seen in the O group.



# Does emotional state affect human performance in spatial cognition tasks?

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Emotion and cognition as two major aspects of human mental life are widely regarded as distinct but interacting. There are many different kinds of interactions which differ in what they imply about the functional organization of the brain and it is a common view that emotional states can diffusely impact higher cognitive functions. The purpose of our study was to investigate the interaction between spatial cognition behavior and emotional state. Therefore we used a virtual version of the Morris Water Task as spatial cognition paradigm and we varied the emotional state of the subjects to influence their task performances.
In the navigation task, subjects had to find a hidden platform, remembered previously. This platform was situated anywhere in a circular water pool (diameter: 20 m) and the pool was placed in the middle of a quadratic room (length: 30 m; height: 2.5 m). To encode the platform's position, subjects could only use four different landmarks (door, group of chairs, couch and window) as external cues (cf. figure 1). Each of the landmarks was placed in the middle of the four walls of the room. During the encoding phase, subjects were on the platform's position and could look around to memorize this place. After memorizing, they were beamed to a start position initiating the navigation task. In the first step, subjects had to rotate at the start position aligning their direction to the place of the hidden platform. As heading error we defined the angle between the real hidden platform vector and the adjusted direction vector. In the second step, subjects should move to the thought place of the hidden platform. As second dependent variable, termed as offset, we measured the Euclidean distance between the subject's end point and the real position of the platform.
To change the emotional state of the subjects, the independent variable, we used evaluated pictures and sounds from the International Affective Picture System (IAPS) and International Affective Digital Sound (IADS) database. With this stimulus material the two most important dimensions of emotion related behavior, valence (pleasant <-> unpleasant) and arousal (calm <-> aroused), could be influenced. For the positive emotional state, subjects were stimulated with pleasant/calm and for the negative one with unpleasant/aroused stimuli.
As result we found a significant impact of the emotional state related to both dependent variables [heading error: $F(1,39)=11.22$, $p<0.01$; offset: $F(1,390)=236.59$, $p<0.001$]. Subjects performed the navigation task in the positive emotional state significantly better than in the negative emotional state.
With this study we could show the interactive relation between emotion and spatial cognition in a virtual Morris Water Task as navigation paradigm.

Abb 1. Screenshot of the virtual Morris Water Task with the four landmarks, each in the middle of a wall.

# Does the Modulation of the Insular Activity Affect Our Emotional Involvement?
# A Real-Time fMRI Study during Emotional Pictures Processing

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The aim of this study was to evaluate the effects of increasing the insular activity on the emotional responsiveness of healthy adults. Real-time functional MRI (rtfMRI) allows for on-line feedback and voluntary control of brain signals; we trained subjects to learn to control their own brain activity in the anterior insula using rtfMRI (3T Siemens Trio, TR 1.5s, TE 30ms, 16 slices, voxel size 3.3x3.3x5 mm3).
Emotional responsiveness was evaluated in terms of local brain activity, subjective valence and arousal during affective pictures presentation.
Nine healthy right-handed adults participated to our study. They were trained to voluntarily control the local BOLD signal of the left anterior insula using the rtfMRI. All subjects underwent a localizer session and a training of four feedback sessions. The sessions consisted of five alternated increase/decrease runs. Each run consisted of a 30s increase/decrease block followed by a 9s block, during which we presented a visual stimulus, that in turn was followed by a 12s evaluation block. During the increase/decrease blocks the subjects had to respectively increase or decrease the local brain activity in the target region of interest. During stimulus presentation blocks, one emotional picture from International Affective Picture Set (IAPS) was presented for 9s. During the evaluation blocks, coming immediately after the presentation of the pictures, the subjects were asked for ratings of the stimuli. Pictures were evaluated in terms of subjective emotional valence and arousal using the Self-Assessment Manikin (SAM, Bradley and Lang 94).
The feedback was the average BOLD signal of the left anterior insula displayed by means of thermometer bars. Online statistical analysis of the fMRI data was performed using Turbo Brain-Voyager and we BrainVoyager QX for offline single subject ROI analysis. SPM2 offline analysis was also performed for group analysis.
All subjects were able to learn to voluntarily increase the activity in the left anterior insula. The strategies reported by the subjects were mainly based on recalling of personal emotional experiences.
Training was found to have effects on subjects emotional responsiveness.
In this work we discuss the implication of these results for clinical applications.

# Differential regulation of synaptic vesicle proteins in serotonin transporter deficient mice - with and without acute stress exposure

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5-HTT deficient mice represent an artificially hyperserotonergic environment, since released 5-HT is not taken back into the presynaptic neuron. The knockout (KO) of 5-HTT in these mice is accompanied by numerous pre- and postsynaptic adaptive changes including adaptive expression changes of different 5HT receptors. Furthermore, 5-HTT knockout mice show an increase in anxiety-like behavior and are less aggressive. These behavioral abnormalities support the notion that 5-HTT KO mice may act as a model for anxiety disorders. In addition these mice show an exaggerated stress response.

To achieve a better understanding of processes involved in altered serotonergic neurotransmission, we analyzed expression levels of synaptic proteins including synaptotagmin (Syt) 1 and 4, syntaxin 1A, and complexin (Cplx) 2 in 5-HTT deficient mice and wild-type littermates. The use of quantitative real time (QRT)-PCR revealed significantly altered Syt 1, Syt 4 an Cplx 2 expression mainly in hippocampus, cortical regions and brainstem of 5-HTT deficient mice compared to wild-type controls, whereas we detected no differences in other brain regions. To investigate mRNA expression levels of these synaptic proteins at the regional and cellular level we used non-radioactive in situ hybridization (DIG-ISH). We showed a specific regional expression pattern of all investigated synaptic proteins in neurons of different murine brain regions. By the use of immunohistochemistry with DIG-ISH slices and using two differently labelled ISH-probes (DIG- and 35S-cRNA) we started to characterize the phenotypic organization of cortical, hippocampal and raphe neurons depending on their synaptic protein mRNA content. Additionally, we found altered expression levels of these proteins in different brain regions after acute immobilization stress in SERT-KO-mice compared to their wildtype liitermates. Thus, our data further support the critical role of different synaptic proteins in the adaptive plasticity of serotonergic circuits especially in response to stress.

# Poster Topic

## T32: Attention

**T32-1A** Attention improves object representation in monkey area V4
*D. Rotermund, K. Taylor, U. Ernst, AK. Kreiter and KR. Pawelzik, Bremen and Paris (F)*

**T32-2A** Dynamic changes of synchrony between neuronal populations in visual cortex are predicted by shifts of attention.
*Y. Smiyukha, S. Mandon, FO. Galashan, S. Neitzel and A. Kreiter, Bremen*

**T32-3A** Attention increases apparent size of moving visual stimuli
*K. Anton-Erxleben, C. Henrich, T. Tzvetanov and S. Treue, Göttingen*

**T32-1B** Transparent motion: attention improves the perception of speed changes but not of direction changes
*V. Kozyrev, A. Lochte, S. Ardid, D. Luxat and S. Treue, Göttingen, Sant Joan d'Alacant (E) and Waltham MA (USA)*

**T32-2B** Variability of attentional modulation of neurons in visual cortical area MT of rhesus monkeys
*F. Pieper, T. Womelsdorf, JC. Martinez-Trujillo and S. Treue, Göttingen, Nijmegen (NL) and Montreal (Canada)*

**T32-3B** Effects of negative emotional arousal states on the neural mechanisms of cognitive control: a functional MRI study
*C. Born, T. Melcher and O. Gruber, Homburg / Saar*

**T32-1C** Visual task performance affects the auditory change detection mechanism
*K. Haroush, S. Hochstein and LY. Deouell, Jerusalem (Israel)*

**T32-2C** Reference frames for covert spatial attention during a visuomotor task
*JC. Martinez-Trujillo and R. Niebergall, Montreal (Canada) and Göttingen*

**T32-3C** Attentional bias in an alcohol-related Stroop and Sternberg task in a non-dependent student sample
*A. Kübler, R. Hester, G. Roberts and H. Garavan, Tübingen, Melbourne (AUS) and Dublin (Ireland)*

# Attention improves object representation in monkey area V4

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Attention is thought to improve processing of selected stimuli. It has been demonstrated that firing rates can be modulated by attention and that their enhancement can improve the signal-to-noise ratio for discriminating stimulus features.

Here we investigate effects of selective attention on object representation using recordings of epidural local field potentials (eLFPs). We trained two monkeys to an extended version of the delayed match to sample paradigm. The animals were required to attend to one of two object sequences presented simultaneously in both hemifields. They had to identify the re-occurrence of the initially presented sample object in the attended object sequence. eLFPs were recorded with an array of 36 epidural electrodes covering parts of area V4 and V1, while the monkeys performed the task.

Analysis of object encoding was done with standard support vector machines trained on eLFP wavelet power coefficients, allowing to estimate the probability of correct classification of object identity. Using all electrodes, we found a classification performance of up to 94% correct (1200 ms window, chance level: 17%) on a trial by trail basis, i.e. the eLFP-data enabled nearly perfect object identification. Almost all stimulus-specific information was concentrated in the gamma frequency range from 40 to 160 Hz. Classification on data from 4 electrodes covering V4 resulted in 56% performance for non-attended stimuli, and 64% for attended stimuli (chance level: 17%). Classification of attention direction on eLFP-signals from the 4 electrodes located above V4 gave 81% performance (chance level: 50%).

We further investigated if encoding improvement is specifically linked to shifts in spectral content, or could be explained solely by increased signal-to-noise ratios (SNRs). We found, that SNR improvements due to attention were small and when removed from the data the classification performance increases were largely conserved.

Our results show that neuronal activity in the gamma band contains a high amount of information on stimulus shape and direction of attention. They indicate that attention improves the distinctiveness of cortical states associated with the processing of different visual stimuli.. The high classification performance on objects and direction of attention suggests that eLFP signals from visual cortex could be useful for brain machine interfaces.

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# Dynamic changes of synchrony between neuronal populations in visual cortex are predicted by shifts of attention.

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Attention is thought to select the most relevant parts of the huge information flow for further processing. On the neuronal level such selection is reflected by the ability of neurons to respond to multiple visual stimuli within their receptive field as if only the attended stimulus would be present. This suggests the existence of a mechanism that allows neurons to enhance the efficacy of those afferents which carry the relevant signals, while suppressing the others.

Here we investigate the hypothesis that selective synchronization of neurons with those afferents representing the attended stimulus could be such a mechanism. Therefore we tested whether synchronization occurs selectively between a population of V4 neurons and those V1 afferents carrying behaviorally relevant information.

We trained a monkey to a demanding shape-tracking task, which required sustained attention to a cued location in visual space. The monkey had to report the reoccurrence of the initial shape in a continuously morphing shape sequence, while ignoring a nearby and similar but behaviorally irrelevant shape sequence. Local field potentials were recorded with an array of chronically implanted epidural electrodes covering part of visual areas V1 and V4, while the monkey performed the task. Both stimuli were arranged to activate two well-separated neuronal populations in V1, and a common population in V4. Hence the separate receptive fields of the two V1 recording sites were contained in the receptive fields of the V4 site. Directing the monkey's attention to one of the two stimuli allowed us to test for corresponding changes of synchronization between the site in V4 and those in V1. Qualitative estimations of the correlations between the recording sites were based on a wavelet analysis.

The results show strong gamma-band synchronization of signals between the V1 site responding to the attended stimulus, and the site in V4. At the same time, much less or no synchronization was observed between the site in V4 and the V1 site representing the ignored stimulus. Switching the monkey's attention from one stimulus to the other reversed the pattern of synchronization. Thus the V4 site synchronized selectively with that site in V1 which represented the attended stimulus. This switch of synchronization occurred even though each stimulus evoked comparable gamma-band activation in both conditions.

In summary, allocation of attention strongly influences the dynamic synchronization pattern between a neuronal population in V4 and its V1 afferents: synchrony is strong with those afferents representing the attended stimulus and weak or absent for others representing non-attended stimuli. Since synchrony is thought to reflect enhanced effective connectivity, our results support the hypothesis that synchronization may serve as a neuronal mechanism underlying selection of behaviorally relevant signals for further processing.

# Attention increases apparent size of moving visual stimuli

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We have previously shown that spatial attention shifts visual receptive fields (RFs) in cortical area MT of rhesus monkeys towards the attentional focus. Because spatial tuning of visual neurons provides the basis of a cortical representation of visual space, this might lead to distortions in the perception of spatial relations. One potential misperception could be an increase of perceived size of attended stimuli. Here we test for such an effect in a psychophysical study with human subjects. To test if the allocation of transient spatial attention increases the perceived size of a stimulus, we adapted an experimental design developed by Carrasco et al. (Nature Neuroscience, 2004) to investigate attentional effects on subjective appearance. While keeping fixation, subjects were presented a brief cue either at the fixation point (control condition) or peripherally to attract automatic attention to the left or right of the fixation point. Then two differently sized moving random dot patterns (RDPs) were presented at the same locations left and right of the fixation point. Subjects were instructed to report if the bigger of the two patterns moved rightwards or leftwards. The timing was such that a subject's attention on one of the RDPs was maximal at the time he/she had to decide which one was bigger: this design allowed us to find the point of subjective equality (PSE) for the perceived size of an attended and an unattended stimulus without allocating attention equally to both of them. We determined the PSE for three different reference sizes (1, 2, and 4 deg diameter at an eccentricity of 4 deg). We found a significant increase in perceived size with the allocation of attention for all reference sizes. This effect ranged from 4% to 12% and was more pronounced for smaller than for larger reference sizes. Since in the present study RFs located on the stimulus borders are the ones that contribute to the attentional effect, this finding suggest that the magnitude of RF shifts decreases with increasing distance from the attentional focus. This is in line with models of the distribution of spatial attention that assume a gradual decrease of attentional modulation around its spatial focus, and with our own physiological observations that RF shifts indeed vary inversely with distance from the attentional focus. In our experimental design an attentional effect on task difficulty might influence the PSE measure: Subjects might prefer to choose the attended stimulus because the direction discrimination is easier to do on it. This difficulty effect and the effect on perceived size add when subjects choose the bigger stimulus but counteract when they are instructed to report on the smaller stimulus. We performed this control experiment and found a smaller effect of attention on perceived size, which was, however, still significant. In summary, our findings demonstrate that the allocation of transient spatial attention onto a visual stimulus increases its perceived size and in addition biases subjects to select this stimulus for a perceptual judgement.

# Transparent motion: attention improves the perception of speed changes but not of direction changes

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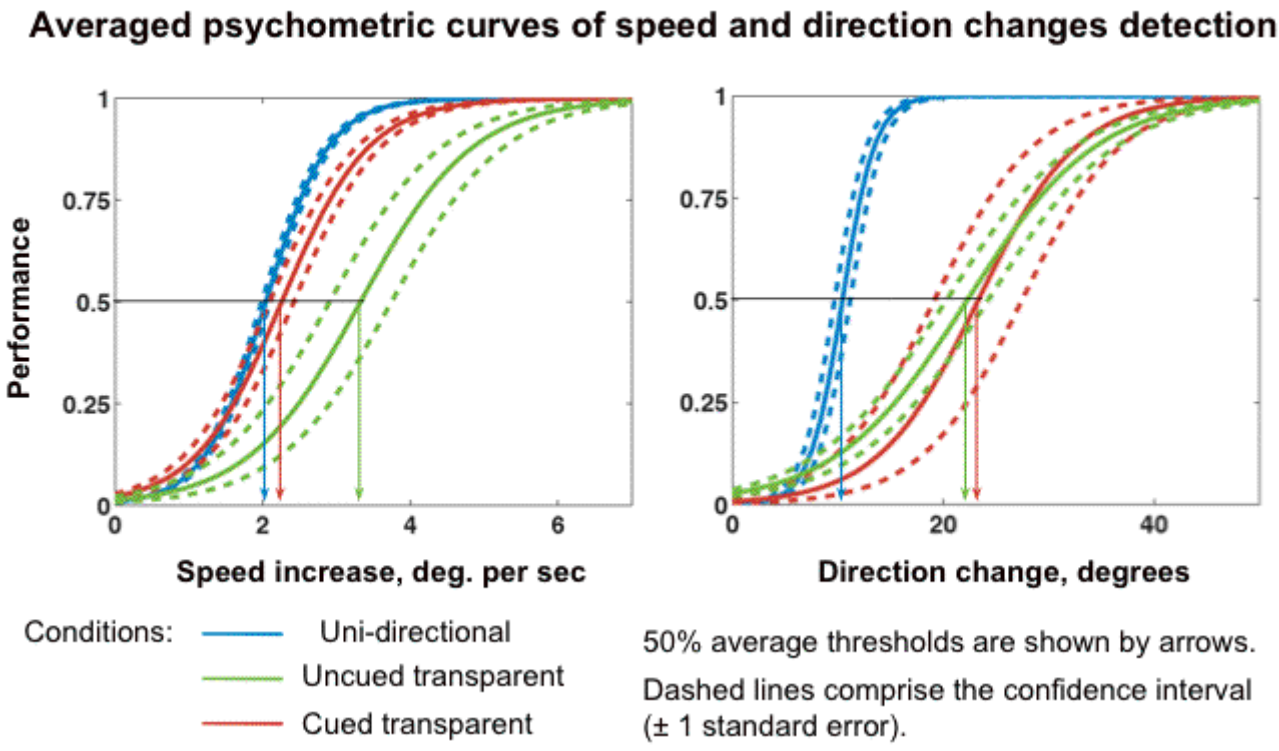
The perception of the individual directions in transparent motion (stimuli that combine two or more directions within the same spatial area) is a difficult task for the visual system. While both spatial and temporal averaging is usually employed to improve performance in motion vision, such approaches need to be applied separately for the different direction components in transparent motion. To elucidate the underlying mechanisms we compared the perception of speed and direction of motion: human observers had to respond to brief changes of either direction or speed in eccentrically presented moving random dot patterns. Uni-directional and transparent bi-directional (directions 90° apart) stimuli were used, and in the transparent conditions subjects were either instructed, which direction would change (cued transparent condition) or this information was not provided (uncued transparent condition). Thresholds in the speed task were similar in the uni-directional and cued transparent conditions (see Figure, left plot). This was not the case in the direction tasks: here thresholds were similar in the two transparent conditions and about twice as high as in the uni-directional condition (see right plot). These data show that the allocation of attention to one of the directions in transparent motion helps to remove the noise introduced by the other direction in speed but not in direction judgments. Therefore speed and direction processing is likely based on mechanisms that differ in if and how they benefit from attention. In addition, we performed pilot experiments where subjects were asked to detect luminance (contrast) or color changes in the cued transparent condition. We found that perception of such changes, unlike of speed or direction ones, could not be reliably associated to a particular moving surface. [The project is supported by the Volkswagen Foundation, grant I/79868.]



# Variability of attentional modulation of neurons in visual cortical area MT of rhesus monkeys

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Attention modulates the gain of sensory responses of neurons in different areas of visual cortex. When attention is directed to a single stimulus inside a neuron's receptive field (RF) its firing rate is increased. This has been shown to reflect a multiplicative gain modulation that is independent of the tuning properties of the neuron. It is unclear, however, whether the strength of attentional modulation is affected by other basic response properties of the neuron. Visual neurons differ in temporal response characteristics, namely in the initial spiking probability after stimulus onset and in whether the later adaptation to steady state firing rates may exhibits a more phasic or tonic course. We hypothesized that these temporal response courses of visual neurons to stimuli in their RF affect the strength and temporal dynamics of attentional modulation and could account for the observed variability of attentional modulation across cells in visual cortex.

To investigate the influence of the temporal response characteristics on attentional modulation we recorded from 120 single neurons in macaque area MT. Monkeys received a spatial cue instructing them to attend either inside or outside the RF of a given neuron while the target stimulus, a random dot pattern (RDP) that moved in the cell's preferred direction, was presented inside the RF. At a random time during the trial the target changed its speed and the animal had to respond to this event. We quantified neuronal response profiles as the ratio between the immediate on-response and the sustained response. Relating the strength and temporal dynamics of attentional modulation to this ratio resulted in two main findings.

First, cells with a highly phasic response showed a delayed onset of attentional modulation compared to cells with a more tonic response. Secondly, we found a significant correlation between the strength of the phasic response and the magnitude of attentional modulation in the sustained response interval: Responses of cells showing a stronger on-response relative to the sustained response showed a stronger attentional modulation in the sustained response period.

These findings suggest that basic response properties of neurons account for substantial proportion of the variability of the strength and temporal evolution of attentional modulation in visual cortex.

# Effects of negative emotional arousal states on the neural mechanisms of cognitive control: a functional MRI study

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Introduction

The aim of the present study was to investigate the influence of negative emotional arousal states on neural mechanisms of cognitive control in two situations of cognitive interference: (a) the overriding of an attentional orienting response towards salient but task-irrelevant low-frequency events (oddballs) and (b) the resolution of cognitive conflict during Stroop-interference. Both situations implicate strengthened attentional processing and require an modulation of cognitive control (Melcher & Gruber, in press). Since the processing of emotionally relevant stimuli arguably occupies attentional ressources, too (Pessoa & Ungerleider, 2004), it was expected that the induction of negative affect would interfere with the mentioned cognitive processes to resolve interference.

Methods

14 neurologically healthy subjects performed an oddball variant of the Stroop paradigm while they underwent fMRI. At the beginning of each trial, an emotionally relevant picture stimulus of the IAPS (International Affective Picture System, Lang et al., 2005), which was rated as either negatively arousing or neutral, was presented. Then, immediately after the picture had disappeared, subjects were to classify word stimuli according to font color by either a left or right button press response. Color-word combinations provided four experimental conditions: during congruent trials, word meaning matched the current font color while during incongruent trials word and color were opposed. During oddball trials, we presented rarely occuring words that bore no relation to color. During baseline trials, word meaning was also unrelated to color but appeared equally often as compared to the prevalent color words.

Results / Discussion

Negatively arousing (compared to neutral) pictures exhibited significant signal increases in numerous emotionally relevant areas, particularly in the amygdala and extrastriate visual cortices. After neutral affect induction, interference from both oddballs and incongruency (i.e. Stroop-interference) exhibited only sparse or even no activation in areas that we have related to control processes during equivalent interference situations in prior studies (Melcher & Gruber, in press). In terms of an interaction effect, the same conditions after negative affect induction showed stronger and more wide-spread activations. This modulation effect was especially pronounced in the posterior inferior prefrontal cortex (inferion frontal junction) and in extrastriate visual areas as well as, particularly for oddballs, in the temporo-parietal transition zone and, patricularly for Stroop-interference, in frontomedian and intraparietal regions.

# Visual task performance affects the auditory change detection mechanism

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While attentional resources are invested in a goal-directed manner, remaining resources enable a constant scan of our environment for potentially important deviant events. Detection of deviation may trigger in turn attention reallocation. In audition, this process is reflected in the mismatch negativity (MMN) event-related potential (ERP). A fundamental debate regards the extent to which this signal, generally considered to be 'automatic', is susceptible to attentional load. Previously, we found that the MMN is robust and its magnitude is only slightly decreased despite heavy visual attentional load (Reviews in the Neurosciences 16: S29-S30 Suppl. 1, 2005). However, in that study even the baseline condition was quite demanding, so a floor effect could not be excluded. In the current study, we increased the load difference between the two conditions. In an "attentional blink" paradigm, participants identified two visual targets within a rapid visual stream, while ignoring background test-signal tones of standard pitch, mixed with infrequent pitch deviants. Load was manipulated mainly by changing the contrast level of the visual stream and the contrast ratio between the targets and distractors. Subtracting the ERP of the standard from that of the deviant sounds elicited the MMN signal. Only mismatch responses for stimuli appearing before the visual targets were analyzed. Task performance decreased significantly with increasing load. Whereas the MMN remained robust with both loads, its amplitude was significantly decreased for the high load condition compared to the low load condition and its latency was significantly shorter. Moreover, in the high load condition, MMN peak amplitude and latency was conspicuously smaller in amplitude in correct trials compared to incorrect trials, reflecting the transient attentional state of the participant. These findings suggest that the auditory change detection process is disturbed by perceptual load and attentional factors.

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# Reference frames for covert spatial attention during a visuomotor task

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Previous studies have demonstrated that attention favors the processing of visual information at attended spatial locations. However, it remains unknown the reference frame in which spatial attention operates. For example it is possible that attention selects object locations in an eye-centered frame of reference, this implies that when the eyes move (e.g., during smooth pursuit eye movements) targets that remain fixed in retinal coordinates will be better attended than other targets that moves relative to the retina. On the other hand, it is also possible that in the same situation, we better attend to targets that remain fixed in space-coordinates, even if those targets are moving relative to the retina. To investigate this issue we tested human subjects' performance on an orientation discrimination task in which they pursued a fixation target and covertly attended to a target Gabor patch that, a) remained fixed in eye-centered coordinates but moved in space-centered coordinates (eye-centered condition), and b) viceversa (space-centered condition). The horizontal, vertical and torsional eye movement components were measured during the task trials and previous to the trials we obtained a measurement of the Listing's plane for each subject. We found that all subjects were able to accurately pursue the fixation target with their eye movements following 3D trajectories that fitted their individual's Listing's plane in both conditions. However, orientation discrimination thresholds in all subjects (n=3) were at least 2 times larger in the space-centered relative to the eye-centered condition. These results suggest that covert attention tracks visual targets more efficiently in an eye-centered relative to a space-centered frame of reference.

# Attentional bias in an alcohol-related Stroop and Sternberg task in a non-dependent student sample

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Introduction: It has been shown that addicts of drugs have an attentional bias towards addiction related stimuli. The emotional Stroop paradigm is most often used to asses this attentional bias. The Stroop task is proposed to get emotionally loaded when word categories related to the disorder are included. When participants are pre-occupied with a drug or behaviour they take significantly longer to respond with the colour of the emotionally related word than a neutral word. Additionally we developed an emotional Sternberg task addressing working memory (WM) when exposed to alcohol related pictures as compared to soft drink pictures. Evidence has emerged that an attentional bias is also present in non-clinical goups such as social drinkers (Cox et al., Alcohol & Alcoholism 2003;38:45-49). Hypothesis: 1. In the Stroop task: slowing of response speed and the average alcohol interference score (derived from RT differences) correlate with measures of drinking behaviour. 2. In the Sternberg task: cue and load effects and an average alcohol interference effect correlate positively with measures of drinking behaviour. 3. Positive correlation between the Stroop and Sternberg average alcohol interference scores. Methods: Participants were 46 university students (Dublin). As subjective measures of drinking behaviour we used the Alcohol Use Disorders Identification Test and the Impaired Control Scale. The Emotional Stroop task included 20 words of the following categories: alcohol-related, music related, and neutral words. We also included a block of coloured 'XXXX's providing a baseline measure of response time. The Sternberg task comprised 2 levels of difficulty: low WM load, with 5 numbers presented in numerically ascending order and high WM load, with random order of numbers. The numbers were presented in white colour either in the foreground of an alcohol-related or a softdrink-related coloured picture. Results: In both tasks we found an alcohol interference effect which correlated positively with the subjective measures of drinking behaviour (Stroop: $r = .32$, $p < .05$; Sternberg: $r = .55$,$p < .001$). The average alcohol interference scores of the Stroop and the Sternberg task were moderately correlated ($r = .47$,$p < .01$). In the Sternberg task a cue effect was only visible in the low WM load. Discussion: Attentional bias and cue-reactivity develops continuously with the individual's history of alcohol consumption, is detectable in the social drinking population and affects automatic processes and WM based information processing. When higher processing resources were required participants were able to overcome the distracting effect of the pictures and were able to more efficiently do so the lower they scored on the subjective measures of drinking behaviour. The data support the use of reaction time to cognitive tasks as a reliable and valid measure of attentional bias and cue-reactivity.

# Poster Topic

## T33: Neuropsychology and psychophysics

**T33-1A** Color Difference, Similarity, and Brightness Measures Based on a Model of Neuronal Color Coding (CC) and Elementary Color (EC) Sensations in Man
*WGK. Backhaus, Berlin*

**T33-2A** Increasing gender-specific differences in haptic object recognition performance over the life-span
*T. Kalisch, M. Tegenthoff and HR. Dinse, Bochum*

**T33-1B** Localization of Auditory Stimuli during Smooth Eye Movements
*K. Königs and F. Bremmer, Marburg*

**T33-2B** The Effect of Echo Roughness on Backward Masking
*S. Schörnich and L. Wiegrebe, Planegg-Martinsried*

**T33-1C** THE EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON PLANNING PERFORMANCE IN THE TOWER OF LONDON TEST
*CA. Dockery, N. Birbaumer and C. Braun, Tübingen*

# Color Difference, Similarity, and Brightness Measures Based on a Model of Neuronal Color Coding (CC) and Elementary Color (EC) Sensations in Man

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The psychophysiological model of color opponent coding (COC) and elementary color (EC) sensations in man (Backhaus, 2006a, b) is extended now by 1) a neuronal model of black/ white (BW) coding. Thus, the model consists of a neuronal color coding (CC) model and of the psychophysical EC model of the six elementary color sensations red, green, blue, yellow, black, and white that constitute our color sensations. The model includes measures and related scales of 2) CC color difference, 3) EC amounts of elementary color sensations, 4) EC color similarity, 5) EC color brightness and color brightness difference. The derived measures allowed the determination of the parameters of (1) the neuronal CC model by best fits of the predictions of color brightness of monochromatic lights to the measured (Judd-corrected) CIE-1924-V(λ)-function of photopic brightness sensitivity. (2) The CC color difference measure, as judged in color discrimination experiments, is derived from the CC model via the Euclidean metric of the three-dimensional color space spanned by three color coding (CC) neuron types (two COC and one B/W coding). (3) The EC amounts of elementary color sensations, as judged in content analytical experiments, provide the three scales red/green, blue/yellow, and black/white that span the EC space. (4) The EC color similarity measure is derived as the sum of the EC amounts of the elementary color sensations, which two color sensations have in common according to mathematical set-theory. (5) The EC color brightness measure is derived as the sum of the specific brightness values of the six elementary color sensations, measured in color induction experiments, multiplied by the respective EC amounts. The EC brightness difference of two color sensations is derived as the difference in the respective EC brightness values. The EC similarity measure, the EC brightness and brightness difference measures (related to aspects of the conscious color sensations) are compared to the neuronal CC color difference measure (related to unconscious electrical excitations). Since the measures rely on different judgment types and different entities of our color vision system, the respective scales are (more or less pronounced) nonlinearly related to each other. Possible applications of the measures and scales of the model of neuronal color coding (CC) and elementary color (EC) sensations in man will be discussed.

Backhaus, W.G.K., 2006a. Psychophysiological simulations of spatial color vision. In: Fünftes Symposium Licht und Gesundheit, 23.-24.2.2006. Eine Sondertagung der TU Berlin und der DGP mit DAfP und LiTG, Hrsg. H. Kaase & F. Serick, Tagungsband, Hauptvorträge, pp. 8-21. Technische Universtät, Berlin (ISBN 3-9807635-0-2).

Backhaus, W.G.K., 2006b. A psychophysiological model of chromatic elementary color sensations in man. In: 5th Forum of European Neuroscience, July 8-12, 2006, Vienna, Austria, Abstracts, A 111.4, p. 41. FENS, Vienna.

# Increasing gender-specific differences in haptic object recognition performance over the life-span

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The human haptic system processes somatosensory inputs from cutaneous as well as from various kinesthetic sources. These inputs are acquired during manual explorative procedures and integrated to derive information about geometrical properties of objects. Under everyday conditions we use our hands and eyes to identify objects, but sometimes it is necessary to identify particular object-features only by haptic impressions and without visual support.

Here we investigated age-related changes in haptic object exploration abilities in 58 healthy subjects of different age ("young": 23.4 ± 2.9 years, "middle-aged": 53.8 ± 1.8 years, "old": 75.0 ± 6.4 years). Haptic performance was quantified by a custom-made test (1), where subjects had to identify cubic objects each in a size that allows a complete enclosure with all fingers of a hand. A total of 17 objects had to be sorted into 5 categories only by the haptic information arising from palpating the objects with the dominant hand. The goodness of haptic performance was estimated by the factors execution time and number of classification errors. Because haptic object identification requires both, sufficient tactile spatial resolution and cognitive abilities (i.e. mental object rotation), we assessed those features by tests on tactile acuity (2-point discrimination task (2) and estimation of absolute touch thresholds (1)) and the non-verbal Raven intelligence test (RSPM).

The major finding of our study was a decline of haptic performance with increasing age, which was more pronounced in female subjects. This held true for both factors of the haptic task, execution time and number of errors. Not unlike the situation observed in previous experiments (3) tactile spatial resolution of the skin, as it was quantified by the 2-point discrimination - and absolute touch threshold, showed a significant age-related decline but here no gender-specific difference was detectable. Middle-aged and old subjects additionally performed the Raven-test, where an age-related decline and a conspicuous gender-specific difference in cognitive performance became apparent.
Comprehensive analyses of individual performances in haptic, tactile and cognitive tasks were based on calculated residual variances (linear correlation of task performance and age) and revealed a significant correlation of individual number of errors in the haptic task and performance in Raven-task. Additional coherency was found for individual execution time in the haptic task and performance in Raven-task. On the other hand no correlation was found for individual tactile acuity (neither absolute touch - nor 2-point discrimination thresholds) and both parameters of the haptic task.

The results indicate that age-related decline of haptic performance occurs in gender-specific manner. Individual tactile acuity seems to account less for this development than cognitive performance in old age.

1. H. R. Dinse et al., ACM Transactions on applied perception 2, 71 (2005)
2. H. R. Dinse et al., Science 301, 91 (2003)
3. H. R. Dinse et al., Ann Neurol 60, 88 (2006)

# Localization of Auditory Stimuli during Smooth Eye Movements

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It is an ongoing debate in multisensory research whether spatial information from different sensory modalities is processed in similar ways by similar neural networks. A number of recent psychophysical studies have shown that the analysis of spatial perception during eye movements allows to successfully analyze sensory spatial processing and its underlying neural basis. Previous studies have demonstrated differences in the spatial localization of visual stimuli briefly presented during smooth pursuit eye movements [1] and during optokinetic nystagmus (OKN) [2]. Visual targets are perceived as being shifted in the direction of the pursuit only in the visual hemifield the fovea is heading for. On the contrary, this spatial shift in perception occurs in the whole visual field during OKN. In our current study we aimed at determining if and how eye-movements influence the perception of auditory as compared to visual stimuli. Specifically, we investigated localization of auditory stimuli during voluntarily controlled (smooth pursuit) and reflexive (optokinetic) smooth eye movements.
15 healthy human subjects participated in this experiment. All reported normal hearing and normal or corrected to normal vision. Experiments were carried out in a light-proof sound attenuated chamber. Subjects performed smooth pursuit or optokinetic eye movements elicited by appropriate visual stimuli. Eye movements were monitored with an infrared eye tracking system (SR Research: Eye Link II). An auditory target (white noise) was presented for 5 ms. Localization during the eye movement conditions was compared to control conditions where subjects had to fixate a stationary target or were allowed to freely move their gaze. The subjects had to indicate the perceived target position relative to a ruler.
Our data clearly indicate that smooth pursuit causes a shift of perceived auditory stimulus location in the direction of the pursuit only when the eyes move towards the auditory target. On the other hand, mislocalization during OKN is shifted in the direction of the slow phase in the whole tested region of space. This mislocalization is temporarily modulated shortly before the fast resetting phase of the OKN. Taken together: as in the visual domain we found differences in localization of briefly presented auditory stimuli during smooth pursuit and OKN. Yet, the spatial and temporal characteristics of the mislocalizations were different in the visual and auditory domain. This suggests that (at least slightly) different neural mechanisms are at play to integrate oculomotor signals and information on the spatial location of visual as well as auditory stimuli.

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[2] Kaminiarz A, Krekelberg B and Bremmer F (2006) Visual localization during optokinetic nystagmus Vis.Res. (in press).

# The Effect of Echo Roughness on Backward Masking

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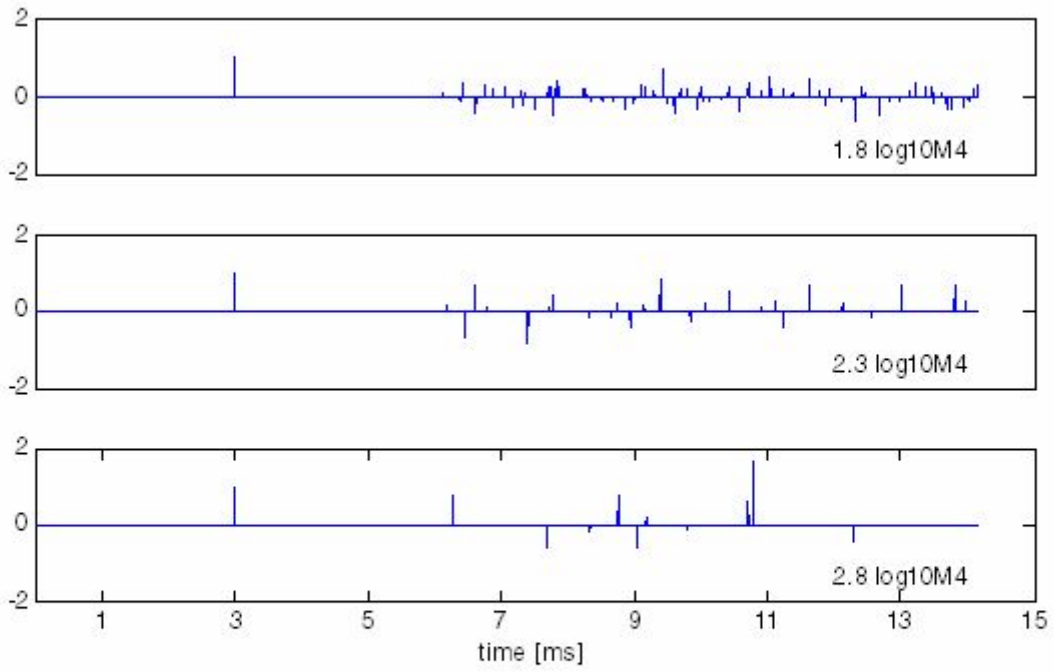
When bats hunt for insects close to vegetation by means of echolocation, one of the problems they encounter is that a multitude of masking echoes originating from the surrounding vegetation will complicate the detection of an insect, as the insect's echo can be confused with the beginning of the masking echo. Present studies about those masking effects either used real objects, whose acoustic impulse responses remained unknown and therefore couldn't be varied in a systematic way, or consisted of playback-experiments with simple maskers.

This study investigates the effects of a complex masker's temporal structure - in this case, its roughness factor (measured as the base 10 logarithm of its 4th Moment) - on the degree of backward masking. It is conceivable that the necessary amplitude for the signal to be detectable for a bat depends on the degree of the masker's roughness, as increasing masker roughness means that it consists of fewer samples with higher amplitudes.

In a two-alternative, forced-choice playback setup, bats are trained to detect a single reflection that occurs shortly before a masker consisting of a 8 ms impulse response. Both the amplitude of the signal and the roughness factor of the masker were varied independently to obtain the signal's detection threshold amplitude for each used roughness factor.




*Examples of a single reflection followed by a impulse response used to generate maskers with different roughnesses (top to bottom: 1.8, 2.3 and 2.8 log10M4).*
*Note how the signal becomes more likely to be part of the masker with increasing masker roughness.*

# THE EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON PLANNING PERFORMANCE IN THE TOWER OF LONDON TEST

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*Weak transcortical direct current stimulation (tDCS) applied over the cortex provides a tool to transiently modify cortical excitability via altering the membrane potential of underlying neuronal populations. The effect can be targeted at different areas of the cerebral cortex. A common feature in patient populations with frontal lobe pathologic abnormalities, such as schizophrenic, depressed and frontal lobe lesion patients, is executive function impairment. A well-known neuropsychological test to evaluate executive function is the Tower of London test (TOL) as it is sensitive in revealing deficits in patient planning performance compared to healthy controls. Neuroimaging studies have shown that brain activation during TOL performance primarily involves the prefrontal cortex.

This study was designed to investigate the effects of tDCS administration over the left dorsolateral prefrontal cortex (DLPFC) on planning performance in the TOL test. It was proposed that anodal tDCS would lead to improved performance, while cathodal and sham tDCS would result in no change in performance. We studied the performance of 15 healthy participants (3 men and 12 women) in the TOL task during and following 15 min. active anodal, cathodal and sham tDCS over three sessions. tDCS did not influence the accuracy of performance under any stimulation condition, however there was a significant improvement in planning performance as indexed by reaction time, for anodal tDCS. Cathodal and sham tDCS did not result in a significant change. Interestingly, the learning effect apparent across sessions was completely suppressed by cathodal tDCS.

Our results indicate that anodal tDCS improves planning performance on the Tower of London test by increasing reaction time while preserving accuracy of response at all levels of task demand. tDCS may provide a tool to improve the planning ability of patients with frontal lobe pathologies by increasing the connections or excitability in pathways on the damaged hemisphere.*

# Poster Topic

## T34: Neuropharmacology

**T34-1A** New potential drugs for treatment of secondary brain injury
*L. Veenman, I. Maniv, A. Shterenberg, W. Kugler, M. Lakomek, I. Marek and M. Gavish, Bat-Galim, Haifa (Israel) and Göttingen*

**T34-2A** Standard antiepileptic drugs fail to block epileptiform activity induced by low magnesium or 4-aminopyridine in rat hippocampal slice cultures.
*K. Albus, A. Wahab and U. Heinemann, Berlin*

**T34-3A** Intrastriatal inhibition of aromatic amino acid decarboxylase prevents L-DOPA-induced dyskinesia: a bilateral reverse in vivo microdialysis study in rats
*K. Buck and B. Ferger, Biberach*

**T34-4A** Effect of "Enriched Environment" during development on adult rat behaviour
*S. Schütte, L. Hoffmann, M. Koch and K. Schwabe, Bremen and Hannover*

**T34-5A** Modafinil inhibits rat midbrain dopaminergic neurons through D2-like receptors
*BP. Klyuch, TM. Korotkova, AA. Ponomarenko, JS. Lin, HL. Haas and OA. Sergeeva, Düsseldorf and Lyon (F)*

**T34-1B** Cocaine and amphetamine dose-dependently increase serotonin and dopamine in memory associated cortical areas: an in vivo microdialysis study in freely moving rats
*ME. Pum, JP. Huston and CP. Müller, Düsseldorf*

**T34-2B** Calcineurin (protein phosphatase 2B) is involved in the mechanisms of action of antidepressants
*C. Crozatier, S. Farley, IM. Mansuy, S. Dumas, B. Giros and ET. Tzavara, Göttingen, Créteil (F) and Zürich (CH)*

**T34-3B** Biogenic amines in the central body of the grasshopper *Chorthippus biguttulus*: Possible modulators of sound production?
*M. Kunst and R. Heinrich, Göttingen*

**T34-4B** Expression of genes related to 5-HT neurotransmission:
Regulation by chronic stress and citalopram in a rat model of depression
*N. Abumaria, R. Rygula, E. Fuchs and G. Flügge, Göttingen*

**T34-5B** *Pharmacological validation of chronic social stress as a model of depressive symptoms in rat*
*R. Rygula, N. Abumaria, C. Hiemke, E. Fuchs, E. Ruether, G. Fluegge and U. Havemann-Reinecke, Göttingen and Mainz*

**T34-1C** Blockade of dopamine D1 receptors in the orbitofrontal cortex improve rat's behavioural flexibility measured in an operant behavioural system
*K. Schwabe and S. Winter, Hannover and Bremen*

**T34-2C** Modulation of GABAergic conductances in cerebellar granula cells by the anesthetic ketamine
*W. Hevers, SH. Hadley, H. Lüddens and J. Amin, Leipzig, Tampa (USA) and Mainz*

**T34-3C** Repeated Exposure to Ecstasy (MDMA) and its Effects on Behavior and Neurochemistry in Male Wistar Rats - Individual Differences.
*Y. Mihov, V. Ludwig and R. Schwarting, Marburg an der Lahn*

**T34-4C** Effect of induced diabetes mellitus, acute morphine or acetazolamide treatment on blood flow increasing caused by cortical spreading depression (CSD) in rat
*K. Nagy, F. Domoki and F. Bari, Szeged (H)*

# New potential drugs for treatment of secondary brain injury

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Traumatic brain injury (TBI) may result from penetrating brain wounds or shock waves, for example due to explosions, accidents, or violence. It is now well understood that the primary injury of TBI is followed for hours and days by a process of secondary injury. Mitochondria are one of the cell organelles affected by secondary brain injury. Our studies indicate that the mitochondrial translocator protein (TSPO) appears to play an important role among the factors leading to neuronal cell death with secondary brain injury. We have shown in vivo that classical TSPO ligands, such as PK 11195, can prevent neurodegeneration due to excitatory amino acids, an important factor in secondary brain injury after TBI. We also found that reducing TSPO expression by genetic manipulation reduced apoptotic levels. In particular, such TSPO knockdown completely prevented apoptosis caused by a major contributor of neuronal cell death, the excitatory amino acid glutamate, and also by Abeta(1-42), one of the causative agents for the neuronal cell death in the neurodegenerative disease of Alzheimer. This indicates that the TSPO plays an essential role in the induction of apoptosis, which is one of the cellular processes leading to neuronal cell death in the process of secondary brain injury. Recently, we have developed compounds that bind with high affinity to the TSPO and block its apoptotic function. These novel compounds reduced basal apoptotic levels in neuronal cells, as well as apoptosis induced by glutamate, which is known to be an important causative agent for secondary brain damage, and also takes part in neurodegenerative diseases. We envision that secondary brain injury due to TBI may be prevented by providing soldiers and paramedics with one of the drugs we have developed, i.e., soldiers and paramedics could carry such medication with them and use it on site. This may reduce the incidence of disabilities presently occurring in the aftermath of TBI suffered from explosions, including terrorist attacks, accidents, and other forms of violence. In addition, such a drug might also find application in the treatment of neurodegenerative diseases.

Keywords: Peripheral-type benzodiazepine receptor (PBR), traumatic brain injury (TBI), neurodegeneration, apoptosis, drug development

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# Standard antiepileptic drugs fail to block epileptiform activity induced by low magnesium or 4-aminopyridine in rat hippocampal slice cultures.

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Hippocampal slice cultures (HSCs) are increasingly used as a model system to study neuronal cell death, neuroprotection and synaptic plasticity. We wondered whether HSCs are suited as a model of epileptiform activity and epileptogenesis which eventually could be exploited for anticonvulsant/antiepileptogenic drug screening.

HSCs with or without entorhinal cortex were prepared from 6 to 10-day old Wistar rats. HSCs were continuously superfused with prewarmed (35-36$^{o}$C) and oxygenated (95%$O_2$/5%$CO_2$) MEM in artificial cerebrospinal fluid. Single unit and population activities were recorded with tungsten microelectrodes and glass pipettes positioned in the dentate gyrus (granular layer), pyramidal layer of areas CA3 and CA1 of hippocampus and layers 3-5 of entorhinal cortex. The $[K^+]_o$ concentration was measured with an ion-sensitive electrode in CA3. A primary afterdischarge (PAD) was induced by electrical stimulation (.1ms, 100Hz, 1s) with a pair of tungsten electrodes positioned in the hilus. The drugs tested were carbamazepine (60-125µM, 24 HSCs 7-56 DIV), phenytoin (40-100µM, 15 HSCs 5-29 DIV), sodium valproate (0.8-2mM, 9 HSCs 19-41 DIV), diazepam (3.5-5µM, 5 HSCs 14-44 DIV), and gabapentin (120-300µM, 3 HSCs 9-11 DIV). Drug concentrations correspond to upper therapeutic-low toxic ranges in plasma of patients treated with the respective AEDs.

Seizure like events (SLEs) consisting of tonic-clonic periods and late clonic-like recurrent activity were reliably induced by either omitting $Mg2^+$ and increasing KCl to 5mM, or by adding 4-aminopyridine (50-200µM, plus bicuculline 1-2µM in some cases). SLEs were synchronous in dentate gyrus, hippocampus and entorhinal cortex and were associated with rises in $[K^+]_o$ in CA3. Unexpectedly none of the drugs tested prevented the induction of SLE or stopped ongoing SLEs, of both types (tonic-clonic, late recurrent), at all ages (DIV) , and in all regions investigated. Clear drug effects were nevertheless present. The latency of occurrence of the first tonic-clonic period increased, tonic-clonic periods as well as single clonic/late recurrent discharges became on average shorter, and the frequency of field potential oscillations during tonic periods decreased. SLE-associated rises in $[K^+]_o$ were on average only slightly reduced under AED treatment. AEDs alone induced a decrease in spontaneous unit activity and a strong reduction of the PAD and the associated increase in $[K^+]_o$. All drug effects were reversible.

Resistance to major AEDs has been reported for tonic-clonic seizures in an intact immature corticohippocampal preparation of rats 7 to 8 days old[1] and for late recurrent discharges in acute slices of adult rat entorhinal cortex and hippocampus[2]. It remains to be seen if these preparations together with HSCs can be exploited for the development of drugs for pharmacoresistant epilepsies.

Literature cited: [1] Quilichini et al. Epilepsia, 2003, 44:1365-1374. [2] Heinemann et al. Epilepsia, 1994, 35(Suppl) S10-S21.
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# Intrastriatal inhibition of aromatic amino acid decarboxylase prevents L-DOPA-induced dyskinesia: a bilateral reverse in vivo microdialysis study in rats

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Dyskinesia consists of involuntary choreiform and dystonic movements, which develop over time after treatment with L-DOPA in Parkinson´s disease. The present study was performed to clarify (1) whether L-DOPA itself triggers dyskinetic behaviour and (2) which role the neurotransmitters dopamine (DA) and noradrenaline (NA) play.

The neurotoxin 6-hydroxydopamine (6-OHDA) was unilaterally injected into the median forebrain bundle of male Wistar rats, followed by L-DOPA treatment (6/15mg/kg, p.o., daily over 3 weeks) to induce stable dyskinetic behaviour. For intrastriatal application guide cannulae aiming at the striata were bilaterally implanted under anaesthesia ipsilateral and contralateral to the side of the 6-OHDA lesion. After a recovery period of 4-5 days concentric microdialysis probes (Microbiotech MAB 4.9.2.PES, 2 mm membrane length, Sweden) were inserted via the guide cannula into the striata and perfused with artificial cerebrospinal fluid for 1 hour (4µl/min). Afterwards different drugs were administered via reverse dialysis on the contralateral side followed on the ipsilateral side for 60 min each: DA (24nmol/60min), benserazide (6nmol/30min) prior to L-DOPA plus aromatic amino acid decarboxylase inhibitor (benserazide) (24nmol plus 12nmol/60min), L-DOPA (24nmol and 2.4nmol/60min), and NA (24nmol and 2.4nmol/60min). Dyskinetic behaviour including axial, limb and orofacial dyskinesia was monitored every 5 minutes.

The 6-OHDA lesion caused an almost complete striatal dopamine depletion (>99%) confirmed by HPLC post mortem tissue analysis. Striatal administration of the drugs on the contralateral side produced no dyskinetic behaviour. L-DOPA in combination with benserazide in the lesioned striatum led only to a slight and transient increase in dyskinesia. In contrast, the same concentration of DA and L-DOPA on the lesioned side induced a pronounced increase in dyskinetic behaviour, which was similar in the maximum level and cumulative scores. However, the DA-induced dyskinesia appeared earlier and was of shorter duration than after L-DOPA treatment. Furthermore, intrastriatal NA induced dyskinetic movement in a dose-dependent manner, which was higher than in the L-DOPA group, both in cumulative scores and in the maximum level.

In conclusion, high levels of DA as well as NA in the striatum are able to switch on dyskinetic behaviour when dopaminergic neurons are degenerated. Moreover, we have shown that L-DOPA itself cannot induce dyskinetic movements.

# Effect of "Enriched Environment" during development on adult rat behaviour

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To improve the well-being of rats in laboratories, enriched housing conditions (enriched environment, EE) are increasingly used, however with different amount of enrichment. In general EE describes a combination of social interaction (e.g., single or group housing) and physical equipment of the cage, two features that are usually intermingled. Recent studies indicate that enriched conditions during development may have a significant impact on rat behaviour and brain neurochemistry, especially on the dopaminergic system. Since behavioural neurobiology is largely based on function of the dopaminergic system there are concerns about using EE for well-being of the rats. Additionally, in these studies the effects of social and environmental EE are usually not differentiated and single housing itself is regarded as a model for certain psychiatric disorders. Thus we here tested effects of different amount of physical equipment during development on cognitive and emotional behaviour of adult rats kept in groups.

Three groups of male Wistar rats (n=5-8) were raised under different EE. For the "high enriched" condition, the rats were raised in a voluminous cage (height: 60 cm, width: 100 cm, depth: 60 cm), which was provided with climbing and nest materials, opportunities to take cover and different toys. The conventional group was raised in standard cages of Macrolon® type IV, likewise the low enriched group. However, these rats were provided with one or two pieces of the equipment used for the high enriched group. From postnatal 70 on rats were behaviourally tested for anxiety behaviour (elevated plus-maze), for spatial learning (four-arm-baited elevated eight-arm-maze), for behavioural flexibility and motivation (operant behaviour system). Finally, locomotor activity and prepulse inhibition of the acoustic startle response was tested with and without challenge with the dopamine receptor agonist apomorphine.

No difference was found in the number of entries into the open or closed arms of the elevated plus maze. However, the high EE group showed higher rearing activity on the open arm compared to the other groups, indicating increased exploratory behaviour under anxiety provoking conditions. Cognitive function in the radial maze task was not affected, but rats of the high EE group needed less time for arm entries, again indicating increased activity on open arms. Additionally, except for higher number of lever presses per time interval, operant behaviour did not differ between the groups. Both, under baseline condition and after apomorphine challenge, groups did not differ in open field locomotor activity. However, while no difference in PPI of startle and startle amplitude was found under baseline condition, in the high EE group startle amplitude was enhanced after administration of apomorphine while PPI was not different between groups.

Altogether, there was no evidence that environmental EE without differences in social EE has an effect on cognitive or emotional behaviour. However, motor activity of rats raised under high EE conditions seems to be enhanced when challenged with cognitive or emotional demand.

# Modafinil inhibits rat midbrain dopaminergic neurons through D2-like receptors

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Modafinil is a well tolerated medication for excessive sleepiness associated with several severe disorders such as Parkinson´s disease, narcolepsy and ALS. The mechanism of its action is elusive. We investigated the modafinil action on identified dopaminergic (DA) and GABAergic neurons in the ventral tegmental area (VTA) and substantia nigra (SN) of rat brain slices. Modafinil (20μM) inhibited the firing of DAergic, but not GABAergic neurons. This inhibition was maintained in the presence of tetrodotoxin and was accompanied by hyperpolarization. Sulpiride (10μM), a D2-receptor antagonist, but not prazosine (20μM, an α1-adrenoreceptor blocker) abolished the modafinil action. Inhibition of dopamine reuptake with a low dose of nomifensine (1μM) reduced the firing of DA neurons in a sulpiride-dependent manner and blunted the effect of modafinil. On acutely isolated DA neurons, modafinil evoked D2-receptor-mediated outward currents, which reversed polarity near the K+equilibrium potential and were unchanged in the presence of nomifensine. Thus, we describe here a novel agonist-like action of modafinil at the D2 receptor, which can explain the differences between classical psychostimulants and modafinil and helps to understand the involvement of different neurotransmitter-systems in the wake-promoting action. We suggest that D2-like receptors are the major if not unique site of modafinil action.

# Cocaine and amphetamine dose-dependently increase serotonin and dopamine in memory associated cortical areas: an in vivo microdialysis study in freely moving rats

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The development of addiction and the establishment of memories may be subserved by common or related mechanism. Despite considerable evidence showing the modulation of neuronal plasticity by psychostimulant drugs, the neurochemical effects of psychostimulant drugs in memory-related brain areas are poorly characterized.

Previous work from our laboratory demonstrated acute effects of cocaine on extracellular serotonin (5-HT) levels in the ventral hippocampus. The present study investigated the acute neurochemical effects of two major psychostimulant drugs - cocaine and d-amphetamine - on cortical areas related to the hippocampus (the entorhinal (EC) and perirhinal cortex (PRC)) and the prefrontal cortex (PFC), which served as a reference area. We used triple-probe microdialysis in freely behaving rats, in combination with HPLC-EC to measure extracellular dopamine (DA) and 5-HT activity. Behavioral data showed, that cocaine (0, 5, 10, 20 mg/ kg; i.p.) and d-amphetamine (0, 0.5, 1.0, 2.5 mg/ kg) dose-dependently activated behavior to a comparable extend. In parallel, cocaine dose-dependently increased DA and 5-HT levels in the EC, PRC, and PFC. The DA response was less pronounced in the EC but not PRC as compared to the PFC. 5-HT was significantly more elevated in the PFC and EC compared to the PRC. D-amphetamine was administered in a dose-range that induced amounts of hyperactivity that were comparable to those found for the tested doses of cocaine. D-amphetamine dose-dependently increased extracellular DA and 5-HT levels in the EC, PRC, and PFC. In contrast to cocaine, d-amphetamine had, with the exception of the 1.0 mg/ kg dose, similar effects on DA levels in the PRC and EC as compared to the PFC. Also, the levels of 5-HT did not differ significantly between the PFC, PRC, and EC. Comparisons of the area under curves (AUCs) of the dopamine responses after cocaine and amphetamine yielded comparable effects for both drugs at the dose ranges used in this study. However, the AUCs of the 5-HT response in the PFC was more pronounced after the application of cocaine compared to amphetamine, while the 5-HT response in the EC and PRC was comparable.

In conclusion, we provide evidence for dose-dependent, acute effects of the psychostimulants cocaine and d-amphetamine on extracellular DA and 5-HT levels in the cortices of the hippocampal formation, the EC and PRC. However, we found different neurochemical response characteristics in these cortices after cocaine as compared to amphetamine. These differences were not reflected by differences in the hyperactivity-inducing properties of these drugs. The neurochemical alterations in cortical areas strongly connected to the hippocampus may contribute to the dysfunctional modulation of memory processes by drugs of abuse, as well as to their leading to the establishment of addiction memory.

# Calcineurin (protein phosphatase 2B) is involved in the mechanisms of action of antidepressants

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Calcineurin (PP2B) is a Ca2+-dependent protein phosphatase enriched in the brain that takes part in intracellular signaling pathways regulating synaptic plasticity and neuronal functions. Calcineurin-dependent pathways are important for complex brain functions such as learning and memory. More recently, they have been suggested to play a role in the processing of emotional information. The aim of this study was to investigate whether calcineurin may be involved in the effect of antidepressants. We first found that chronic antidepressant treatment in mice leads to an increase of calcineurin levels in the hippocampus. We then studied the behavioral and molecular responses to fluoxetine of mice with a genetic overactivation of calcineurin in the hippocampus (CN98 mice). We observed that CN98 mice are more sensitive to the behavioral effect of fluoxetine and desipramine tested in the tail suspension test. Moreover, the basal expression of growth factor BDNF and subunit 1 of AMPA glutamate receptor, GluR1, both of which are modified after chronic antidepressant administration, are altered in the hippocampus of CN98 mice. These results suggest that calcineurin-dependent dephosphorylation plays an important role in the mechanisms of action of antidepressants, providing a new starting point for developing improved therapeutic treatments for depression.

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# Biogenic amines in the central body of the grasshopper *Chorthippus biguttulus*: Possible modulators of sound production?

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Acoustic communication of grasshoppers in the context of mate finding, courtship and rivalry is controlled by the central body complex. Activation of muscarinic acetylcholine receptors (mAChRs) in the central complex has been demonstrated to stimulate specific singing in various grasshopper species including *Chorthippus biguttulus*. Investigation of the second-messenger pathways activated by mAChR's showed that these are positively coupled to the AC- and the PLC -pathway.

To gain a more thorough understanding of how the internal state or the mood of an animal influences the motivation to perform this behavior, we started to investigate the role of biogenic amines in the central body. As a start we mapped several biogenic amines in the central body using immunocytochemistry and compared their distribution with that of mAChRs. All the biogenic amines tested so far ( Histamin, Serotonin, Dopamin and Octopamine) are present in the central body, especially in the upper division, a region in which mAChR related immunostaining is also most prominent. These anatomical data indicate that biogenic amines are potential modulators of acoustic communication in insects.

Pharmacological experiments, in which biogenic amines are injected into the central body neuropile of restrained but otherwise intact grasshoppers indicated an excitatory role for Dopamin and Octopamine and an inhibitory role for Tyramine in the control of sound production.

# Expression of genes related to 5-HT neurotransmission: Regulation by chronic stress and citalopram in a rat model of depression

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Selective serotonin-reuptake inhibitors (SSRIs) increase extracellular serotonin (5-HT) but the molecular mechanisms underlying their therapeutic actions are not yet fully understood. We investigated effects of the SSRI citalopram on expression of 5-HT-related and stress-upregulated genes that were previously identified in the dorsal raphe (DR) nucleus of stressed and unstressed male rats [1]. Animals were chronically stressed using the resident-intruder paradigm on a daily basis for five weeks, and the daily citalopram treatment (30mg/kg/day) started after the first week of stress and lasted for four weeks. At the end of the experiment, individual DR nuclei were isolated by the punching technique. Real-time PCR and Western blot analysis demonstrated that tryptophan hydroxylase (TPH) protein expression was upregulated by chronic stress and normalized by citalopram although mRNAs for TPH 1 and 2 were differentially affected. Neither stress nor citalopram did significantly change serotonin transporter mRNA, but citalopram decreased 5-HT1A autoreceptor mRNA in stressed animals only. Citalopram reversed the stress-induced upregulation of mRNA for synaptic vesicle glycoprotein 2b and CREB binding protein. Chronic social stress increased the expression of SNAP-25 mRNA and protein, but citalopram had no significant effect on SNAP-25 expression. These findings indicate that in the DRN of chronically stressed rats, citalopram restores normal 5-HT-biosynthesis, modulates feed-back mechanisms mediated by 5-HT1A and normalizes expression of distinct genes and proteins involved in the synaptic-machinery and neuroplasticity. Similar mechanisms may contribute to the therapeutic-actions of citalopram in patients.

[1]Abumaria N et al. (2006) Identification of genes regulated by chronic social stress in the rat dorsal raphe nucleus. Cell Mol Neurobiol 26: 145- 162.

# *Pharmacological validation of chronic social stress as a model of depressive symptoms in rat*

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Recently we have developed a model of chronic social stress in rats where stressed animals showed anhedonia and motivational deficits [1]. The changes were reversible by treatment with antidepressant drug citalopram [2]. These observations prompted us to suggest that chronic social stress paradigm in rats can serve as a valid model of depressive symptoms. The present study was designed for further pharmacological validation of this model. Male Wistar rats were subjected to chronic social defeat, as described previously [1], and in separated experiments treated with antidepressant drug reboxetine (40 mg/kg/day), neuroleptic haloperidol (2 mg/kg/day) and anxiolytic diazepam (1 mg/kg i.p.). Reboxetine and haloperidol were administered chronically. The drugs were administered orally, in drinking water. Diazepam was administered acutely at the end of 5 weeks period of chronic social stress. The drug monitoring performed at the end of the experiment allowed determination of plasma levels of each drug and their metabolites. The effects of social stress and the treatments were investigated in behavioural paradigms such as sucrose preference, forced swim test, open field test and elevated plus maze. Cooperational study assessed the influence of stress and drug treatments on protein and gene expression. [4]. After four weeks of oral treatment the plasma concentrations of reboxetine and haloperidol were similar to those reported in human patients treated with therapeutically effective doses [3]. Four weeks of daily treatment with antidepressant reboxetine reversed the majority of adverse effects of chronic social stress. Treatment with haloperidol worsened the adverse effects of chronic social stress having effects similar to stress on reward and motivation related behaviours. Treatment with diazepam reduced the anxiety related behaviours in control animals. However had no beneficial effects on socially stressed individuals. Diazepam had no effects in force swimming and sucrose tests. The results of present study provided the proof for the specificity of antidepressant treatment in reversing socially induced changes in rats. The present data confirm predictive validity of the chronic social stress as a model of depressive symptoms in rats.

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# Blockade of dopamine D1 receptors in the orbitofrontal cortex improve rat's behavioural flexibility measured in an operant behavioural system

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Dopamine neurotransmission in the orbitofrontal cortex (OFC) is regarded as important for the regulation of behavioural flexibility and for the neural representation of reward value. We here compared the effect of systemic and local blockade of dopamine D1 and D2 receptors in the OFC on rats' behavioural flexibility measured in an operant behaviour system. Rats were trained in a two lever equipped Skinnerbox. The number of lever responses for reward was nine responses for the efficient and 25 responses for the inefficient lever. Once the rat continuously used the efficient lever the demand reversed, i.e., the efficient lever became the inefficient lever. Ten reverses or 60 min ended the trial. Once rats had learned the task, i.e., had repeatedly reached criterion of ten reverses per 60 min, they were intraperitoneally injected with the DA D1 receptor antagonist SCH23390 (40 µg/kg), the DA D2 receptor antagonist sulpiride (10 mg/kg) or vehicle (phosphate buffer saline; 2 ml/kg) in randomized order. After systemic blockade of the DA D1 receptor rats switched marginally faster to the efficient lever after reverse of demand. However, at the same time rats switched more often back to the inefficient lever, indicating increased impulsivity, although the effect of vehicle injection was similar. Additionally, SCH23390 injection decreased the number of reverses within 60 min, which may point to a motivational deficit and/or decreased motor function. Instead, systemic blockade of DA D2 receptors by injection of sulpiride did not affect behavioural function in this task.

A second group of rats were stereotaxically implanted with bilateral guide cannulae aiming at the OFC under chloralhydrate anaesthesia. After three days of recovery, training continued until the rats reached baseline performance again. Rats were then bilaterally microinjected with 3 µg/0.5 µl SCH23390, 3 µg/0.5 µl sulpiride or vehicle (0.5 µl only). The effects of local injection of SCH23390 more or less reproduced the effects of systemic injection, i.e., faster switching to the efficient site after reverse, indicating faster recognizing the switch in demand. The number of reverses within 60 min also decreased, whereas the number of switches back to the inefficient lever was not affected. Similar to the systemic injection, local injection of sulpiride did not affect responding in this task.

These data indicate that DA D1 receptors in OFC are involved in the ability to perceive the reverse earlier thus affecting rats' behavioural flexibility and detection of reward value.

# Modulation of GABAergic conductances in cerebellar granula cells by the anesthetic ketamine

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The inhibitory neurotransmitter γ-aminobutyric acid (GABA) mediates most effects through GABA-gated $Cl^-$-channels ($GABA_A$Rs) composed of five out of the 19 known subunits (α1-6, β1-3, γ1-3, δ, ε and ρ1-3, π, θ). $GABA_A$Rs mediate the effects of many anesthetics, including barbiturates and general or volatile anesthetics with their potencies being related to their GABAmimetic action. One intravenous anesthetic with unique pharmacological and clinical manifestations is ketamine, known as non-competitive NMDA-receptor antagonist (Ki ~ 400 nM). Whereas the role of NMDA receptors in ketamine's action has been well-established, there is little evidence of ketamine interacting with the primary receptor-target of most anesthetics, the $GABA_A$Rs.

Here, using oocyte expression, we show that ketamine at anesthetically relevant concentrations modulates and directly activates a subset of $GABA_A$Rs containing α6β2δ and α6β3δ receptor subtypes expressed abundantly in granula neurons within the adult cerebellum. The high sensitivity to ketamine is unique to α6β2δ and α6β3δ $GABA_A$Rs based on comparative studies expressing α1β2γ2, α1β2δ , α4β2δ , α4β2γ2 , and α6β2γ2 $GABA_A$R subtypes. Present understanding is, that in cerebellar granula cells α6β2δ receptors are localized extrasynaptically and are responsible for a tonic background conductance, revealed only when blocked by a GABA antagonist. In *in situ* experiments using patch-clamp recordings in cerebellar slices from adult rats and mice, high μM concentrations of ketamine potentiate the tonic GABAergic conductance present in granula neurons even in absence of exogenously applied GABA. All effects are blocked by the competitive $GABA_A$R antagonist bicucculline or by the α6-specific inhibitor furosemide and were absent in cerebellar slices from $\alpha6^{-/-}$ or $\delta^{-/-}$ knockout mice. To differentiate GABA independent intrinsic effects of ketamine from a potentiation of the low GABA concentrations present in cerebellar slices, we established a preparation of acutely dissociated granula neurons. Here, the tonic conductance was absent. Ketamine at high μM concentrations only potentiated GABA induced currents, an effect most pronounced at low GABA concentrations.

Despite the fact that ketamine is widely used to prevent interference with the GABAergic system our electrophysiological and pharmacological data indicate that cerebellar $GABA_A$Rs containing α6- and δ-subunits are potentiated by ketamine. Despite its high affinity for the NMDA-receptor, ketamine is used in high doses during anesthesia (1-5 mg/kg in humans, 10 - 50 mg/kg in rodents), suggesting these $GABA_A$Rs may contribute to the atypical effects of ketamine.

# Repeated Exposure to Ecstasy (MDMA) and its Effects on Behavior and Neurochemistry in Male Wistar Rats - Individual Differences.

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Ecstasy (3,4-methylenedioxymethamphetamine, MDMA) is a popular illicit recreational drug. In humans, long-term consumption can be followed by various psychological changes and deficits (e.g. verbal and visuo-spatial memory deficits). This study addresses the issue of whether and to what extent these effects depend on subject-inherent factors related to personality. Since such relationships cannot be tested experimentally in humans, animal models are required.

Male albino Wistar rats were screened in the elevated plus-maze (EPM), prior to drug application. On five consecutive days, these animals received subcutaneous injections of either D,L-MDMA (5 mg/kg) or saline once daily. Upon each application, behavior was monitored for 60 minutes in an activity box to gauge the development of behavioral sensitization. After two days without treatment, all rats were challenged with 5 mg/kg MDMA to test for the expression of sensitization (an established test in research on addiction). Several days later, they were again tested in the elevated plus-maze and also in a novel object test (reactivity to novelty, short-term memory). Finally, striatal and cortical brain tissues were analyzed post-mortem for their contents of biogenic amines to relate neurochemical with behavioral variables.

Repeated application of MDMA significantly decreased serotonin concentration in the ventral striatum, irrespective of basal anxiety-like behavior in the EPM. However, individual differences in this screening test affected the behavioral consequences of MDMA, as measured in the novel object test and the second EPM.

Repeated application of MDMA also induced behavioral sensitization. The data indicated that detecting sensitization was dependent on the type of behavioral measure used (i.e. locomotion, rearing) and the time window after drug application. Furthermore, statistical analysis revealed a relationship between sensitization and measures of serotonin metabolism in the ventral striatum.

# Effect of induced diabetes mellitus, acute morphine or acetazolamide treatment on blood flow increasing caused by cortical spreading depression (CSD) in rat

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Cortical spreading depression (CSD) is a transient perturbation of the cortical neuronal activity and cortical blood flow (CBF). Although several vasoactive substances of various origins are thought to be implicated in CSD-related transient changes in CBF, the mechanism has never been satisfactorily elucidated. Hyperemia observed during CSD or seizures are, at least in part, mediated through CGRP receptors. The sensory system, including the CGRP containing fibers is strongly affected in diabetes mellitus. We tested the hypothesis that acute morphine exposure and inhibition of the carbonic anhydrase (CA) or experimentally induced diabetes mellitus, would affect the CSD-induced changes in CBF. Male Wistar rats weighing between 250 and 420g were used. Diabetes was induced by a single i.p. dose (65 mg/kg) of streptozotocin (STZ) and the CSD measurements were performed 8 weeks later. In anaesthetized animals CSD were repeatedly induced by topical application of KCl and CSD- related CBF changes were recorded by laser Doppler flowmetry. In diabetic rats (n=5) CSD- induced an elevation in CBF that peaked at 301±31% whereas it was 297±30% in the controls (n=5). In spontaneously breathing rats (n=6), after 3 consecutive CSD morphine was given i.v (1 and 5 mg/kg). At low does of the drug, the CSD-related CBF changes were not different (peak flows: 243±14% vs.238±26% above baseline) from the controls. The significant depression in the blood flow elevation after 5 mg/kg could be attributed to the depressed ventilation because in the ventilated group (n=6) even the high morphine dose did not affect the CBF. After administration of acetazolamide (n= 8, 10 and 20 mg/kg, iv) the CSD related CBF changes were depressed (276±24% in control, and 175±30% and 168±22% after 10 and 20 mg/kg, respectively). Our experiments confirm earlier observations indicating that CSD related CBF changes are intact after impairment of the sensory neuronal function. Our data with the morphine and acetazolamide treatment give new information on the nature of the CSD-related CFB changes.

# Poster Topic

## T35: Disorders of the nervous system

**T35-1A** Behavioral investigations in the *dt*$^{sz}$ hamster, a model of idiopathic paroxysmal dystonia
*M. Hamann, M. Bennay, M. Gernert, K. Schwabe, M. Koch and A. Richter, Berlin, Düsseldorf, Hannover and Bremen*

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**T35-21C** FUNCTIONAL CHARACTERIZATION OF MICE LACKING THE DOPAMINE TRANSPORTER - A MOUSE MODEL FOR ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)
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# Behavioral investigations in the *dt*$^{sz}$ hamster, a model of idiopathic paroxysmal dystonia

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The pathophysiology of various types of hereditary dystonia, a common movement disorder, is still unknown, hampering the development of rational therapeutic strategies. Basal ganglia dysfunctions play a critical role in this movement disorder. There is evidence that primary dystonia is accompanied by affective disorders, e.g., depression and enhanced anxiety, pointing to the possibility that limbic structures might be involved in the pathophysiology of idiopathic dystonias. Interestingly, previous investigations in the *dt*$^{sz}$ mutant hamster, which represents a unique model of stress-inducible paroxysmal dystonia, demonstrated changes in limbic structures. To elucidate the possible functional relevance of these alterations, we performed behavioral analyses, i.e., elevated plus maze, a modified open field for investigating anxiety-related behavior, and acoustic startle response (ASR) as well as prepulse inhibition (PPI) of ASR investigations in mutant and control hamsters for investigating dysfunctions of sensorimotor information processing.

In the elevated plus maze, no significant differences were detected between *dt*$^{sz}$ and control hamsters. In the modified open field, the control animals stayed significantly longer in the inner, i.e. less safe, region of the open field than mutant hamsters ($p=0.003$). The PPI of *dt*$^{sz}$ hamsters was not only significantly reduced compared to controls ($p=0.026$), but the ASR was even facilitated by the prepulse.

The present results of a moderately enhanced anxiety-related behavior and an abnormal sensorimotor gating of the ASR are in accordance with previous data of alterations in limbic structures and demonstrate that dysfunctions within the limbic system may be critically involved in the manifestation of stress-inducible dystonic episodes in this animal model. Furthermore, the present data may reflect a deficit in processing and prioritizing incoming sensory information and are in line with findings of a co-occurrence of idiopathic dystonia and affective disorders in human patients.

# BLOCK OF DRUG TRANSPORTER ACTIVITY AND EFFICACY OF ANTIEPILEPTIC DRUGS IN HUMAN EPILEPTIC HIPPOCAMPUS AND TEMPORAL CORTEX

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Ectopic expression of multidrug transporter proteins (Pgp, MRP1, and MRP2) in astrocytes or neurons may be one reason for antiepileptic drug (AED) resistance in patients suffering from mesial temporal lobe epilepsy. To find out whether Pgp and MRPs contribute to drug resistance we tested whether inhibition of transporter activity by verapamil (40 µM) and/or probenecid (400 µM) affected self-sustaining AED-resistant ictaform activity induced in slices of both resected hippocampus (dentate gyrus) and temporal cortex (laminae II-III/V). Here we report that block of drug transporter activity rarely reverses carbamazepine- or valproate-resistant activity in slices from sclerotic hippocampi (2 out of 27 slices in 2 out of 15 patients) but suppresses such activity in 53% of slices from non-sclerotic specimens (8 out of 15 slices in 5 out of 7 patients; $p<0.01$). In the temporal cortex a reversal of AED-resistance is also rare (3 out of 19 slices in 1 out of 9 patients) but a reduction of activity is more frequently seen than in the hippocampus ($p<0.01$). Our preliminary data on localization of drug transporters show that these are present in slices of the temporal cortex and both sclerotic and nonsclerotic hippocampus, except for the dentate gyrus. In contrast, monitoring multidrug transporter activity by using the calcein extrusion essay reveals functional drug transporters mostly in slices of the temporal cortex (12 out of 15) but rarely in the hippocampus (3 out of 9). Further experiments are required to explain the different effects of drug transporter inhibition in sclerotic and nonsclerotic hippocampus as well as the different transporter activity in hippocampal and cortical tissue.

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# Rapid eye movement sleep behaviour disorder, obstructive sleep apnea syndrome and stimulus sensitive myoclonus in a patient with pontine lesions - a case report -

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**Introduction:** REM sleep behaviour disorder (RBD) is characterized by variable loss of muscle atonia and excessive phasic EMG activity during REM sleep associated with enactment of unpleasant dreams. RBD mainly affects men older than 50 years and is strongly associated with neurodegenerative diseases. Furthermore, RBD can be facilitated by treatment with various psychotropic drugs.

**Patient:** A 79 year old patient showed complex motor behaviour during sleep including screaming and attacking his wife while dreaming. Events occurred since the intake of venlafaxine in addition to mirtazapine three months before admission for treatment of depression and insomnia.

**Methods:** We performed a polysomnography (PSG), routine EEG, waking EEG-EMG-video documentation, MRT, ECG, routine laboratory parameters, physical examination, the “Sniffin´ Sticks Screening12 Test” , the blink reflex and neuropsychological testing.

**Results: PSG** revealed typical signs of RBD and distinct periodic limb movements. Frequent desaturations arose mainly in NREM 1 and during REM sleep (AHI: 16).

**Routine EEG** showed a slow occipital alpha activity and no signs of focal slowing or epileptic potentials. However, photic stimulation elicited generalised, symmetric jerks accompanied by vocalizations and a feeling of increased intra-abdominal pressure. The patient was fully conscious and adequately reacting.

**EEG-EMG-video documentation** revealed no epileptic discharges. Initial jerks consistently occurred with a latency of about 200 ms and increased in length, intensity and duration in the course of photic stimulation. Like in the routine EEG, the patient was fully conscious.

**Other findings:** The MRT displayed bilateral cerebellar and pontine white matter lesions. A CCT, accomplished in August 2005, displayed signs of multiple medullary infarctions in both cerebral hemispheres. The early component of the blink reflex (R1) was slightly elongated on the right side, consistent with an ipsilateral pontine lesion. “Sniffin´ Sticks Screening12 Test” and routine laboratory parameters were normal. The ECG showed multiple VES next to sinus arrhythmia and first degree A-V block. Physical examination uncovered extrapyramidal signs but no full parkinsonism was present. Neuropsychological diagnostic revealed an attention deficit, disturbances in psychomotor and executive function as well as mild deficits in the visuoconstructional domain.

**Conclusions:** In our patient who has a history of cerebrovascular events and distinctive pontine lesions as ascertained by MRT and neurophysiology, RBD was probably facilitated by augmentation of the antidepressive medication. The exaggerated startle response provoked by photic stimulation is similar to reflex jerks seen in patients with hyperekplexia and might be due to a disinhibition of thalamocortical motor pathways. Symptomatic RBD as well as hyperekplexia have been described in association with brainstem pathology. On the other hand, RBD could have been enhanced by the newly diagnosed obstructive sleep apnea syndrome which was successfully treated by continous positive airway pressure (CPAP).

# Anitdyskinetic effects of the $K_V7$ channel opener retigabine in an animal model of levodopa-induced dyskinesia

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The idiopathic Parkinson syndrome is a common neurodegenerative disease, in which a progressive degeneration of dopaminergic neurons in the substantia nigra leads to decreased striatal dopamine levels. Considering the patient profile, levodopa in combination with decarboxylase-inhibitors (e.g. benserazide) represents still the therapeutical "gold standard" in many cases. However, many patients develop dyskinesias after long-term treatment. The pathophysiology of these spontaneous involuntary dystonic and choreatic movements is unclear, but an increased activity of striatal projection neurons seems to play a critical role. These neurons express $K_V7$ channels, which cause a hyperpolarization after voltage-dependent activation. As the $K_V7$ channel openers retigabine and flupirtine proved to be antidystonic in an animal model of paroxysmal dystonia, we examined their potential antidyskinetic action in an animal model of levodopa-induced dyskinesia.

Dopamine-denervating lesions were performed by unilateral injections of 8 µg 6-OHDA in the left medial forebrain bundle of female Sprague-Dawley rats. All rats were tested for amphetamine-induced rotational behaviour 2 weeks after the injection. Rats showing >4 ipsilateral rotations/min over a 90-minute period were presumed to have a striatal dopamine depletion of more than 90%. At 4 weeks post lesion, 2 groups started to receive chronic treatment with either 20 mg/kg levodopa and 15 mg/kg benserazide or vehicle for 20 days. For rating, dyskinesia was classified into three subtypes (limb, axial and orolingual) and scored from 0 (=absent) to 4 (=permanent, not suppressible) over 200 minutes and every 30 minutes. For drug testing, retigabine (2.5 and 5 mg/kg) was administered additional to levodopa and vehicle respectively. Effects of drug action in comparison to vehicle controls were detected by adding up the severity scores of each observation time.

Retigabine reduced the severity of dyskinesia significantly from the 110. to 140. minute of observation after intraperitoneal (i.p.) administration of 2.5 mg/kg ($p<0.05$) and 50, 80 (each $p<0.05$) and 110 ($p<0.01$) minutes after i.p. injection of 5 mg/kg. Preliminary data on flupirtine, an analogue of retigabine, supported the antidyskinetic efficacy of $K_V7$ channel openers in the rat model. The higher dosage of retigabine caused marked hypolocomotion and ataxia during the first hour of observation.

The results of our study suggest that openers of $K_V7$ channels are interesting candidates for the treatment of levodopa-induced dyskinesia. Both, retigabine and flupirtine, are known to be well tolerated by patients. Additionally, dyskinetic patients, who often suffer from painful muscle distortions, could benefit from the analgesic actions of these compounds. Further studies have to clarify whether the effects of retigabine and flupirtine are only due to their actions on $K_V7$ channels and if these drugs are able to delay the development of levodopa-induced dyskinesias.

# Examination of new lines of transgenic mice localizes age-dependent LTP deficits to the soluble ectodomain of Amyloid Precursor Protein

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Alzheimer´s Disease is a neurodegenerative disorder, pathologically identified by plaque-forming deposits of Abeta, a cleavage product of Amyloid Precursor Protein (APP). But the physiological role of APP and its secreted fragments is still elusive. This might be of special intrerest, because the typical decline of memory function in the disease precedes amyloid deposition and is closely correlated with synapse loss (DeKosky and Scheff, 1990). APP is a transmembrane protein with a multitude of cellular signalling functions and can be subjected to several cleavage pathways, leading to fragments having signalling functions themselves. Apart from the Abeta part, a soluble N-terminal ectodomains (APPsα, APPsβ) and a C-terminal intracellular fragment (AICD) can be generated.

Whereas it is becoming clear that Abeta plays a role in synapse function, much less is known about physiological and synaptic functions of the other cleavage products of APP. To elucidate the role of APP and its fragments in synaptic function, we used two lines of transgenic mice, both lacking wildtype APP, but expressing either the part forming the soluble extracellular domain (APPsα) or an APP construct lacking the last 15 C-terminal amino acids (APPΔCT15). By means of recordings of extracellular field potentials in the CA1 region of acute hippocampal slices of aged mice of the two transgenic strains as well as the complete APP-KO strain, we investigated the effects of the deletions on basal synaptic transmission, short-term plasticity and long-term potentiation (LTP). Basal synaptic function was assessed by investigation of response strength to different intensities of stimulation of the Schaeffer collaterals and by paired pulse stimulation. Short-term plasticity and long-term potentiation were assessed by theta burst stimulation.

Neither the complete KO mice nor the two transgenic strains expressing only parts of APP showed altered basal synapse function, compared to WT littermates, as determined by comparison of input-output strength and responses to paired pulse stimulation. The rate and level of LTP-induction were unaltered in both transgenic strains, compared to their wildtype littermates. Aged APP KO animals, however, displayed a significant reduction of LTP induction level still after 60 min. Post-tetanic potentiation also was unaltered in the two transgenic strains, but slightly impaired in aged APP KO animals.

The deficit being confined to the line lacking the whole protein, but not to the line expressing solely the APPsα fragment, revealed an involvement of the soluble ectodomain of APP in induction and maintenance of long-term-potentiation in old age, whereas lack of the intracellular domain of APP did not seem to affect the ability to perform LTP. This hints to a role of APPsα in synaptic plasticity, raising the question whether the soluble part of APP also plays a role in the genesis of Alzheimer´s Disease, e.g. when it might be lacking as a consequence of altered cleavage balance.

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# Synaptic remodeling in trasgenic mice expressing wild-type or mutant human amyloid precursor proteins

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Previous reports in transgenic mouse lines demonstrated that the human amyloid precursor protein (hAPP) had considerable morphogenetic impact upon neocortical pyramidal cells. Wild-type hAPP at physiological level in yeast artificial chromosome transgenic mice (APP-YAC mice) induced dendritic thickening and increases segment density in the proximal and intermediate arbor fields with a simultaneous, moderate withdrawal of the dendritic trees. In contrast, high solube Abeta level leading to Abeta depositions - a characteristic hallmark of Alzheimer's disease - has been reported to result in less branched and more withdrawn apical arbours with a dramatic decrease in spine density in the neocortex of a mouse line expressing mutant hAPP (Tg2576 strain). The present study shows that the altered dendritic phenotype of principal cells is accompanied by remodeling of synaptic pattern as well. Electron microscopic analysis showed that synaptic density significantly increased in APP-YAC mice when compared to controls that suggested a putative effect of hAPP on inter-neuronal contacts. Light microscopic analysis showed that cholinergic bouton density increased whereas no changes were observed regarding monoaminergic innervation. In contrast, in Tg2576 mice with elevated Abeta level and plaque load investigations using immunohistochemistry for specific vesicular glutamate transporters suggested that intra- and interhemispheric afferents declined whereas thalamocortical afferents remained seemingly unaffected. Similarly, varicosity spacing on anterogradely labelled pyramidal cell axons decresed in these animals. At the same time, cholinergic bouton density was higher in Tg2576 mice when compared to controls similarly to findings in APP-YAC transgenic mice. These bidirectional changes in the number of inter-neuronal contacts resulted in a principally unchanged overall synaptic density in Tg2576 as shown by electron microscopical investigations. The above results suggest a putative role for hAPP in synaptic reconstructuring and a rather selective destruction of intra- and interhemispheric afferents at elevated Abeta level and plaque load. The findings are completed by the quantitative analysis of perisomatic inhibition of neocortical principal cells in both strains.

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# Age Dependent Effects of Isolation Induced Changes of Behavioural and Hormonal Characteristics in Mice

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Social isolation of rodents is one of the animal models to investigate the neurobiological background of psychopathological processes. In dependence on the duration of isolation housing characteristic changes in behaviour and neurotransmission (Schiller et al. 2004) are observed corresponding to depressive and/or impulse control deficit behaviour seen in humans. Of special interest are effects of 6 weeks housing on social behaviour where a crucial role of fertility is suggested if littermates were supposed to isolation.

To elucidate the influence of littermate's age, 3 weeks and 5 weeks old male mice were housed in isolation or group cages for 1, 3 or 6 weeks. At the end of housing behavioural tests (1. running wheel, 2. open field, 3. plus maze, 5. social intruder) were performed. Immediately after the social intruder test trunk blood was collected for RIA corticosterone and ELISA testosterone determination.

Mice 3 respectively 5 weeks of age at the beginning of experimental housing develop a different pattern of behavioural and hormonal changes induced by short and long term isolation housing. Younger mice demonstrate more prominent behavioural activation after one week of isolation with enhanced running wheel and plus maze activity as well as remarkable motional behaviours during the open field and social intruder test in comparison to moderate short term isolation effects in the older mice. After long term isolation housing in young mice emotional behaviour during the plus maze and the social intruder test remains remarkable in comparison to older mice where emotional behaviour rather decreased.

Behavioural characteristics were discussed considering the decreasing blood corticosterone in young mice and increasing corticosterone in old mice in the one hand and the different blood testosterone in young and old mice on the other hand.

Schiller L. Donix M., Jähkel M. Oehler J.: Serotonin 1A and 2A receptor densities, neurochemical and behavioural characteristics in two closely related mice strains after long-term isolation. Progress in Neuro-Psychopharmacology & Biological Psychiatry 30 (2006) 492-503.

# Gene Expression in Early CMT1A Pathogenesis Studied in the Murine Model PMP22$^{tg}$C61

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A duplication of the PMP22 gene is the most frequent cause of Charcot-Marie-Tooth 1A neuropathy (CMT1A), though the initial molecular events in the pathogenesis still remain obscure.

The transgenic mouse strain PMP22$^{tg}$C61, that carries 4 copies of a human YAC-clone encompassing the complete human PMP22 gene, shows a doubling of PMP22 mRNA expression level and closely mimics the human disease (1). This includes mild axonal demyelination and appearance of numerous myelin-phagocytosing macrophages in the peripheral nerves. The use of the hemizygous murine model C61 allowed us to study the early molecular events in the pathogenesis of CMT1A.

As a result of a preliminary analysis, we observed a peak of abnormal overexpression in total PMP22 mRNA at embryonic stage 15.5 to birth (P0). In order to identify genes differentially expressed immediately after this PMP22 overexpression peak, expression profiling has been carried out at P0 by means of Affymetrix GeneChips. The analysis was also extended to P7, to establish whether the initial deregulations in gene expression would continue during myelination. At least 11 highly deregulated genes have been identified at P0; at P7 the number of abnormally regulated genes has increased to 44. Array results have been validated by Real Time RT-PCR.

Few highly significant genes, that show differential expression either at P0 and/or at P7 have been selected for further analyses. As first approach, the expression profile at different developmental stages has been described by Real Time RT-PCR in hemizygous transgenic mice compared to wild type littermates. In vitro and in vivo studies of the localisation, as well of the putative function of the top candidate genes have been proposed and are currently ongoing.

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1. Huxley et al., Hum. Mol. Genet. 7, 1998, 449-458)

# Altered Posttranslational Cleavage of the Proteoglycan Neurocan Coincides with the Appearance of Granule Cell Dispersion in Temporal Lobe Epilepsy Patients

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Temporal lobe epilepsy (TLE), one of the most common neurological disorders in humans is often pharmacoresistant and has to be treated surgically by resection of the affected hippocampus. One key pathological finding of TLE is Ammon's horn sclerosis (AHS) which is characterized by neuronal loss in the CA1 and CA3 subfields of the hippocampus and by a widening of the granule cell layer of the dentate gyrus, termed granule cell dispersion (GCD). Upregulation of the proteoglycan neurocan in the brain extracellular matrix has been shown to accompany GCD formation in an animal model of TLE. In order to investigate the role of neurocan in the development of GCD in humans, we evaluated the expression of neurocan mRNA in surgically resected hippocampi from TLE patients by conventional in situ hybridization (ISH) and fluorescence in situ hybridization (FISH): We found strong neurocan mRNA expression in the cornu ammonis, dentate gyrus and hilus of TLE patients and controls. Double-labeling of neurocan mRNA with NeuN or GFAP by FISH and immunocytochemistry showed an exclusive neuronal localization of neurocan mRNA and quantitative real time RT-PCR analysis revealed a good correlation of neurocan mRNA expression with the extent of GCD. On the protein level we found an altered posttranslational processing of neurocan in relation to AHS: After translation the full length form of the neurocan protein (250 kDa) is cleaved into an N-terminal fragment (130 kDa) and a C-terminal one (150 kDa). Western blot analysis revealed that the levels of the N-terminal fragment were significantly increased in TLE cases with severe AHS as compared to controls, whereas the expression of the C-terminal form declined. In addition, we found a good correlation of the levels of the N-terminal neurocan fragment with the extent of GCD. Similar results were obtained for the neural cell adhesion molecule L1, an important interaction partner of neurocan: the full length form of L1 (220 kDa) and a cleavage product (80 kDa) were mainly found in patients with a high degree of AHS and L1 levels strongly increased with the severity of GCD. Taken together, these results show that the composition of the extracellular matrix is severely altered in hippocampi of TLE patients and that these changes may facilitate the development of the GCD.

# Generation of epileptiform activity requires sclerotic and intact networks

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The mesial Temporal Lobe Epilepsy (MTLE) syndrome is among the most prevalent forms of partial epilepsies, however, network structures and dynamics involved in the generation of seizures are still fairly unknown. MTLE is associated with severe changes in the hippocampal network histology, termed hippocampal sclerosis and recurrent epileptic seizures occurring in temporal brain areas. Since MTLE is usually pharmacoresistant, the only possibility to suppress seizures is the surgical resection of involved brain areas. To advance less invasive therapy options it is necessary to understand on which time scale processes initiating epileptic seizures act and if this initiation is confined to the histologically changed hippocampal areas or additionally involves other brain areas.

We addressed these questions using a model for MTLE in mice in which a single unilateral injection of kainate into the hippocampus induces histological changes comparable to human hippocampal sclerosis. We recorded recurrent epileptiform activity (EA) in the injected and in the contralateral, intact hippocampus and measured inter-hippocampal coherence to investigate generation processes of epileptiform events (EE) on a timescale that would allow direct intervention. Coherence decreases considerably in high frequency bands several seconds before the onset of EEs, indicating an early decoupling of the ipsilateral hippocampus from the contralateral, intact hippocampus during seizure initiation. To investigate if the histologically changed ipsilateral hippocampus could be the generator of EEs, we prepared slices from kainate injected hippocampi and recorded with multielectrode arrays. Surprisingly, the most sclerotic areas of the ipsilateral hippocampus could neither generate nor sustain EA, while hippocampal slices distant from the injection site with apparently intact histology could sustain EA. However, spontaneous EA could not be observed there either, so the network structure generating EA still unknown. Our results suggest in contrast to previous assumptions, that sclerotic areas of the hippocampus are not sufficient to generate EA, but that the interaction with presumably healthier areas is necessary. The initiation of EEs may involve a rather complex network, instead of the homogeneously damaged area central to the affected region. This challenges the classical definition of an epileptic focus and inspires further diagnostic investigation on the interaction of sclerotic tissue with healthy brain areas in humans.

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# Green Fluorescent Protein (GFP) transgenic rats: A new tool for neurotransplantation

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The principal goal of neuroregenerative strategies is to restore the anatomy and the function of damaged neural circuitries. While stem cell transplantation is considered a promising therapeutic approach, knowing the fate of transplanted cells using appropriate markers is essential. GFP transgenic animals (Inoue et al, Biochem Biophys Res Commun 329 (2005) 288-295) express GFP ubiquitously and can be used as transplanted cell resource for motoring donor's cellular fate during migration and differentiation phases in vivo.

A rat model of Parkinson's Disease (PD) is based on unilateral injections of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle of the rat, resulting in a complete loss of nigral dopaminergic (DAergic) neurons and leading to a depletion of dopamine within the relevant striatum. After the lesion, primary cell suspensions rich in DAergic neurons, derived from dissociated ventral mesencephalon (VM) of E14 rat embryos, are transplanted into the lesioned striatum. Intrastriatal grafts of dopaminergic neural progenitor cells can reinnervate the striatum and restore, at least partly, lesion-induced behavioural deficits. In this study, VM cells of E14 (CRL=10-11mm) GFP Lewis rats were transplanted into the striatum of lesioned Spargue Dawley rats.

Taken in consideration the fact that the donor and the recipient belong to two different rat strains, special focus was set on the survival of the grafts, expression of GFP and how the survival correlates with the immunosuppression.

Transplanted animals were divided into two groups. One group received immunosuppression the other not. Half of the animals of each group were sacrificed in two weeks after transplantation (short cell survival) and the other in four weeks (long term survival). Lesion and transplantation effects were evaluated with drug-induced rotations after 6 weeks of lesion and 2 and 4 weeks after transplantation.

This is an ongoing study and the evaluation of survival, functional and structural integration of the grafts as well as graft GFP expression analysis will be presented in the conference. However, preliminary data suggested that GFP rats might serve as an excellent tool for studying neural stem plasticity in the transplantation paradigm.

# Application of Exogenous Reelin Attenuates the Development of Granule Cell Dispersion in a Mouse Model for Temporal Lobe Epilepsy

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A characteristic structural abnormality of the epileptic hippocampus is the widening of the granule cell layer termed granule cell dispersion (GCD). Recently we have shown that the development of GCD correlates with a decreased expression of the extracellular glycoprotein reelin. Moreover, neutralization of reelin by chronic infusion of a function-blocking antibody into the adult mouse hippocampus leads to a local GCD-like effect, suggesting that a loss of reelin causes GCD in the epileptic hippocampus (Heinrich et al., J. Neurosci. 26, 2006). Based on these findings we designed an experiment to prevent the development of the GCD in a mouse model of temporal lobe epilepsy. We performed unilateral intrahippocampal injection of kainic acid (KA) in adult mice known to induce GCD formation within fourteen days. One day after KA injection, when the initial status epilepticus had ceased, recombinant reelin was chronically infused into the hippocampus using osmotic minipumps over a period of fourteen days. A control group was treated with reelin-free medium. A third group of mice was injected with KA only. After a survival time of eighteen days, the animals were perfusion-fixed and the width of the granule cell layer (GCL) was measured in cresyl violet-stained hippocampal tissue sections. The average width of the GCL was 129 ± 4.3 µm in the reelin-treated group vs. 144 ± 7 µm in the control group. The third group of mice with KA injection alone displayed an average GCL width of 159 ± 2.5 µm. In order to check the stability of the reelin protein under our experimental conditions, we performed Western blot analysis of reelin which hat been incubated at 37°C for fourteen days. We detected the three reelin isoforms (400, 320 and 180kDa) only, but no degradation products. Taken together our data show that the continuous application of exogenous reelin into the epileptic hippocampus can partially compensate the reelin loss and thus confirm the crucial role of this protein for GCL maintenance. (Supported by the DFG: TR3).

# Investigating the potential of embryonic ventral mesencephalon stem cells to differentiate into dopaminergic neurons after in *vitro* expansion.

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Neural precursor cells from embryonic ventral mesencephalon (VM) tend to lose their developmental potential to differentiate into dopaminergic (DAergic) tyrosine hydroxylase (TH) positive neurons in *vitro* after expansion.

The aim of the study was to evaluate in *vitro* parameters and techniques to promote DA survival, differentiation and, at a later stage, functional integration.

VMs of E12 (CRL=6-7mm) and E14 (CRL=10-11mm) Spargue Dawley rat embryos were removed and dissociated. Cells were plated at a concentration of 25,000 cells/cm² in 48-well, precoated with 0.01% poly-L-ornithine (PLO) plates and expanded as a monolayer or as neurospheres in untreated cell culture flasks in medium containing FGF2, EGF and heparin. Monolayer cultures were expanded for one week and differentiated for one more week in medium containing FCS and ascorbic acid. Neurospheres expanded cells were passaged once per week mechanically or enzymatically for three weeks. Every week passaged, cells were plated on PLO coated plates and either fixed immediatelly or differentiated for one week. Immunofluorescence staining for TH, ß-tubulin III, dopamine transporter, MAP2, GABA, GFAP, NeuN, GAD65 were used to analyze and characterize the developmental stage of the cells as well as their neuronal differentiation. A detailed analysis of the proliferation and of the differentiation profiles of rat embryonic E12 and E14 cells will be presented at the meeting demonstrating that in *vitro* expansion protocols indeed exert a significant influence on DA survival and differentiation.

# Up-Regulation of Vascular Endothelial Growth Factor mRNA in the Epileptic Mouse Hippocampus is Accompanied by Increased Blood Vessel Formation

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The impairment of neurogenesis in Ammon's horn sclerosis, which is often associated with temporal lobe epilepsy (TLE), has recently been described in the resected hippocampi of patients suffering from this neurological disorder (Fahrner et al., Exp. Neurol. 2006). Unilateral intrahippocampal injection of kainic acid (KA) in adult mice induces an epileptic focus associated with the major histopathological features of TLE including the observed loss of neurogenesis. In order to elucidate the molecular mechanisms involved in the regulation of neurogenesis we investigated potential changes in the expression of vascular endothelial growth factor (VEGF), a potent neurogenic and angiogenic molecule, in the KA-injected hippocampus. We performed a postlesional time course analysis at two days, four days, eight days and, for long term effects, at eighty days after KA-injection. Using in situ hybridization we found a constitutive expression of VEGF mRNA in pyramidal cells of the cornu ammonis and in the granule cell layer of the dentate gyrus, which did not change during the whole time period. In addition, we observed a transient upregulation of VEGF mRNA expression in the ipsilateral hilus peaking at eight days after KA injection. In order to identify the newly appearing VEGF mRNA-positive cells, we performed fluorescence in situ hybridization (FISH) for VEGF mRNA and combined it with immunolabeling for NeuN (as neuronal marker) or GFAP (as astrocytic marker). Most of the hilar VEGF mRNA expressing cells were double labeled with GFAP indicating that reactive astrocytes synthesize VEGF mRNA in the epileptic hippocampus. Since VEGF is also a strong angiogenic factor, we investigated possible alterations in blood vessel distribution in the ipsilateral hippocampus. Using immunolabeling for CD 31, a marker for adult and embryonic endothelial cells, we observed an increase in the blood vessel density in the epileptic hippocampus. Taken together, our results show that there is an upregulation of VEGF mRNA synthesis in the epileptic mouse hippocampus possibly leading to the observed changes in the blood vessel system. (Supported by the DFG: TR-3).

# In *vivo* characterization of embryonic dopaminergic neurons derived from transgenic mice ectopically expressing Otx2 in the anterior hindbrain

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Organizing centres emit signalling molecules that specify different neuronal cell types at precise positions along the anterior-posterior and dorsal-ventral axes of neural tube during development. Otx2 gene is critical for the specification and regionalization of forebrain and midbrain regions. In transgenic (tg) mice, ectopically Otx2 expression in the anterior hindbrain using a knock-in strategy into the En1 locus results in a caudally shift of the midbrain-hindbrain organizer. This leads to an expansion of the midbrain dopaminergic (mid-DA) neuronal population and to a decrease of the hindbrain serotonergic cell group. These changes are preserved in adulthood, and the additional ectopic dopaminergic neurons also project to the striatum.
The purpose of the study is to evaluate the functional restorative properties of the ectopically expanded mid-DA neurons (tgP) in vivo. tgP and the respectively wild type (wtP) area as well as ventral mesencephalon (VM) from Otx2 Tg (tgA) and wild type (wtA) E13 mouse embryos were explanted. Tissue was dissociated and transplanted into the striatum of 6-hydroxydopamine unilaterally lesioned immunosuppressed rats. Lesion and transplantation effects were evaluated by drug-induced rotations. In apomorphine-induced rotations, both VM groups showed a significant compensation 5 weeks after transplantation. Furthermore, we saw a strong tendency of compensation in the tgP group, whereas no such an effect could be seen in wtP. A complete compensation was already observed in amphetamine-induced rotations, not only in the tgA and wtA groups, but also in the tgP group. wtP group showed no signs of recovery. Morphological and stereological analysis showed a significantly higher DA cell survival as well as bigger graft volumes in the wtA group as compared to all other graft groups. tgA and tgP groups showed a significantly higher DA cell survival as compared to the wtP group. In conclusion, ectopically Otx2 expression in the anterior hindbrain results in caudally expanded mid-DA neurons that demonstrate similar functional properties like their counterparts of the VM.

# Differentiation of Endogenous Neural Precursor Cells in The Rat MCAO Model of Stroke

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Recent studies have revealed that the adult rat brain has neuroregenerative capacities after various insults like trauma or ischemic processes. The Subventricular Zone (SVZ), physiologically involved in the constant renewal of the olfactory bulb interneurons, has been identified as the main source of these newborn cells but precises mechanisms of stroke-associated neurogenesis are still largely unknown.

The aim of this study was to analyze and quantify the various newly generated cell populations originated from the SVZ and produced in response to the ischemic lesion.

Focal stroke was induced in adult rats by 60-minute right middle cerebral artery occlusion (tMCAO). Cell proliferation, neurogenesis and acquisition of defined phenotypes were assessed with bromodeoxyuridine (BrdU) labeling and immunostaining for cell type-specific markers.

# Activation of Astrocytes by Ciliary Neurotrophic Factor (CNTF) Reduces Granule Cell Dispersion in a Mouse Model of Temporal Lobe Epilepsy

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Granule cell dispersion (GCD) in the dentate gyrus is a characteristic feature of Ammon's horn sclerosis (AHS) which often accompanies temporal lobe epilepsy (TLE). GCD is considered to be a local migration defect, but the mechanisms underlying its development are rather unclear. We have recently shown that the appearance of a radial glial scaffold coincides with GCD formation in TLE patients and in a mouse model of TLE (Fahrner et al., 2006; Heinrich et al., 2006). In order to investigate whether glial cells indeed play a role in GCD development we used a mouse model of TLE and combined it with pre-activation of astrocytes. To this end, we injected ciliary neurotrophic factor (CNTF, a potent activator of glial cells) into the hippocampus of adult mice 48 hours prior to the injection of kainate (CNTF preconditioning). Injection of kainate (KA) alone is known to induce focal epileptic seizures and GCD. The width of the granule cell layer (GCL) was measured sixteen days after KA injection in cresyl violet stained hippocampal sections of CNTF-preconditioned mice and in a control group treated with saline prior to KA injection. While these mice had a mean GCL width of 172 ± 5.0 µm, in CNTF-preconditioned mice the GCL width was significantly reduced by 44 percent (97 ± 3.94 µm; t-test $p<0.001$). The specific activation of hippocampal astrocytes by CNTF treatment was confirmed by real-time RT-PCR analysis. Two days after CNTF-injection we found a strong up-regulation of GFAP mRNA expression indicating that the preconditioning results in an activation of astrocytes. Taken together our results show that CNTF-mediated activation of astrocytes has an attenuating effect on GCD formation. In order to further investigate the role of astrocytes in GCD development, we are currently investigating mouse mutants of the CNTF pathway. Preliminary results confirm the crucial role of astrocytes in GCD development.

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# Anti-Nogo-A-specific antibody treatment fosters recovery of manual dexterity from unilateral spinal cord injury in adult monkeys

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In rodents, neutralization of the neurite growth inhibitor Nogo-A was shown to improve motor recovery from spinal cord lesion. Is this true for primates? The recovery of manual dexterity following unilateral cervical lesion at C7/C8 level was analyzed in 12 adult monkeys: in six monkeys an anti-Nogo-A-specific antibody was delivered intrathecally, whereas a control antibody was infused in the other six monkeys. Following lesion, the hand ipsilateral to the lesion was first paralyzed, followed by progressive motor recovery reaching a stable level of performance after a few weeks. The performance of manual dexterity of control antibody treated monkeys was inversely related to lesion size as assessed by a static precision grip test ("Modified Brinkman board"). In contrast, anti-Nogo-A antibody treated monkeys irrespective of lesion size recovered faster and better. In addition three monkeys were tested for their capacity to perform a dynamic precision grip test ("Rotating Brinkman board"). For this challenging task requiring prehension of moving objects an anti-Nogo-A antibody treated monkey recovered considerably better than two control antibody treated monkeys. Such improvement of recovery parallels enhanced sprouting of corticospinal axons caudal and rostral to the lesion in anti-Nogo-A treated monkeys.

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# Upregulated expression of N-type voltage-dependent calcium channels (Cav2.2) in experimental autoimmune optic neuritis detected by MRI and immunochemistry

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Optic neuritis is one of the most common clinical manifestations of multiple sclerosis (MS), a chronic inflammatory disease of the central nervous system (CNS). In female Brown Norway (BN) rats, myelin oligodendrocyte glycoprotein (MOG) - induced experimental autoimmune encephalomyelitis (EAE) affects the optic nerve (ON) in more than 90% of immunized animals, leading to inflammation, demyelination and degeneration of axons. The precise mechanisms of this ON damage are not fully understood, but likely to involve excess accumulation of calcium ($Ca^{2+}$) ions into axons. One of the possible routes of entry of $Ca^{2+}$ ions under pathological conditions is via voltage-dependent Ca channels (VDCC). Since manganese ($Mn^{2+}$) ions also enter cells via VDCCs and cause signal enhancement in T1-weighted magnetic resonance images (MRI), $Mn^{2+}$- induced signal enhancement can be used as a marker of influx of $Ca^{2+}$ ions via VDCCs. In this study we tested specific blockers of L-type (Cav1.2 and Cav1.3), N-type (Cav2.2) and P/Q-type (Cav2.1) VDCCs, diltiazem (20mg/kg), ω-conotoxin GVIA (10µg/kg) and ω-agatoxin IVA (20µg/kg), respectively. The blockers were applied intraperitoneally or intravenously (IV) to MOG-immunized animals which had optic neuritis as detected by MRI. Ratios of T1-weighted signal intensities before and after application of $MnCl_2$ (0.05mmol/kg) were calculated and compared between groups as well as to controls, which comprised immunized animals not treated with any blocker. Statistical significance was assessed using one-way ANOVA followed by Dunnett's test. A statistically significant decrease of the $Mn^{2+}$-induced MRI signal enhancement typically seen within the ONs during optic neuritis, was detected after IV application of ω-conotoxin GVIA, a specific blocker of N-type VDCCs. Furthermore, an increased expression of N-type VDCCs in demyelinated parts of the ONs was established by immunohistochemistry for α1B, the pore-forming subunit of N-type VDCCs. Based on these results, we hypothesize that the upregulated expression of N-type (Cav2.2) VDCCs in inflamed ON leads to an increased calcium influx during MOG-induced optic neuritis.

# Cortical remodelling in an animal model of multiple sclerosis

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Multiple sclerosis (MS) research traditionally focused on white matter pathology, but recent studies revealed important involvement of cerebral cortex in MS patients. The most widely used animal model of MS, experimental autoimmune encephalomyelitis (EAE), shows disseminated lesions in the spinal cord white matter but cerebral cortex is only rarely affected. We therefore developed a focal EAE model where demyelinated lesions are targeted to the cerebral cortex resulting in cortical lesions resembling those observed in MS patients. Lesions were stereotactically targeted to cerebral cortex by injection of pro-inflammatory mediators in rats immunized with myelin oligodendrocyte glycoprotein (MOG). Repetitive lesioning was performed four times at intervals of ten days. Thereafter, animals were assessed with respect to inflammation, demyelination, remyelination and axonal/neuronal pathology. Furthermore, distribution of synaptic markers in demyelinated cortex was analysed. Studying mechanisms of grey matter pathology in our newly developed focal EAE model will contribute to a better understanding of repair and plasticity during disease and recovery in MS.

# MeCP2 deficiency impairs synaptic plasticity but does not modulate hippocampal hypoxia-sensitivity

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RETT syndrome is a neurodevelopmental disorder caused by mutations in the X chromosome-linked *MECP2* gene that encodes for a transcriptional regulator, methyl CpG binding protein 2 (MeCP2). Among the target genes controlled (silenced) by MeCP2 are the growth factor BDNF and $GABA_A$ receptor subunits. Cortical/hippocampal synaptic plasticity, i.e. paired-pulse facilitation and long-term potentiation, are known to be reduced in Rett animal models. Rett patients suffer from respiratory irregularities resulting in episodes of reduced arterial oxygen levels, i.e. hypoxia. So far it is, however, unclear whether such frequent hypoxic episodes induce adaptation of the hypoxic responses of highly vulnerable neuronal networks such as the hippocampal circuitry. Therefore, changes in basal synaptic function, plasticity and hypoxic responses of the hippocampal formation were quantified. Systematic comparison of adult male and female mice (WT and MeCP2 mutants) revealed intact basal synaptic function and a reduced synaptic plasticity (paired pulse facilitation) in MeCP2 KO males, but not in heterozygous females. Inducing hypoxia-induced spreading depression, a model for ischemic stroke and for the assessment of hypoxia-susceptibility, did not reveal any obvious changes in the characteristic electrophysiological spreading depression parameters. Also the intrinsic optical signal of hypoxic spreading depression (an increase in light reflectance/scattering), the propagation speed of spreading depression and the invasion of the hippocampal formation were undistinguishable among wildtype mice and MeCP2 mutants. Hypoxia-induced synaptic failure was similar in all groups and complete within 2 minutes after oxygen withdrawal. Upon reoxygenation, synaptic function recovered fastest in MeCP2 KO males and slowest in WT females. In conclusion, anoxic responses appear unchanged in male MeCP2 KO mice and heterozygous females. The frequent hypoxic episodes resulting from insufficient breathing periods do neither impair basal synaptic function, nor does an obvious adaptation to hypoxia seem to take place as hypoxic responses of the hippocampal formation are unchanged in MeCP2 deficiency.

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# Multi electrode recordings in brain slices of substantia nigra pars reticulata

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Parkinson´s disease is characterized by loss of dopaminergic neurons and extrapyramidal motor symptoms. In various models of Parkinson´s disease an altered firing pattern of basal ganglia output neurons has been observed. These changes include increased bursting activity and increased synchrony of neurons.

To further investigate these changes, we established multi electrode recordings of rat substantia nigra pars reticulata (SNr) slices. The GABAergic neurons of the SNr convey the final output signal of the basal ganglia to the thalamus. Their main input consist of glutamatergic fibers from the nucleus subthalamicus and GABAergic fibers from the striatum, both modulated by dopaminergic neurons of the substantia nigra pars compacta. Physiologically, SNr neurons are tonically active, like most other basal ganglia nuclei. Changes in firing are associated with movement.

300 µm parasaggital slices of SNr were placed on multi-electrode arrays, a grid of 64 planar electrodes, and spontaneous action potentials recorded. Using this technique, we were able to measure drug induced changes in firing rate, bursting, oscillation and synchrony of up to 40 neurons simultaneously.

The Parkinson state was modeled (a) by inhibiting dopaminergic neurons using the selective neurotoxin MPP+ and (b) using dopamine receptor antagonists such as haloperidol, which are known to induce Parkinsonian symptoms in humans.

Most drugs produced a heterogeneous response pattern with some neurons being activated, others inhibited or unaffected. Some neurons started firing, others were silenced. Bursting, oscillations and synchrony between pairs of neurons were also altered by dopamine depletion.

Taken together, important electrical changes in basal ganglia activity known from in vivo recordings, can also be observed in the slice preparation. Multi-electrode arrays make it easier to assay correlation between pairs of neurons and the slice preparation allows better access for pharmacological interventions. We therefore believe that new insights as to how dopamine depletion leads to the characteristic motor symptoms of Parkinson's disease can be obtained from this model.

# Deletion of MeCP2 depresses GABAergic synaptic transmission in early postnatal stage of mouse

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Rett syndrome is a debilitating neurodevelopmental disorder caused by mutations in the X-chromosomal gene of the transcriptional repressor methyl-CpG-binding protein 2 (MeCP2). MeCP2-mutant mice have been generated to study the molecular mechanisms of the disease. It has been suggested that imbalance in synaptic transmission was the underlying cause for the behavioral abnormalities. It remained unclear, however, which transmitter and receptor systems are predominantly involved, and when the cellular defects become apparent. In the present study, we used a molecular biological, biochemical and electrophysiological approach to test whether deletion of MeCP2 gene would lead to an impairment of inhibitory synaptic transmission in the brainstem respiratory network of younger mutant mouse (P7), long before the manifestation of characteristic symptoms (>P20). Reverse transcriptase-polymerase chain reaction (RT-PCR) revealed a relatively impaired expression of several GABAA receptor subunits mRNA, but not of glycine receptor subunits, in brainstem neurons of MeCP2 mutant mice. Quantitative immunoblot analysis in the same region, confirmed a significant decrease in the expression of GABAA alpha2 receptor subunit and a mild increase in the expression of GABAA alpha1 receptor subunit. Patch clamp experiments in brainstem acute slices showed that the frequency and the amplitude of the spontaneous IPSCs were significantly decreased in respiratory network of MeCP2-KO compared to WT mice. Subsequent experiments showed that both frequency and amplitude of miniature GABAergic PSCs were substantially reduced in the KO mice. Furthermore, local application of the GABAA receptors agonist muscimol, but not glycine, revealed lower amplitude of the postsynaptic response in the MeCP2 deficient mice. Thus, we conclude that, despite the inconspicuous phenotype at this age, MeCP2 mutation already leads to a depression of GABAergic, but not glycinergic inhibitory synaptic transmission in the respiratory network. These results suggest that GABAergic synapses are a primary target of the MeCP2 gene regulation during postnatal development. The early presence of defects in inhibitory neurotransmission may provide a rationale for the timing of future therapeutic interventions in Rett syndrome patients.

# Visualisation of alpha-synuclein aggregates in living cells using a PDZ-domain suggests increased toxicity of C-terminal truncated alpha-synuclein.

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It is commonly accepted that aggregates of alpha-synuclein play an important role in the pathogenesis of Parkinson's disease and other neurodegenerative disorders. To further study alpha-synuclein aggregation and its effects on cell function and viability we developed a new technique to visualize alpha-synuclein in living cells, based not on EGFP fusion proteins but on the specific interaction of a PDZ domain and its binding domain.

Since alpha-synuclein is much smaller than EGFP, fusion constructs carry a high risk of EGFP interfering with alpha-synuclein folding and protein-protein interactions. To reduce this risk, alpha-synuclein was labelled with a 6 amino acid PDZ binding domain and coexpressed with a PDZ domain fused to EGFP. The following variants of alpha-synuclein were used: wildtype, A30P, A53T and aSyn 1-108 (amino acids 1-108), which is lacking the C-terminus.

Using this approach, EGFP aggregates were observed with all alpha-synuclein variants. They colocalized with immunostaining for alpha-synuclein and were observed mainly in the perinuclear region and close to the plasma membrane. The timecourse of aggregate formation was followed by time-lapse imaging. Aggregates were most frequently observed with aSyn 1-108 and least frequently with wildtype. Using EGFP fusion constructs, aggregates were observed with aSyn 1-108 but not with wildtype.

Cell viability was measured using propidium-iodide, annexin V and trypan blue staining.

Coexpression of alpha-synuclein and particularly aSyn 1-108 with PDZ-EGFP was associated with a higher proportion of unhealthy and dying cells.

In summary, our results indicate that the specific interaction of PDZ domain and PDZ binding domain can be a useful tool to label protein aggregates with higher sensitivity and less interference compared with EGFP fusion constructs.

Moreover, C-terminal truncation of alpha-synuclein leads to a higher rate of aggregates and cell death, consistent with previous *in vitro* data that suggested that the C-terminus of alpha-synuclein shields the NAC region and prevents aggregate formation.

# Brain endogenous IκB kinase complex modulates course of demyelination

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The IκB kinase (IKK) activates the transcription factor NF-κB, which is critical for the regulation of inflammatory and immune responses. NF-κB has been implicated in the pathogenesis of many acute and chronic neurological disorders such as Multiple Sclerosis. However, little is known about the molecular mechanisms and pathways regulating brain demyelination and the possible role for NF-κB in this process. For that reason, we used Cre/loxP-mediated gene targeting in mice to investigate the brain-specific function of IKK2 during demyelination induced by cuprizone.

Mice with brain-specific deletion of IKK2 (IKK2fl/fl Nestin Cre) were highly protected against cuprizone-induced demyelination of the corpus callosum after both acute (5 weeks) and chronic (10 weeks) treatment. The myelin preservation was accompanied by significantly reduced infiltration and accumulation of microglia/macrophages and by lower astrocytic gliosis in the acute phase of treatment. Protected mice further showed reduced levels of IL-1β, TNFα, CCL3, CXCL10 and CCL2 mRNA in their brains. In contrast, oligodendrocyte-specific deletion of IKK2 (IKK2fl/fl MOGiCre) did not inhibit neither toxic demyelination nor remyelination. Our results indicate a critical function of IKK2 during demyelination of the brain.

# Getting a grip of CLN3: A Transposomics Approach to Juvenile Neuronal Ceroid Lipofuscinosis

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Juvenile Neuronal Ceroid LipoFuscinosis is a pediatric neurodegenerative disease with lethal outcome and no theraphy. The phenotypical lysosomal accumulation of autofluorescent material and neuronal death is caused by mutations in the CLN3 gene. To understand the molecular mechanisms of the disease it is a prerequisite to gain information about the functional CLN3. Unfortunately, thus far the function and even the localization of CLN3 has remained enigmatic, mostly due to the absence of reliable antibodies and clearly non-functional terminal CLN3 fusion proteins.

In this project we have approached the visualization problem by labeling human CLN3 gene with random insertions of different fluorescent protein variants and epitope tags using a transposase enzyme. The generated CLN3 clones with different insertion sites were screened for their localization in HeLa and cerebellar granular cells, and the best performing clones were used for further studies. Using this library we were able to investigate the properties of CLN3, such as its localization, trafficking, oligomerization, and interaction partners.

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# Flupirtine as a neuroprotective add-on therapy in a rat model of autoimmune optic neuritis

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Multiple sclerosis (MS) is a chronic autoimmune disease of the CNS characterized by inflammation, demyelination and axonal and neuronal loss. IFN-β is one of the standard MS therapies, reducing the frequency and severity of relapses by its immunomodulatory properties. In chronic progressive disease stages, the clinical effects of IFN-β are rather unsatisfactory. In these disease stages, the continuously progressing neuronal and axonal degeneration are the most relevant histopathological findings for persisting neurological impairments in MS patients, indicating the importance of an additional neuroprotective therapy strategy.
In our present study, we evaluated the neuroprotective properties of flupirtine, a non-opioid analgetic, in combination with IFN-β-1a in a rat model of myelin oligodendrocyte glycoprotein (MOG)-induced optic neuritis. If flupirtine was applied, we observed a significant reduction in the apoptosis of retinal ganglion cells (RGCs), the neurons that form the axons in the optic nerve (ON). Treatment with IFN-β-1a substantially decreased inflammatory infiltration and demyelination within the ON. Only the combination therapy resulted in a significant improvement of the visual function of the animals determined by in vivo recordings of visual evoked potentials (VEPs). Furthermore, flupirtine also protected RGCs from degeneration in the non-inflammatory animal model of ON transection. Although flupirtine was previously shown to increase the survival of cultured cortical neurons by up-regulation of the anti-apoptotic protein Bcl-2 (Perovic et al., Eur J Pharmacol 1996), we can exclude an up-regulation of Bcl-2 to be relevant for the flupirtine-mediated protection of RGCs. Western blot analyses revealed that the application of flupirtine did not change the expression of Bcl-2 in in vitro and in vivo experiments. Another possible mechanism of flupirtine-mediated neuroprotection is the activation of potassium channels (Jacob et al., Br J Pharmacol 1997; Martire et al., 2004, J Neurosci). Considering the low rate of side-effects that occur during a long-term treatment with flupirtine in patients suffering from chronic pain make this drug an interesting candidate for a neuroprotective add-on therapy in MS patients.

# BAG1 reduces huntingtin aggregation

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Huntington´s disease results from a polyglutamine expansion within the huntingtin protein. Nuclear and cytosolic huntingtin aggregates are observed in affected cells during disease. However, their relevance for the pathology is not understood, yet. The co-chaperone BAG1 binds to Hsp70 as well as the proteasome, is essential for neuronal survival and differentiation, and has been shown to bind to mutant huntingtin inhibiting its toxicity. Here, we show that BAG1 is capable of reducing mutant huntingtin aggregation. This effect is dependent on BAG1-Hsp70 interaction and proteasomal activity. Furthermore, BAG1 influences huntingtin´s subcellular distribution. We present BAG1 as an interesting therapeutic gene for treatment of Huntington´s disease, modulating the balance between mutant huntingtin refolding and degradation.

# Studying mutant SOD1 detoxification mechanisms in intact single cells

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Mutant superoxide dismutase 1 (mtSOD1) causes amyotrophic lateral sclerosis (ALS) in a dominantly inherited manner. The mechanism for mtSOD1 toxicity remains unknown. Two main hypotheses are the impairment of proteasomal function and chaperone depletion by misfolded mtSOD1. Here, we employed FRET/FLIM and biosensor imaging to quantitatively localize ubiquitination as well as chaperone binding of mtSOD1, and to assess their effect on proteasomal and protein folding activities. We found large differences in ubiquitination and chaperone interaction levels for wild-type SOD1 versus mtSOD1 in intact single cells. Moreover, SOD1 ubiquitination levels differ between proteasomal structures and cytoplasmatic material. Hsp70 binding and ubiquitination of wt and mtSOD1 species are highly correlated, demonstrating the coupled upregulation of both cellular detoxification mechanisms upon mtSOD1 expression. Biosensor imaging in single cells revealed that mtSOD expression alters cellular protein folding activity but not proteasomal function in the neuronal cell line examined. Moreover, we identified the co-chaperone BAG1 as a novel binding partner of mutant SOD1, using both FLIM in neuronal cell culture and biochemical approaches in BAG1/SOD1G93A double-transgenic mice. Together, these results provide for the involvement of altered chaperone function in the mechanism of mtSOD1 toxicity.

# Modulation of rat hippocampal neurons by $H_2O_2$-mediated oxidative stress

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Superoxide released from dysfunctioning mitochondria is converted to $H_2O_2$, which modulates redox-sensitive proteins. Such redox signaling occurs under pathophysiological conditions, but it is also part of normal signaling. To identify putative signaling pathways involved in such redox signaling, we analyzed the $H_2O_2$-mediated responses of hippocampal neurons. Oxidation of the redox-sensitive dyes hydroethidium and dichlorofluorescein confirmed the membrane permeability of $H_2O_2$ in cultured neurons and acute slices, thus $H_2O_2$ may not only act at its generation site, but may affect neighboring cells as well. Application of 1-5 mM $H_2O_2$ postponed the onset of hypoxic spreading depression, but did not depress basal synaptic function or plasticity. Mitochondria depolarized only slightly in response to 1 mM $H_2O_2$, directed mitochondrial motility was arrested, and cellular NADH as well $FADH_2$ were apparently directly oxidized. Sharp electrode recordings revealed a hyperpolarization of CA1 pyramidal neurons paralleled by a decrease in input resistance, suggesting that $H_2O_2$ activates $K^+$ channels. In cultured hippocampal neurons low concentrations of $H_2O_2$ (0.2 mM) moderately increased the intracellular $Ca^{2+}$ concentration. This $Ca^{2+}$ rise was not prevented by $Ca^{2+}$-free solution, mitochondrial uncoupling by 1 µM FCCP or $Fe^{2+}$-chelators. Yet it was depressed by 1-5 µM thapsigargin, 10 µM ruthenium red or 20 µM dantrolene. Ryanodine applied in low concentrations (1 µM) mimicked the $H_2O_2$-evoked $Ca^{2+}$ transients, while higher concentrations (25 µM) depressed them. In conclusion, low levels of $H_2O_2$ release $Ca^{2+}$ from the endoplasmic reticulum via ryanodine receptors. Such modulation of $Ca^{2+}$ sequestration by redox state and ROS levels as well as the redox-dependent activation of $K^+$ channels could play a pivotal role in the sensing of metabolic disturbances and the adjustment of neuronal function to oxidative stress.

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# Distinct changes in the spatial and temporal expression pattern of Connexin30, Connexin43 and Connexin36 in the hippocampus of Borna Disease Virus infected rats

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Neonatal infection of the rat brain with the Borna Disease Virus (BDV) leads to a progredient degeneration of dentate gyrus (DG) granule cells, resulting in distinct behavioural deficits. Since cellular mechanisms underlying these changes are still unidentified, and since gap junctional intercellular communication (GJIC) is a known mechanism for the regulation of neuronal differentiation and survival, we analysed here the epression of the astroglial and neuronal gap junction connexins (Cx) Cx30, Cx43 and Cx36 in the Lewis rat hippocampus before the onset of DG-degeneration at 4 weeks post infection (w.p.i.), and at 8 w.p.i., when DG-degeneration is almost complete.

As revealed by Western Blot analysis we detected a downregulation of Cx30 and Cx43 at 8 w.p.i., which could be confirmed on the mRNA level by RT-PCR, were a slight but non-significant decrease in the expression of all three connexins at 4 w.p.i. and a significant decrease at 8 w.p.i. was detected.

Immunocytochemistry revealed a diverse region specific pattern of changes in Cx expression. Thus in the BDV infected rats 4 w.p.i. Cx30 was reduced in all subregions of the hippocampal formation, whereas Cx43 was also reduced in the outer layer (OL) and the CA3-region, but was induced in the hilus (HI) of BDV infected animals. At 8 w.p.i. Cx30 was induced in OL and HI, but reduced in DG and CA3-region, whereas Cx43 was reduced not only in OL, and CA3-region, but also in HI. With regard to Cx36 we found at 4 w.p.i. a reduction in the DG, and at 8 w.p.i. in DG and CA3-region.

In conclusion our results demonstrate for the first time the highly dynamic changes of the Cx expression pattern in the rat hippocampus following BDV infection, and its correlation to BDV dependent primary and secondary changes in hippocampal structure. Our findings suggest an important role of BDV dependent changes in GJIC for virus dependent degeneration and for the resulting functional deficits.

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# Erythropoietin improves behavioral readouts of cognition: Influence on neuroplasticity and neuronal survival

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Several studies of ourselves and others have shown that erythropoietin (EPO) is capable of improving cognitive function in rodents as well as in man under physiological and pathological conditions, including neurotrauma, schizophrenia, or multiple sclerosis. We are presently exploring the molecular/cellular mechanisms of EPO-induced effects on cognition. Using a standardized cryo-lesion model of the right parietal cortex of juvenile (4 week-old) mice, we found global neurodegenerative changes occurring many months after the lesion. Interestingly, EPO prevented behavioral abnormalities, cognitive dysfunction and brain atrophy when given every other day for 2 weeks after this acute brain lesion. Work will be presented to explain mechanisms of the observed atrophy and the EPO action, e.g. effect on neuronal survival, synaptogenesis, neurogenesis and expression pattern of various markers involved in pathogenesis of neurological disorders, e.g. GABAergic genes. In addition, we are investigating how EPO influences functional synapse formation and network activity in vitro. The prominent EPO effects on neuroprotection and, in particular, on neuroplasticity will open new avenues for treatment of neurological and psychiatric diseases.

# The functional roles of neuroligins and neurexins in the postnatal maturation of synaptic function.

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The functional maturation of neuronal synapses is one essential step in the development of neuronal networks. Autism is a debilitating neurodevelopmental disease at which the dysregulation of functional synaptic maturation has been proposed to be the underlying cause for the disease. Recently, it has been demonstrated that a sub-population of autistic patients showed mutations in the human neuroligin genes NLG3, and NLG4. Neuroligins are postsynaptic cell adhesion proteins which interact with the presynaptic cell adhesion proteins α- and β-Neurexins (Nrx). The transsynaptic NLG/Nrx interaction has been proposed to play a central role in the process of functional synaptic maturation, although it remained unclear, which transmitter and receptor systems are predominantly involved. For this reason, different neuroligin and neurexin-mutant mouse models have been generated to study the molecular mechanisms of the disease. In the present study we investigate the functional role of NLG and Nrx on acute brain slice of NLG4-KO mouse and a truncated Nrx 2α/2β-mutant mouse. Although NLG4-KO mice are viable, fertile and behaviorally normal, it revealed changes in spontaneous synaptic activity in brainstem respiratory network. The same also apply to the truncated Nrx 2α/2β-mutant mouse. Further electrophysiological experiments in combination with immunocytochemical and biochemical methods will give us more insight about the functional roles of NLG and Nrx in the postnatal maturational process of synapse.

# The role of cellular prion protein for neuronal survival under autoimmune inflammatory conditions

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Multiple Sclerosis (MS) is an inflammatory demyelinating disease in which the critical role played by neuronal pathology has only been recently recognized. In order to investigate neuronal pathology in MS we have studied myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE) in C57Bl/6 mice.

Bilateral optic neuritis was found to be present in 80% of immunized mice. This was characterised by a demyelination of the optic nerve, accompanied by an infiltration of both T cells and macrophages, from the onset of EAE. Axonal damage was also observed at this time, although a significant loss of retinal ganglion cells (RGCs), whose axons form the optic nerve, did not occur until 21 days after the onset of EAE.

Prion diseases are characterised by neurodegeneration associated with the conformational alteration of the cellular prion protein (PrPc) into an abnormal isoform of the protein (PrPSc). PrPc is predominately neuronally expressed and although it has been implicated in a wide range of cellular processes including cell signalling, adhesion and differentiation, its normal function remains largely unknown. As it has also been shown to confer neuroprotection in a growing number of paradigms, we are interested in determining whether or not PrPc may also play a neuroprotective role in EAE. Western blot analysis demonstrated that an upregulation of PrPc protein occurs following the onset of clinical EAE. We have then proceeded to use both knockout mice and transgenic mice overexpressing PrPc, in order to further study the putative neuroprotective role of PrPc within the context of EAE.

# Alterations in neurons of the enteric nervous system in the large intestine of transgenic mice expressing alpha-synuclein from the Platelet-derived Growth factor (PDGF) promoter

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Accumulation of alpha-synuclein is involved in the pathogenesis of neurodegenerative disorders such as Lewy body- and Parkinson's disease. Parkinson's disease is characterized by muscular and autonomic dysfunctions. Some of these are manifested in the gut. Constipation and difficult defecation are the most common gastrointestinal symptoms among Parkinson's disease patients; moreover the development of a megacolon has been described in some cases [1]. Alpha-synuclein aggregationss have been reported in neuronal cells of the enteric nervous system in the large intestine of Parkinson's disease patients, which seemed to be correlated with degenerative changes [2; review].

To investigate further the role of alpha synuclein in the pathogenesis of these disorders, transgenic mice expressing alpha-synuclein under the control of PDGF promoter were generated [3]. In the present study we examined the large intestine of transgenic (tg) and non-transgenic (n-tg) mice by light microscopy, immunohistochemistry/LSCM and ultrastructural analysis. From each colon we investigated a region near the caecum and another region close to the anus. Cryosections of each region and mouse were hematoxylin-eosin stained and the circumference of the colon from tg mice was compared with n-tg controls. The comparison of the circumference of the colon from tg and n-tg mice demonstrated that tg mice had a significantly enlarged circumference of both, the caecum-close and the anus-close region (7%±2%, $p<0.01$ and 15%±2%, $p<0.001$, respectively). This alteration is more prominent in the region close to the anus than near the caecum. Double immunohistochemical staining of the cryosections with alpha-synuclein and neurofilament specific antibodies was performed. The immunohistochemical analysis showed an increased expression of alpha-synuclein in neurons of the enteric nervous system of transgenic mice. Neurons contained inclusions positive for alpha-synuclein and neurofilament antibodies. Ultrastructural examination of neuronal cells proved electron dense inclusions both in the cell bodies and neurites. These inclusions are composed of fine granular and fibrillar material. An increased number of vacuoles were found near these inclusions. Additionally, damaged mitochondria with lacking cristae and matrix material have been detected. According with the study of [4], these results suggest that abnormal accumulation of alpha-synuclein could lead to mitochondrial alterations that may result in cell death.

In conclusion, transgenic mice expressing the alpha-synuclein from PDGF promoter are a good model for studying alterations in nerve cells of the enteric nervous system by Parkinson's disease.

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# Epileptic seizures and hippocampal damage after cuprizone-induced demyelination in mice

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Epileptic seizures have been described in different animal models of demyelination and occur in demyelinating diseases of the central nervous system such as multiple sclerosis (MS). A causal relationship between MS and seizures was assumed, since the prevalence of seizures in MS is about three to six times that in the general adult population (Poser & Brinar, 2003). The exact mechanisms why myelin deficiency can lead to seizures are not known, but may involve axonal pathology and resultant alterations in neuronal excitability. The cuprizone model of demyelination in mice resembles myelin deficiency in MS. To our knowledge spontaneously occurring seizures have been described early in this model (Kesterson & Carlton, 1970), but have not been characterized in any detail yet. In the present study, we used continuous EEG/video monitoring to record seizures occurring after chronic demyelination of C57BL/6J mice fed for 12 weeks with 0.2% cuprizone. Furthermore, we histologically examined demyelination patterns in the brain and especially in the hippocampal formation. In search for morphological correlates of seizures, we quantitfied neuronal density and area of the hilus and paid especial attention on signs of neuronal damage. To ensure viewing a possible process of neuronal pathology, we histologically examined mice after 6 and 12 weeks of cuprizone treatment and 5 weeks after a 12 week period of treatment compared to untreated but equally handled control mice. We observed three kinds of epileptiformic discharges in cuprizone-treated mice: in all mice we recorded epileptiform spike-wave formations in the EEG. Furthermore, most of the animals developed generalized, tonic-clonic seizures upon stress-inducing stimuli. In one out of 15 mice, spontaneous seizures could be observed and recorded. Histological studies showed - apart from the known demyelination of the corpus callosum - massively affected hippocampal structures and cortex regions. Hippocampal alterations were associated with decreased neuronal density in the hilus of the dentate gyrus and neuronal damage in CA1 and CA3 regions of the pyramidal cell layer and in the granula cell layer and the hilus of the dentate gyrus. Our study suggests that the seizures occurring in the cuprizone model are a consequence of neuronal degeneration in the hippocampal formation that develops as a consequence of the impaired myelination in this region. In view of the role of the dentate gyrus in epileptogenesis, such hilus damage could be causally involved in the paroxysmal alterations observed after prolonged treatment with cuprizone. Based on the present data, the potential role of the hippocampal formation for seizures occurring in different models of CNS demyelination should be examined.

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# Effect of chronic implantation of neurostimulation electrodes into the central piriform cortex on epileptogenesis and behavior in the rat kindling model of temporal lobe epilepsy

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Chronic neurostimulation is widely used for treatment of neurological disorders, e.g. high frequency stimulation in patients suffering from movement disorders. However, in pharmacoresistant epilepsies chronic neurostimulation of brain regions is in its infancy. Observations in the kindling model of temporal lobe epilepsy proved that, probably due to profound biochemical changes, chronic implantation of metal electrodes in limbic structures such as amygdala has clear pro-epileptogenic effects in rats in the absence of any stimulation. The central piriform cortex (cPC) has not yet been investigated in this respect. Because the cPC is an important therapeutic target in models of temporal lobe epilepsy, it is highly important to perform a safety study of chronic implantation of metal electrodes in this brain region.

We therefore chronically implanted platinum-iridium electrodes bilaterally into the cPC of female Wistar rats in addition to a stainless steel kindling electrode placed into the right basolateral amygdala. Considering the above mentioned study on the amygdala, we started the kindling procedure four weeks after implantation of the electrodes. Rats with kindling electrode but without cPC electrodes served as controls. Numerous kindling parameters were investigated. However, preliminary data indicate that neither kindling development nor seizure thresholds differed significantly between rats with electrodes implanted in the cPC and controls. Because temporal lobe epilepsy is prone to show comorbidity with psychiatric disorders, we additionally investigated the rats behaviorally after kindling. The following tests were used: the open field test and the elevated plus maze test for evaluating anxiety-related and exploratory behavior, and the Morris water maze test for evaluating spatial learning behavior. The data revealed a reduced anxiety-related behavior in rats with chronically implanted electrodes in the cPC when compared to controls. However, preliminary data did not indicate intergroup differences in learning behavior.

In contrast to previous observations of pro-epileptogenic effects in response to chronic implantation of electrodes in the amygdala, the present findings failed to show pro-epileptogenic effects of electrodes in the cPC. Because the cPC is known to be an interesting target for therapeutic manipulation in models of temporal lobe epilepsy, the present data is important to document the safety of chronic implantation of neurostimulation electrodes into this limbic structure. However, the behavioral data call for further investigations for risk assessment on the one hand, but on the other hand also for further investigations for evaluation of the therapeutic potential associated with reduced anxiety-related behavior in response to chronic implantation of electrodes in the cPC. This is especially interesting considering the comorbidity of psychiatric disorders in a subgroup of epileptic patients.

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# HISTOLOGICAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF SHORT AND LONG TERM IMPLANTATION OF AUDITORY MIDBRAIN ELECTRODES IN THE COLLICULUS INFERIOR

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Auditory brainstem implants with electrodes positioned in the Cochlear nucleus are used for the auditory rehabilitation of patients with neural deafness [1]. Tonotopic stimulation of the higher auditory areas, such as Colliculus inferior (IC), are currently under investigation. In acute experiments electrically in IC evoked potentials can be registered at the auditory cortex using surface and/or deep insertion electrodes and compared with acoustically induced cortical signals [2,3]. We test the safety and functionality in chronic experiments with and without stimulation.

In collaboration with Cochlear Ltd. (Sydney), a 4 mm 20-channel rod electrode was developed, electrode contacts being arranged in a circle on a rod at 200 µm intervals corresponding with frequency-band laminae in IC. In chronic experiments this type of electrode inserted in IC. Parallel to a non-stimulated control group daily stimulation with commonly used speech processor and impedance measurements are performed. After 3 months stimulation the brain was fixed for histological analyses.

Electrode insertion in IC proved to be easy and reproducible. The behavior of the implanted cats did not change in any way with or without electric stimulation. First experiments with electrical stimulation in IC with a during speech processor a little above hearing threshold evoked no avoidance reaction. There is no visible pain reaction. On short loud sounds neonatally and adult deafened animals react with attention and visual searching. Impedances stayed stable before and after electrical stimulation (0.008 - 0.04 C/m²) independent of current level and pulse width at about 10 kOhm. Electrically evoked potentials of this electrodes are congruent of the acoustically ones. Histological analyses of IC showed a damage zone limited around the insertion channel. There was a thin endothelial cell layer and a few new capillaries around the electrode track. Tissue behind the damage-zone appears to be undamaged and healthy. There was no chronic inflammation.

More long-term animal studies with and without stimulation are running. The implantation of this multi channel electrode in IC has to be considered safe. It is improbable that the speech processing strategies of the cochlea implant can also used in the IC. Therefore optimum stimulation parameters must be investigated in further acute and chronic experiments. The goal is auditory rehabilitation in patients suffering from neural deafness more efficiently than surface electrodes places on the Cochlear nucleus.

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# Messenger RNA and protein expression of components of the Nrf-ARE signaling pathway in post mortem tissue of Amyotrophic Lateral Sclerosis (ALS) patients

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Oxidative stress and inflammation are of major relevance as pathomechanisms in Amyotrophic Lateral Sclerosis (ALS). The aim of the present study is to investigate the role of the Nrf-ARE signaling pathway in the ALS-related selective degeneration of motor neurons.

Nrf (Nuclear erythroid 2 related factor; Nuclear respiratory factor) 1 and - more potently - Nrf2 are basic region leucin-zipper transcription factors. After activation and translocation to the nucleus, they bind to the antioxidant response element (ARE), a regulatory enhancer region within gene promoters. This regulates the expression of more than 200 genes involved in the cellular antioxidant and anti-inflammatory defense. Among them are classical phase 2 detoxification enzymes such as NAD(P)H quinone oxireductase and glutathione, enzymes, which are necessary for glutathione biosynthesis, extracellular superoxide dismutase, glutamate-6-phosphate-dehydrogenase, heat shock proteins and ferritin, furthermore pro- and antiinflammatory enzymes such as cyclooxigenase-2 (COX-2), inducible nitric oxide synthase (iNOS) und heme oxigenase-1 (HO-1).

In the present study we investigated the mRNA and protein distribution of Nrf1, Nrf2, the endogenous Nrf2-inhibitor Keap1 and the transcriptional co-activator PGC1-α (peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1α) in post mortem tissue (primary motor cortex, spinal cord) of ALS-patients and control tissues at the cellular level using in situ hybridization and immunohistochemistry to find out whether disease-specific alterations of the elements of this pathway occur in ALS. This could further support the hypothesis that the Nrf2/ARE pathway represents a novel therapeutic target in neurodegenerative disorders.

# Fibroblast-growth factor 2 (FGF-2) has antidepressant properties in an animal model of depression

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Depression is a disorder of the representation and regulation of mood and emotion. Recent evidence suggests an involvement of tfibroblast growth factors (FGFs) in mood disorders. Specifically, several members of the FGF family have been shown to be dysregulated in individuals with major depression. In addition, treatment with antidepressants has been reported to cause increased mRNA levels of FGF-2 in the forebrain. We, therefore, hypothesized that FGF-2 may play an important role in depression and/or in the action of antidepressants. We have used olfactory bulbectomy (OB), an established animal model of depression, and FGF-2 deficient mice to investigate putative antidepressant properties of FGF-2 and the involvement of FGF-2 in the actions of antidepressive drugs.

FGF-2 applied intraventricularly for four times and at a concentration of 40 ng/mouse generated antidepressant-like effects in bulbectomized mice as well as in the tail suspension test. FGF-2, similar to the antidepressants amitriptyline and citalopram, attenuated neurodegeneration in the piriform cortex (Pir) and posterolateral cortical nucleus of the amygdala (PLCo). FGF-2, like the antidepressants amitriptyline and citalopram, also attenuated depressive-like behaviour. Following OB, FGF-2 knockout mice, similar to wildtype littermates, developed behavioural alterations, as well as neurodegeneration in the Pir and PLCo. In contrast to bulbectomized wildtype mice, treatment with amitriptyline, however, failed to reverse this behaviour in FGF-2 deficient mice.

Our data suggest that FGF-2 administered to bulbectomized mice can (1) exert antidepressant effects and (2) partially reverse behavioural and cellular changes induced by OB. Moreover, FGF-2 seems to be an essential mediator in the action of antidepressant drugs. Together, these results suggest that FGF-2 has antidepressive properties and that it may represent a potential target for antidepressive treatments. This raises the possibility that drugs that selectively stimulate the production of FGF-2 could represent a novel class of antidepressants.

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# *In vivo* voltage sensitive dye imaging in a mouse model of a human genetic epilepsy.

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Measuring in vivo electrical activity of a neuronal population with high spatial and temporal resolution is crucial for understanding patho-physiological processes leading to seizure generation and increased susceptibility. Using the voltage sensitive dye RH 1691 and a fast imaging system (Fuji Deltaron HR1700) we measured optically at 200 Hz spontaneous and whisker evoked electrical activity in the primary somatosensory cortex of mice anesthetized with Urethane (1.5 g/kg). We compared heterozygote mice carrying a point mutation (R43Q) in the gamma2 subunit of the GABAA receptor (Petrou et al, unpublished data) and wild type mice. The G2R43Q mouse strain was chosen since the same point mutation causes absence epilepsy and/or febrile seizures in humans (Wallace et al. Nat.Genet., 2001). The point mutation in heterozygote mice results in decreased membrane targeting of the GABAA receptor, reduced amplitude of miniature inhibitory postsynaptic potentials (mIPSP), and altered kinetics (Tan et al., unpublished results). Heterozygote mice with this knock in gene display spontaneous absence seizures and a significantly reduced seizure threshold when challenged with pentylentetrazole (PTZ, Davies et al., unpublished results). Imaging experiments were performed in the somatosensory cortex of male adult mice (average p49) as it has been implicated in the genesis of absence seizures in other rodent models of absence epilepsy (the GAERS rats, Pinault et al.J.Physiol.2003).

Spontaneous neuronal population activity was measured before and after intra peritoneal injection of PTZ (40mg /kg, at this dose wild type animals do not show tonic-clonic seizures). Spontaneous electrical activity was greater in heterozygote mice than in wild type mice (ANOVA, $p = 0.019$, $n = 24$). Injection of PTZ significantly increased the spontaneous activity in wild type and G2R43Q mice, $p < 0.01$ and $p = 0.01$, respectively, (ANOVA, $p = 0.0046$, $n = 24$). In order to measure evoked neuronal responses we stimulated the D2 whisker using a piezzo bimorph presenting 2 stimuli with different amplitudes. Stimulus responses before administration of PTZ were not significantly different, however PTZ administration enhanced the stimulus response significantly more in G2R43Q mice than in wild type controls. This effect was bigger for stronger whisker stimuli. The observation that stimulus evoked activity before PTZ administration is comparable indicates that the changes in excitability can be compensated. However, a challenge with PTZ reveals the difference between wild type and G2R43Q mice also for stimulus responses. The increased spontaneous and evoked electrical cortical population activity in G2R43Q mice is consistent with increased seizure susceptibility to a seizure inducing agent.

# Distribution of leucine-rich-repeat-kinase-2 (LRRK2), a novel member of the Ras/GTPase superfamily, in the developing and adult murine brain

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The leucine-rich-repeat-kinase 2 (LRRK2) belongs to the family of ROCO proteins, a novel group of the Ras/GTPase superfamily. The LRRK2 gene was recently found to have multiple mutations that are causative for autosomal dominant inherited forms of Parkinson's disease (PD). These mutations have recently been described as one of the most common hereditary forms of late onset PD. The expression pattern of the non-mutated LRRK2 in the embryonic and adult brain, however, is only fragmentarily known. Here, we describe the widespread distribution of LRRK2 mRNA in the embryonic and adult mouse CNS.

Expression of LRRK2 can be found as early as embryonic day 10.5 in the CNS and is maintained until adulthood. Within the embyonic brain, the level of LRRK2 expression was highest in proliferative regions of the cortex, like the subventricular zone. In addition, LRRK2 mRNA was observed in the cerebellar plate, medulla oblongata and the dorsal horn of the spinal cord. Moreover, LRRK2-mRNA was found in peripheral developing organs, such as kidney, lung and interdigital zones of the paws.

In the adult brain, LRRK2 mRNA is not only restricted to areas known to be involved in PD, such as the striatum and substantia nigra. Like its homologue LRRK1, LRRK2 expression is not limited to the CNS, but also occurs in many peripheral tissues, as e.g. kidney, lung, testis. Since mutations in LRRK2 have been linked to PD and LRRK2 may be involved in apoptotic cell death, we tested whether LRRK2 is up-regulated in the brain areas after MPTP-intoxication. Similar to parkin, but different from to α-synuclein, no up-regulation of LRRK2 was found in this animal model of PD.

Concerning the distribution in the murine CNS an involvement of LRRK2 in both apoptotic cell death and proliferation can be suggested. However, downstream signalling pathways of LRRK2 are still unknown.

# Profiling the Transcriptomic Consequences in the Embryonic Mouse Brain of Neuregulin-1 Deficiency as a Schizophrenia Model

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Schizophrenia is a devastating disorder of the human brain with a worldwide prevalence of about 1 %. The psychopathological phenotype is characterized by positive symptoms like hallucinations and delusions and negative symptoms like affective flattening, social withdrawal and thought disturbances. Neuropathology has elucidated numerous subtle changes in the architecture of the schizophrenic brain, the most robust of which include a substantial loss of dendritic neuropil and of certain GABAergic markers in the supragranular layers of the dorsolateral prefrontal cortex (dlPFC), deficits in neuron numbers in corresponding thalamic nuclei and reductions in transcriptomic oligodendrocyte markers. Lacking signs of neurodegeneration, these deficits are supposed to be of developmental origin. It is believed that manifestation of the disease, nonetheless, only occurs in early adulthood due to the consumption of a neuronal reserve at this age.

Disease state concordance of monozygotic twins reaches 50 %, indicating a strong, but complex genetic contribution to the etiology of schizophrenia. The large number of suggested susceptibility genes has converged to about ten candidates in recent years. Among them neuregulin-1 (Nrg-1) is particularly suggestive due to its genetic linkage and its described biological functions. Nrg-1 has been found to have roles in synaptic plasticity, guidance of GABAergic interneuron precursor migration, thalamocortical wiring as well as myelination. Albeit suggestive, none of these processes has conclusively been shown to play a role in the pathophysiology of schizophrenia.

We therefore aim to investigate ab initio the transcriptomic consequences of deregulations of Nrg-1. To this end, we are pursuing a microarray approach using the heterozygous pan-Nrg-1 knock-out mouse[1] as a model system. We are profiling in turn the transcriptomes of pools of defined tissue samples from the frontomedial cortex (prelimbic/infralimbic PL/IL region), from corresponding anterior midline thalamic nuclei as well as somatosensory cortex and the corresponding ventrobasal thalamic complex of the Nrg-1+/- mouse and its wildtype siblings on embryonic day 15.5 (E15.5). The PL/IL cortex is believed to be the mouse homolog of the human dlPFC. E15.5 was chosen, because at this time the generation of upper layer cortical neurons and thalamocortical wiring are well underway. To reduce temporal and spatial variability of the collected tissue samples, we performed Theiler staging on the embryos and delineated the regions of interest in the embryonic brain by whole-mount in-situ hybridization with regional marker genes and by axonal tracing. Through bioinformatic analysis of the microarray data, we hope to identify gene sets that are specifically deregulated as a consequence of Nrg-1 deficiency in the affected frontomedial as compared to the "non-affected" somatosensory thalamocortical system. Our focus is on covariant and functionally related gene sets, which might indicate cellular systems potentially related to the pathomechanism of schizophrenia.

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# Statin-mediated neuroprotective effects on retinal ganglion cells after acute ischemia/reperfusion involve increase of αB-crystallin and Hsp27 expression and modulation of caspase levels

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Statins are 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitors broadly used to treat hypercholesterolemia. Clinical trials demonstrated that statins markedly reduce incidence of cardiovascular and cerebrovascular events in patients with coronary artery disease, regardless of serum cholesterol levels. Recent data suggest that statin therapy has pleiotropic effects, including protection against acute and chronic neurodegeneration following ischemic stroke, Alzheimer's disease and inflammatory central nervous system (CNS) pathologies. Mechanisms of statin action involve the improvement of endothelial function and suppression of inflammatory reactions. In addition, statins increase levels of stress proteins after an optic nerve lesion. However, there is a lack of studies related to the effects of statins on delayed neuronal death, and potential targets remain unexplored. Emerging evidence supports an important role for caspases in neuronal death following retinal ischemia/reperfusion injury. The aim of this study was to evaluate putative mechanisms of statin-mediated neuroprotection on retinal ganglion cells (RGCs) after acute retinal ischemia/reperfusion and the role of caspases as putative targets of statins in the CNS. We used a model of transient global retinal ischemia by transient elevation of intraocular pressure above systolic blood pressure. Statins simvastatin, mevastatin, pravastatin and lovastatin (0.2-5 mg/Kg) were delivered subcutaneously 1, 24, 48 and 72 hours after retinal ischemia. The numbers of RGCs surviving the insult were determined after specific labelling of flat mounted retinae 10 days after injury, and specific caspase expression was quantitated by RT-PCR and Western blot analysis. Simvastatin enhanced RGCs survival by 40.9% 10 days after ischemia/reperfusion. Pravastatin and lovastatin improved RGCs survival after ischemia by 39.6% and 23.7%, respectively. αB-crystallin levels were significantly increased after ischemia/reperfusion and this increase was further potentiated by statin treatment. Hsp27 levels were increased after 24 h of reperfusion. In addition, statin treatment significantly reduced caspase 2 levels in the retina 3 to 6 hours after the ischemic insult. Results of this study support a potential use of statins as therapeutic agents and the role of cell specific caspases in neuronal degeneration following transient retinal ischemia. Administration of statins may constitute a suitable approach to treat neurodegenerative and ocular diseases including diabetic retinopathy and glaucoma.

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# Tau-induced reorientation of microtubules polarity leads to axonal traffic jams and neurodegeneration

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At the forefront of tauopathies research are the attempts to understand the molecular and cellular cascades that lead to the pathologies, in particular Alzheimer's disease (AD). It is generally accepted that the cascade generated by tau overexpression culminates in impaired organelle transport leading to neurodegeneration. Several mechanisms have been proposed to link tau overexpression and impaired axoplasmic transport. Nevertheless, the underlying mechanisms are not clear.

Here we developed a novel cellular platform and used confocal microscope imaging to address the above questions. For the study, we overexpressed human wild-type tau or double mutant tau (containing both mutations K257T and P301S) fused with cerulean. In order to label polymerizing microtubules we overexpressed the plus end microtubule tracking protein EB3-GFP. For imaging of anterograde and retrograde transport we imaged cherry-SNAP-25 and the fluid phase endocytotic marker - sulphorhodamin 101 respectively, and for imaging of mitochondria we used the mitotracker -RPAC.

Overexpression of cerulean-tau generates three abnormal phenotypes characterized by: (1) The transient appearance of MicroTubules Organizing Center-Like Structure (MTOCLS) in the axon. The MTOCLS locally disrupted the axonal transport and spontaneously disappeared within 48-72 h. (2) chaotic reorientation of the MTs. This led to massive mitochondria and vesicular "traffic jam". (3) The accumulation of MTs at the submembranal domain, concomitant with condensation of organelles within the axon core followed by neurodegeneration.

To conclude, we unraveled a new mechanism that underlies the aberrant transport and neurodegeneration characteristic of tau overexpressing neurons, namely the reorientation of MTs polarity and their displacement.

# Suitability of a food-carrying task to evaluate lesion-dependent deficits in decision-making in rats (*Rattus norvegicus*)

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Rodents are widely used as models for human diseases. Functional assessment of disease pathophysiology comprises several levels of investigation, ranging from molecular to behavioral studies. With respect to behavior, it is important to choose behavioral tasks which are relevant to regional deficits.

In both rats and humans, medial prefrontal areas including the orbitofrontal and anterior cingulate cortex are involved in planning and preparation of movements as well as in reward-based decision-making. In order to evaluate chronic deficits in decision-making after lesioning the medial prefrontal cortex, we have adapted a food-carrying task so far used in the field of behavioral ecology. Because longitudinal studies covering most of an animal's life span detect not only disease-related effects but also processes of normal aging, we compared decision-making of two age groups.

In this experiment, hungry rats (*Rattus norvegicus*, Wistar albino) are provided with food pellets in a meander maze (curved alley, 10 m long) which is attached to their home cage. The distance between food and home cage varies randomly between 1 and 10 m. Rats have to decide between carrying the food to the cage or eating it directly at the food source. We tested rats at the age of four (n=10) and twelve months (n=10). The behavior of the rats was recorded and statistically analyzed.

During the training phase, the young rats learned significantly faster to accept food in a novel environment than the old animals. In the testing period, young rats carried significantly more food than old rats which tended to eat at the food source. Three old animals did not even carry food to the cage when it was only 1 m away. Young rats moved faster while carring food and it took them less time to eat one food pellet, compared to the old rats. In both groups, we found a significant negative correlation between food distance and probability of food-carrying, which is in line with previous experiments reported in the literature. When rats carried one food pellet only, the number of food-carrying events decreased exponentially with increasing distance between food and home cage. This exponential function was less obvious when rats carried two or three food pellets, and changed into a Gauss-function when they carried four pellets at once. The number of foraging tours was negatively correlated with food-carrying probability in both groups. Anxiety (measured in the elevated plus maze) and spatial working memory (spontaneous alternation in the Y-maze) was not correlated with food-carrying behavior.

We conclude that the food-carrying task is a suitable test to investigate decision-making in rats, because it is not sensitive for changes of anxiety or working memory. Old and young animals behave significantly different, which should be considered in a longitudinal study.

# Axotrophin a RING-variant domain protein acts as E3-ubiquitin-ligase and ubiquitinates the microtubule-associated protein tau

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A number of neurodegenerative diseases among others Alzheimer´s disease (AD) are characterized by aggregation and accumulation of misfolded proteins. These protein aggregates are partly ubiquitinated. Tau protein, which aggregates during AD, is poly-ubiquitinated in AD brain to some extent.
In order to find modifiers of tau aggregation and posttranslational modification we screened for proteins, which interact with tau protein. In previous experiments we showed that the tau protein interacts with the protein axotrophin of unkown function.
Affinity-purified antibodies against axotrophin C-terminus labeled tau protein aggregates in AD brains.
Axotrophin harbours a C4HC3 zinc-finger-like motif in the C-terminus, which is referred to as Ring-variant domain and has been implicated in protein ubiquitination. Recombinant expression and refolding of the C-terminus of axotrophin allowed us to test the E3-ubiquitin-ligase activity. We found that axotrophin shows E3-ubiquitin-ligase activity in combination with several E2 enzymes and becomes autoubiquitinated. Ubiquitination of tau protein but not KLC1, another axotrophin-interacting protein, was mediated by axotrophin.
Further investigation of ubiquitination effects on the protein tau will give more insights about the E3-ubiquitin-ligase axotrophin and especially its role in AD.

# Tau phosphorylation in hibernating hamsters: Selective vulnerability of cholinergic basal forebrain neurons - Implications for Alzheimer's disease

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Major hallmarks of Alzheimer´s disease are neurofibrillar tangles made up by 'paired helical filaments' (PHFs) consisting of hyperphosphorylated microtubule-associated protein tau. Tangle formation selectively affects certain neuronal types and systematically progresses throughout numerous brain areas which reflects a hierarchy of neuronal vulnerability and provides the basis for the neuropathological staging of disease severity. Mechanisms underlaying this selective neuronal vulnerability are still unknown. We showed previously that reversible PHF-like phosphorylation of tau occurs during obligate hibernation in European ground squirrels (Arendt et al., 2003, J Neurosci 23: 6972-6981).

Here we extend these findings to facultative hibernators such as Syrian hamsters (*Mesocricetus auratus*) forced into hibernation. Immunoperoxidase labelling and Western blot analysis revealed phosphorylated protein tau with the monoclonal antibody AT8 in all investigated torpid animals. AT8-immunolabelling diminished during arousal and was absent in euthermic hamsters in the medial septum/diagonal band (MSDB) complex, the striatum, the neocortex and the hippocampus. Combined immunofluorescence labelling of AT8 and the cholinergic markers choline acetyltransferase (ChAT), vesicular acetylcholine transporter or the low-affinity neurotrophin receptor p75 revealed phospho-tau in a large portion of cholinergic cells in the MSDB from torpid hamsters. On the contrary, AT8-staining in the MSDB was not found in glutamatergic and GABAergic axonal endings immunolabelled for the vesicular glutamate transporter 2 and the vesicular GABA transporter, respectively. Moreover, triple labelling revealed that parvalbumin-containing GABAergic basal forebrain neurons were devoid of tau hyperphosphorylation and confirmed AT8-staining in numerous cholinergic neurons.

Taken together, we demonstrated in the basal forebrain projection system that cholinergic neurons are selectively affected by PHF-like phosphorylated tau while GABAergic neurons are largely spared which shows strong parallels to the situation in AD. Formation of PHF-tau in these neurons apparently does not affect their function as pacemaker for terminating hibernation. We conclude that although formation of PHF-like phosphorylated tau in mammalian brain follows a certain hierarchy, affecting some neurons more frequently than others, it is not necessarily associated with impaired neuronal function and viability. These data indicate a more general link between PHF-like phosphorylation of tau and the adaptation of neurons under conditions of a "vita minima".

# Tau-protein hyperphosphorylation in hibernating hamsters (*Mesocricetus auratus*)

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Neurofibrillary tangles (NFT) are a major hallmark of Alzheimer's disease (AD). These tangles consists of intracellular accumulation of paired helical filaments (PHF) that result from the aggregation of hyperphosphorylated protein tau. It is still unknown whether the abnormal phosphorylation of tau is a primary event of AD pathogenesis, a direct consequence of AD pathology or if it may rather represent an epiphenomenon. Hyperphosphorylation of tau implicates the dissociation of tau protein from axonal microtubules and promotes the disorder of axonal transport and the aggregation of tau and consequently the formation of PHF and NFT. We analysed the epitope specific tau phosphorylation in distinct brain-regions of syrian hamsters (*Mesocricetus auratus*) in different hibernation stages and found that hibernating animals form PHF-like phosphorylated tau during torpor, which will be dephosphorylated immediately after arousal. Furthermore, we investigated the tau gene and protein expression including an analysis of the tau isoform expression pattern to characterise the hibernation induced tau phosphorylation of these animals and reveal differences and parallels to the tau pathology of Alzheimer's disease.

# Shift in spike patterns in projection neurons of the mouse lateral amygdala following status epilepticus

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Fear and anxiety-related behaviors are commonly reported in patients suffering from temporal lobe epilepsy (TLE). Moreover, experimental models of TLE have revealed a regulation of intrinsic neuronal properties by seizure activity. Most studies have been focusing on the hippocampal formation, and therefore we thought it timely to investigate neuronal properties in the amygdala, a region of critical importance during TLE.
For this purpose, we have used the pilocarpine-mouse model of TLE in which 7 to 9 weeks old mice underwent multiple systemic administration of pilocarpine, a muscarinic agonist, at a dose adjusted to 320 mg/kg. Injections were repeated every 30 minutes until *status epilepticus* was generated. Two months thereafter, slices were prepared from pilocarpine-treated mice and from age-matched saline-injected mice as a control. Electrophysiological recordings using the whole-cell patch-clamp technique were performed *in vitro* from projection neurons in the lateral amygdala (LA), which were identified as such by an established battery of electrophysiological and morphological criteria.
The results obtained after comparing experimental data from saline-injected mice with those from the pilocarpine-injected mice outlined three major lines of findings. First, electrotonic membrane properties, in particular resting membrane potential and input resistance, were not affected. Second, no difference was detected with regards to the duration of fast action potentials. However, third, the pattern of action potentials generated upon depolarizations was substantially altered in neurons of epileptic mice. In particular, the frequency of repetitive spikes was increased, and spike frequency adaptation, representing a typical feature of adaptive behavior to maintained depolarizing stimuli in control neurons, was replaced by non-adapting spike activity in epileptic neurons. Voltage-clamp analyses of the membrane current generating the slow afterhyperpolarization (sIAHP) after spike series showed a relative decrease in epileptic animals compared with controls, suggesting a regulation of small conductance potassium channels involved in adaptive behavior.

These results indicate a reduction in adaptive electrogenic behavior in LA neurons which may contribute to the spectrum of excitatory events characterizing TLE. Moreover, they are indicative of specific membrane mechanisms relating to calcium and/or voltage-dependent potassium channels.

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# Absence of Ret signaling in mice causes progressive and late degeneration of the nigrostriatal system

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Pathological changes in the dopaminergic system account for a number of devastating illnesses including schizophrenia, psychosis, addiction or the most well known Parkinson's disease (PD). Loss of substantia nigra (SN) neurons and the underlying nigrostriatal pathway (NSP) they form is one of the hallmarks of PD, however, the precise mechanisms involved in the maintenance and the demise of SN neurons are poorly understood.

Neurotrophic factors such as GDNF (signaling via Ret) and BDNF (signaling via TrkB) were reported to have protective and rescuing properties on dopaminergic neurons and GDNF is currently tested in clinical trials of PD, but the results are so far conflicting. The physiological roles of GDNF and BDNF in the dopaminergic system are unknown. Null mutant mice for GDNF, BDNF and their signaling receptors do not survive to adulthood preventing the genetic analysis of their roles in long-term DA neuron survival.

To investigate the physiological requirements of Ret and TrkB for the establishment and maintenance of the NSP, we generated mice with dopaminergic- and whole nervous system - specific Ret and TrkB ablations that are compatible with postnatal survival of the mice. We find that Ret, but not TrkB, regulates long-term maintenance of the nigrostriatal dopaminergic system. Ret ablation by DAT-Cre causes histopathological defects that recapitulate some important features of PD, including progressive and late loss of a fraction of dopaminergic neurons in SN, marked degeneration of dopaminergic nerve terminals in striatum and occurrence of neuroinflammatory processes. Interestingly, like in PD, we observed specific loss in SN but not in the neighboring dopaminergic ventral tegmental area, a greater degree of axonal loss than cell body loss (consistent with a "dying back" process) and reduced evoked dopamine release in striatum. However, we found no evidence for alpha-synuclein accumulation in SN neurons, no decrease in the total amount of dopamine in the striatum and no behavioral alterations, consistent with a presymptomatic phase of nigral pathology.

Our data establish Ret as a critical regulator of long-term maintenance of the NSP and suggest Ret conditional mutants as useful tools for gaining insights into the molecular mechanisms involved in the development of PD. In particular, combining this model with other genetic and toxin models of NSP pathology could help develop better animal models to mimic with more accuracy the human pathology.

# Apoptosis Inducing Factor (AIF) is essential for neuronal cell death in vitro and in vivo

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Introduction:

Delayed neuronal cell death is a major hallmark of acute brain injury and a primary target for neuroprotective strategies. Apoptosis-inducing factor (AIF) promotes caspase-independent apoptosis upon translocation to the nucleus. The aim of the current study was to investigate the role of AIF for neuronal cell death.

Mehtods&Results:

Here we show that AIF translocates to the nucleus and is associated with apoptotic DNA damage in primary neurons and immortalized HT22 hippocampal neurons after oxygen-glucose-deprivation or exposure to glutamate. In order to provide evidence for the essential role of AIF in glutamate-induced neuronal cell death we employed AIF targeted small interfering RNA (siRNA) to downregulate the protein. Both, mRNA levels and protein levels were reduced to less than 20 % in primary neurons or HT22 cells after AIF-siRNA treatment for 48 h. Moreover, RNA interference (RNAi)-mediated downregulation of AIF provided profound protection against excitotoxic cell death in primary neurons and HT22 cells. In animals models of stroke and traumatic brain injury rapid AIF translocation to the nucleus was associated with apoptotic nuclear morphology and DNA damage, and occurred prior to cytochrom c release in injured neurons. Furthermore, mutant mice expressing AIF at low levels showed significantly reduced brain damage following ischemic or traumatic brain injury.

Conclusion:

These results provide compelling evidence for the primary involvement of AIF in neuronal cell death in vitro and in vivo and suggest that therapies targeting caspase-independent cell death signaling may provide neuroprotection following acute brain injury or other pathological conditions where programmed cell death is prominent.

# PIFITHRIN PROTECTS BRAIN TISSUE AFTER TRAUMA WITH A WIDE THERAPEUTIC WINDOW BY INHIBITION OF p53 AND ACTIVATION OF NF-kB

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Acute and chronic neurodegeneration, e.g. following brain injury or Alzheimer's Disease, is characterized by programmed death of neurons. The present study investigates potential neuroprotective effects of the p53 inhibitor pifithrin (PFT) in a model of traumatic brain injury (TBI) in mice. After TBI p53 protein rapidly accumulated in the injured brain tissue and translocated to the nucleus of damaged neurons, whereas NF-kB transcriptional activity simultaneously declined. Post-traumatic neurodegeneration correlated with the increase in p53 levels and was reduced by PFT. Inhibition of p53 and its downstream targets, e.g. Bax, resulted in the concomitant increase of NF-kB transcriptional activity and upregulation of NF-kB target proteins, e.g. x-chromosomal-linked inhibitor of apoptosis (XIAP). Strikingly, reduced brain tissue loss and significantly improved brain function after trauma was observed in animals treated with the p53 inhibitor PFT even when treatment was delayed up to 6 h after trauma. Inhibition of the NF-kB target protein XIAP abolished the neuroprotective effect of p53-inhibition in cultured neurons exposed to camptothecin, glutamate, or oxygen glucose deprivation. In conclusion, delayed neuronal cell death after trauma is mediated by a p53-dependent cell death program that also involves inhibition of NF-kB survival signaling. Hence, the pronounced neuroprotective effect of the p53 inhibitor PFT is attributable to the inhibition of programmed cell death and concomitant stimulation of programmed cell life in neurons.

# Neural activity of the ventral lateral nucleus around active electrode contacts in movement disorders

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Aside from the classical indications for deep brain stimulation as in the case of Parkinson´s disease and Essential tremor there are further clinical entities, which possibly respond to electrostimulation with chronic implanted macroelectrodes. Established target structures, mainly based on empirical evidence, are the ventral intermediate nucleus of the thalamus (Vim), the globus pallidus internus (GPi) and subthalamic nucleus (STN). There has been agreement that for the treatment of tremor the Vim is the best target area. In tremor-dominant Parkinsonian patients Vim is only a treatment option in the initial stages of the disease, in later stages the STN is preferable, especially if the subthalamic afferents to the thalamus (H1/H2, zona incerta) were inhibited. For rigidity and akinesia STN or GPi is the target structure with potential benefit for the patients. In a series of 16 patients with tremor as the main symptom (Essential Tremor, Multiple Sclerosis, tremor-dominant Parkinson´s disease, Dystonic tremor, Spinocerebellar ataxia with intension tremor) chronic macroelectrodes were stereotactically implanted into the ventral lateral nucleus (VIM). Intraoperatively, a microdrive attached to a ZD-frame allowed five simultaneous trajectories 2.3 mm apart. Methods for recording single cell potentials and microstimulation were used to study the pattern of activity. Postoperative stereotactic radiography was used to determine the stereotactic position of the most effective electrode contacts relative to the midcommissural point (MCP). Retrospective analysis of the spike pattern around the active contacts, where stimulation resulted in disappearance of tremor with a strong rebound effect after the stimulator was switched off, enabled the delimitation of the effective VIM margins and the differentiation of electrophysiolocigal properties. In the future, detailed analysis of the basal ganglia output system based on microrecording and microstimulation intraoperatively and meticulous follow-up studies of motor performance post-surgery might lead to a deeper understanding of the basal circuitry and might allow to establish more specific target areas.

# The up-regulation of acetylcholinesterase and butyrylcholinesterase activity in rat brain are related to behaviour disturbances in the course of experimental breast cancer.

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Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are esterases involved in acethylcholine degradation significant in cognitive functions disturbances. AChE is localized mainly in neurons and BChE in glia, endothelial cells and neurons. Both enzymes are also theraputic targets. Cognitive impairment associated with malignancy may result from remote effects of cancer, paraneoplastic syndromes like limbic encephalitis or cerebellar degeneration. In breast cancer adjuvant chemotherapy is suggested to produce cognitive impairment not linked to malignancy - related factors.

The aim of this study was to correlate the activity of AChE and BChE in brain regions in the course of experimental neoplastic disease with behavior of breast cancer bearing rats.

Material and methods: The brains of female Wistar rats were sectioned macroscopically in frontal, temporal and occipital lobes, cerebellum and brainstem one and two weeks after breast cancer transplantation. The activity of both esterases were estimated spectrophotometrically in tissue homogenates. The activities were expressed as units per milligram of protein. The behaviour tests were based on open-field, T-maze and elevated-plus maze and video-monitored. The software built-up by the authors used intuitive algorithms.

Results: AChE activity was increased in cerebellum during first ($p<0.05$) and second week of the tumor growth ($p<0.05$) and after 2 weeks in brainstem ($p<0.05$). BChE was elevated after one week in brainstem ($p<0.05$), cerebellum ($p<0.05$), frontal ($p<0.05$) and temporal lobes ($p<0.05$). Abnormal motor behavior and spatial disorientation were evidenced.

Conclusions: The up-regulation of esterases involved in acethylocholine degradation coexists with motor activity impairment and spatial disorientation in the course of experimental breast cancer.

# Anti-GAD and IA-2 antibodies in neuroimmunological diseases.

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Organ-specific autoantibodies may be present in patients with neurological autoimmune disorders. Among them anti-beta cells have particular importance because of associated diabetes. Untill now anti-GAD (Glutamic Acid Decarboxylase) have been found in up to 80% of patients with type 1 diabetes mellitus and such neurological disorders like stiff-person syndrome or epilepsia partialis continua. Anti-insulinoma-associated protein-2 (IA-2) were detected in 37% of type 1 diabetes patients. The aim of this study was to analyze the incidence of anti-GAD and IA-2 antibodies in patients with neurological autoimmune diseases.

Material and methods: Fifty one patients with neurological autoimmune disorders (36 females, 15 males) were examined. In this group 30 patients had multiple sclerosis, 18 - myasthenia gravis, 1 patient - coexisting multiple sclerosis and myasthenia gravis, 2 patients - Guillain - Barre syndrome. In 9 patients with multiple sclerosis diabetes was diagnosed

( 6 - insulin-dependent diabetes mellitus - IDDM), in myasthenia gravis in 9 cases ( 5 IDDM ) and 1 patient with Guillain - Barre syndrome had diabetes (NIDDM) as well.

Results: We have found anti - GAD antibodies were positive in 5 (56%) patients with coexisting multiple sclerosis and diabetes, and 1 patient (11%) with myasthenia gravis and diabetes, no patient with Guillain Barre and diabetes had anti-GAD antibodies. Anti IA2 antibodies were positive and presented as high levels only in one patient with multiple sclerosis and diabetes.

Conclusion : Anti-beta cells antibodies studied were specific for coexistence of diabetes and multiple sclerosis and to a lower extend myasthenia gravis. No presence of anti-GAD or anti-IA2 antibodies was revealed in non-diabetic patients with autoimmune neurological disorders.

# The depletion of Tumor Necrosis Factor - alpha in macrophages of migraine patients.

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Sterile inflammation of dura matter during migraine attack is suggested as one of pathomechanisms basing on observed increase in serum interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor (TNF). The secretion of cytokines in migraine patients is triggered by calcitonin gene-related peptide (CGRP). On the other hand tumor necrosis factor alpha as well as interleukin-1, interleukin-6 and interleukin-8 may promote hyperalgasia - a clinically important symptom associated with migraine attack. Those observation compose the evidence for the use of non-steroid anti-inflammatory drugs (NSAID's) in migraine attacks treatment.

The aim of this study was to examine the expression of tumor necrosis factor alpha in monocytes of migraine patients.

Material and methods: Twenty migraine patients (aged 39±11) (16 females, 4 males) were included in the study. Ten healthy subjects (7 females, 3 males) were used as controls. The migraine patients were examined neurologically and assessed according to QMV, HimQ, MIDAS and MIGSEV scales. The heparinized blood was used for monocytes separation. Following meglumini amidotrizoas (Uropolinum) - Ficoll centrifugation the monocytes were isolated with the use of magnetic labeling system (MACS, Miltenyi Biotec). Monocytes underwent further immunostaining with the use of anti-TNF antibodies (Bender System). The final color obtained in result of peroxidase-DAB reaction was estimated using ImageJ software.

Results: We have found decreased expression of tumor necrosis factor alpha in monocytes originating from migraine patients compared to controls ($p<0.001$). There were no statistically significant differences between patients with and without aura.

Conclusion: The depletion of TNF in circulating monocytes may be result of its release and increased serum level.

# Lysosomal degradation and alpha-synuclein aggregation and toxicity

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Parkinson disease (PD) is characterized by the loss of dopaminergic neurons in the substantia nigra and the deposition of eosinophilic, fibrillar, proteinaceous aggregates known as Lewy bodies (LBs). Aggreagted alpha-synuclein is the major component of LBs. LBs are found in other so called synucleinopathies, such as Dementia with LBs (DLB) as well. Degradation of alpha-synuclein molecules has been widely studied for the proteasomal degradation system. However, we and other have shown that that another degradation pathway - lysosomal degradation - is involved in alpha-synuclein degradation. Here we show that lysosomal degradation marker are present in LBs and are dysregulated in the temporal cortex of patients with DLB. Furthermore, inhibition of lysosomal degradation in a cell culture model of alpha-synuclein aggregation substantially alters alpha-synuclein aggregation and toxicity. Thus, we hypothesize that the lysosomal degradation pathway participates in alpha-synuclein aggregation and degradation, and thereby influencing its toxic function.

# ERP COMPUTATIONAL METHODS IN TINNITUS DECOMPENSATION

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Neural correlates of psychophysiological tinnitus models in humans may be used for their neurophysiological validation as well as for their refinement and improvement to better understand the pathogenesis of the tinnitus decompensation and to develop new therapeutic approaches.

In our work we make use of neural correlates of top--down projections, particularly, a recently introduced synchronization stability measure, together with a large--scale continuum evoked response potential (ERP) model in order to study and evaluate the tinnitus decompensation.

By introducing these tools we follow an inverse--forward mathematical scheme that allows for our study's validation and at the same time brings light regarding the connection between the top--down adaptive resonance theory and the Jastreboff model of Tinnitus.

# Dehydroepiandrosterone sulfate is neuroprotective in a focal cortical cold lesion model

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Dehydroepiandrosterone and its sulfate (DHEAS) are sex hormone precursors. They may function as neurotrophic or neuroprotective factors to protect central nervous system neurons against a variety of insults, including excitotoxicity. The present study evaluated the effects of DHEAS and 17beta-estradiol (E2) in a focal cortical cold lesion model, in which DHEAS and equimolar E2 were administered either as pretreatment (two injections 1 d and 1 h before lesion induction) or as posttreatment (immediately after lesion induction). The focal cortical cold lesion was induced in the primary motor cortex by means of a cooled copper cylinder placed directly onto the cortical surface. One hour later, the animals were killed, the brains were cut into 0.4-mm-thick slices, and the sections were stained with 1% triphenyltetrazolium chloride. The volume of the hemispheric lesion was calculated for each animal. The results demonstrated that the lesion area was significantly attenuated in both the DHEAS- and E2- pre- and posttreated groups and that, in the presence of letrozole, a nonsteroidal aromatase inhibitor, no neuroprotection was observed, suggesting that the beneficial effect of DHEAS on the cold injury might depend on the conversion of DHEAS to E2 within the brain. It is concluded that even a single posttraumatic administration of DHEAS may be of substantial therapeutic benefit in the treatment of focal brain injury with vasogenic edema.

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# Neuroprotective effects of repeated transient global ischemia and of kynurenine adminsitration induced by four-vessel occlusions on hippocampal CA1 neurons

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The hippocampal CA1 subfield is a brain region that is particularly sensitive to hypoxia. Although this subfield is selectively vulnerable to ischemic injuries manifested in delayed neuronal death (DND), the mechanism leading to neuronal degeneration is not fully understood. Burda recently reported that a second pathophysiological stress, applied within a suitable time, offers an opportunity for salvaging neurons in the CA1 region against DND (Neurochem. Res., 30: 1397-1405, 2005). In our study, NeuN immunohistochemistry was applied to detect survival CA1 neurons, while Fluoro-Jade B staining was used to evaluate the number of injured neurons after interventions resulting in transient global ischemia. Four groups of animals were used: 1: intact controls; 2: sham controls (2 vertebral arteries coagulated (2VAC), but 2 carotids sham-operated); 3: 2VAC + 2 carotids occluded (2CA) for 10 min; 4: 2VAC + 2CA (10 min) + 2 days later, a repeated 2CA (5 min). In group 3 (2VAC + 2CA (10 min)), marked cell destruction was found in the CA1 subfield: only 36.4% of the CA1 neurons survived. However, in group 4 (5-min second ischemic insult), the proportion of surviving cells in the CA1 region was 59.3%. There was
no significant difference in CA1 cell loss between groups 1 and 2. Our findings suggest that the second ischemic stress, 2 days after the first ischemia induced by 2VAC + 2CA can be efficient in the prevention of DND. Neuroprotective effect was also found in four-vessel occlusion models after kynurenine (i.v.) administration.

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# Salicylate and pure tone trauma and band noise trauma induce Tinnitus in a rat behavioural model

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Recently we could show the temporary presence of tinnitus in a rat behavioural model using high dosages of salicylate injections (350 mg/kg body weight, see also Rüttiger et al. 2003). As an alternative model we could demonstrate that also acoustic trauma of either pure tone (10 kHz, 118 dB SPL for 2 hours) or broadband noise stimuli (8-16 kHz band noise, 119 dB SPL, 2 h) led to permanent hearing loss and tinnitus, correlated with a differential change in the expression of activity dependent genes in cochlear spiral ganglion neurons, midbrain auditory nuclei and auditory cortical area AI.

The question was whether the tinnitus sensation induced by salicylate and noise trauma was founded on the same or different mechanisms. Rats were exposed to a traumatic acoustic stimulus and examined for temporary and permanent hearing loss and the development of tinnitus sensation. After evidence for a profound tinnitus manifestation, animals were injected with salicylate (350 mg/kg body weight) and retested for tinnitus sensation and general hearing function.

First results point to a non additive process of tinnitus generation and hearing impairment after combined salicylate and acoustic trauma treatment, suggesting similar mechanisms for tinnitus generation - most likely an impairment of adequate cochlear function.

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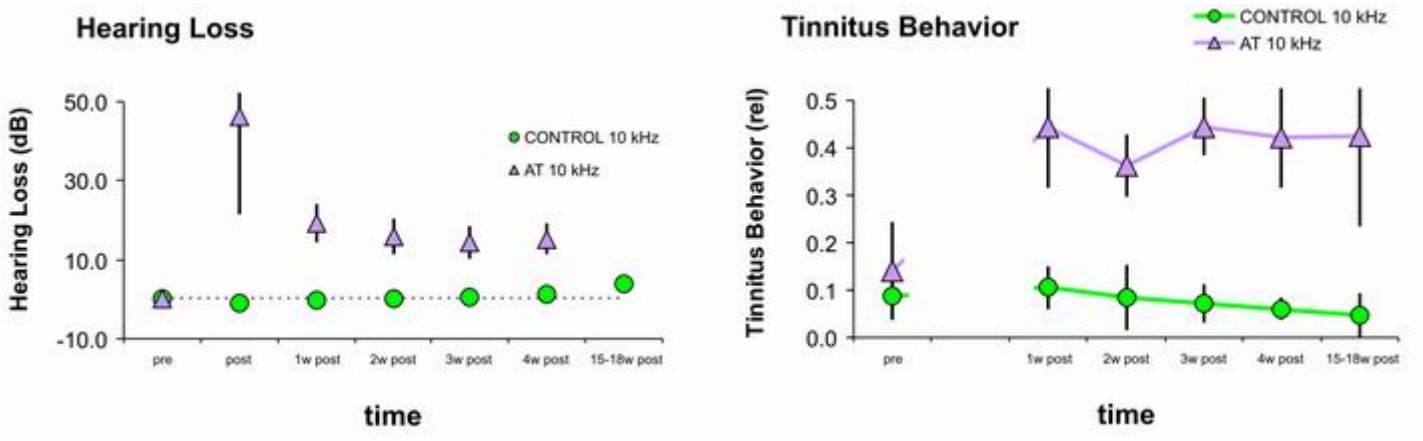

# Soluble Amyloid Assemblies: Mechanism of Formation, Biological Activity and Novel Aromatic Peptide Agents for Inhibition

Anat Marom-Frydman[1], Meirav Recther[1], Irit Shefler[1] and Ehud Gazit[4]

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Alzheimer's disease (AD), a progressive neurodegenerative disease for which there is no cure or effective treatment, is the leading cause of dementia in aged humans. The clinical hallmarks are progressive impairment in memory, language deterioration, and poor judgment. In the past several years a large body of evidences has established a contributory role for β-amyloid peptide (βA) in AD. In contrary to the initial hypothesis that mature aggregated forms of βA are the most toxic, recent studies indicate that the globular βA oligomers of about 50kDa (referred to as globulomers) are the major toxic specie. Therefore, an attractive therapeutic strategy for AD will be to block the early stage of misfolding and aggregation of soluble βA peptide. Towards this end, we have rationally designed a dipeptide inhibitor, EG30, which contains an aromatic residue and a β-breaker element. The inhibitor was designed to interact with the peptide through aromatic interactions and to interfere with the assembly into β-sheet rich fibrillar structures. We demonstrated that the EG30 has a highly valuable pharmaceutical profile such as low molecular weight, high stability in aqueous solution, serum stability and trans-membrane transport. It has an efficient oral bioavailability and penetration through the blood-brain barrier and it was proven to be safe both in vitro and in vivo. We showed the potency of the peptide in inhibiting amyloid aggregation, and in preventing its cytotoxicity. NMR analysis shows indications of in volvement of EG30 with the aromatic core of the βA. Moreover, we observed prevention of cognitive decline in AD model transgenic (tg) mice that were treated with EG30. This was in agreement with a significant reduction of amyloid plaques in the transgenic mice's brain. Thus, we suggest that EG30 interferes with the initial molecular recognition processes that are involved in the early stages of the bA fibrillization, and consequently blocks the transition of the assemblies into the toxic b-sheet globulomers structure.

# Approaches to auditory Brain-computer interfaces for communication with Locked-in patients without vision

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Many people with severe motor paralysis (e.g. as a consequence of amyotrophic lateral sclerosis; ALS) need muscle-independent communication technologies. Brain-computer interfaces (BCIs) are systems that translate electrical activity of the brain into signals controlling external devices. For complete locked-in patients (CLIS) the existing BCIs are not feasible, because they rely on vision which is often impaired or lost in these patients. Usually the sense of hearing is uncompromised in these patients, thus different approaches to auditory BCIs are tested in our studies. It has been shown that that healthy participants and locked-in patients can control BCIs on the basis of Sensorimotor rhythms (SMR) and the P300 component of the event-related potential when provided with visual feedback [1, 2]. An accuracy of 70 % correct is the minimum for satisfactory communication [3]. Hinterberger at al [4] demonstrated that regulation of slow cortical potentials is achieved when provided with auditory feedback. The current study investigated auditory stimulation (P300) and feedback (SMR) in healthy subjects and one locked-in patient.
Eight healthy participants were trained to self-regulate their SMR amplitude by auditory feedback. An increase in SMR amplitude was represented by harp sounds and a decrease by bongo sounds. All users participated in three sessions consisting of 510 trials. During a trial the user had to produce either harp or bongo sounds as instructed by a computer-generated voice. Performance in the last session ranged from 39.61 to 78.63 %. Three out of eight participants performed over 70 % correct in the last session. Six participants significantly increased their performance. This suggests that an auditory SMR BCI is feasible, but users may need longer training than often provided with visual feedback.

Following [5] we presented a locked-in patient without residual muscular movement, who was not able to use a BCI based on vision, with an auditory BCI. When confronted with the words 'ja', 'nein', 'weiter' and 'stop' (yes, no, pass, end) and asked to focus either on 'yes' or on 'no', he had a prominent P300 which he subsequentially used to answer questions

With this study we demonstrated the feasibility of both the auditory SMR- and P300 BCI. A downside of auditory BCIs is first, that it takes longer to acquire self-regulation as compared to vision and second, that items for selection can only be presented serially. We are currently developing an auditory BCI spelling program for locked-in patients.

1.Birbaumer et al, Nature, 1999.
2.Kübler et al, Neurology, 2005.
3.Kübler et al, Biofeedback - A Practitioner's Guide, 2003
4.Hinterberger et al, Exp Brain Res, 2004
5.Sellers and Donchin, Clin Neurophysiol, 2006.

# Calcium binding protein secretagogin expression in the human hippocampus is restricted to pyramidal neurons and independent of concomitant Alzheimer disease pathology.

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The pathological findings in Alzheimer's disease (AD) are partly attributed to alterations in calcium-binding protein (CBP) functions. We evaluated immunoreactivity of secretagogin, a recently cloned CBP, in hippocampus and adjacent entorhinal cortex of 30 neuropathologically examined post mortem brains (female, 18; mean age, 79.8 ±15.1 years). The study group consisted of 15 cases fulfilling the criteria for high probability of AD according to the NIA-Reagan Institute Criteria and 15 cases with no to medium probability. Sections were incubated with secretagogin-specific antibodies and the number of immunoreactive neurons as well as staining intensities in both neurons and neuropil were assessed. Both cellular and neuropil immunoreactivity were restricted to subiculum and Ammons horn. Cellular immunoreactivity was further restricted to pyramidal neurons and showed a hierarchical distribution: the mean percentage of immunoreactive neurons was highest in sector CA3 (64.41%), followed by CA2 (44.09%), CA4 (34.38%), CA1 (10.9%), and the subiculum (2.92%; $P<0.001$, except CA2-CA4, $P>0.05$), while it did not differ significantly between groups with different degrees of AD pathology. Double staining for both tau and secretagogin (immunofluorescence) revealed that both proteins rarely co-localize since 5.3% of tau and 2.9% of secretagogin positive neurons, respectively, showed such co-localization and secretagogin. The pattern of secretagogin immunoreactivity resembles that of calcium sensor proteins as it is restricted to a subset of neurons and therefore secretagogin could serve highly specialized tasks in neuronal calcium signalling. The lack of an association between tau burden and the density of secretagogin expressing neurons together with the low co-localization rate of both tau and secretagogin in the human hippocampus, suggest that secretagogin expressing neurons are largely resistant to neurodegeneration in AD.

# FUNCTIONAL CHARACTERIZATION OF MICE LACKING THE DOPAMINE TRANSPORTER - A MOUSE MODEL FOR ATTENTION-DEFICIT/HYPERACTIVITY DISORDER (ADHD)

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Attention-deficit/hyperactivity disorder (ADHD) is a common neurobehavioral disorder of childhood onset that can include elements of inattention, hyperactivity and impulsive behavior. Although dysregulation of different neurotransmitter systems such as the dopaminergic, noradreneric and serotonergic system has been postulated to be involved in ADHD, it is commonly believed that the dopaminergic system is preferentially implicated in the etiology and pharmacotherapy of this disorder. Brain-imaging studies have documented both structural and functional pathological changes in frontal-subcortical-cerebellar circuits. In the subcortical structures associated with ADHD, the striatum has been of particular interest.

All monoaminergic neurotransmitters probably involved in the pathophysiology of ADHD are affected by synaptic proteins, since they influence the release of dopamine, serotonin and noradrenaline. Mice with a genetic deletion of the dopamine transporter (DAT-KO mice) demonstrate several key features of ADHD, such as hyperactivity, cognitive impairment and paradoxical calming responses towards MPH. We found altered expression levels of different synaptic proteins in the DAT-KO compared to WT control mice. Most of the differences were found in striatum and cortical regions, which implies modulated dopaminergic networks in this mouse model for ADHD. Beyond this, we showed a specific regional expression pattern of these synaptic proteins in neurons of different murine brain regions and we characterized these neurons especially in striatal regions using colocalization studies. To learn about the identity and individual roles of these neurons will help us to understand how the striatum processes its inputs, and how pathology in striatal circurity may participate in the development of symptoms of human psychiatric diseases, potentially also of ADHD.

# Thalamic LFP and scalp EEG in patients with Parkinson's disease

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To investigate the pathophysiology of Parkinson's disease (PD), most research focuses on basal ganglia dysfunction. However, the main output from the basal ganglia is via the thalamus and corticothalamic feedback constitutes the primary source of synapses in the thalamus. We therefore focus on the thalamocortical interplay.

During the surgical intervention in six patients, local field potentials (LFP) were recorded from motor thalamic nuclei VA and VLa. Simultaneously, EEG was recorded from several sites on the scalp. The highest thalamocortical coherence was found in the 4-9 Hz theta frequency band. Thalamocortical theta coherence reached 70% and was maximal with frontal scalp sites on both hemispheres. In the 13-18 Hz beta frequency band, maximal coherence was lower but localized on the central scalp ipsilateral to the site of thalamic LFP recording.

To define abnormality in the EEG of PD patients, we recorded whole-head EEG from 21 patients and from 26 healthy, age-matched controls. On average, the patient group exhibited a higher dominant peak and more spectral power over the whole frequency range (2-100 Hz) and the dominant peak was shifted towards frequencies in the theta range. In LORETA topographic maps of statistical activation differences in theta and beta, high t-values appeared dominantly in fronto-insulo-temporal areas.

The high thalamocortical coherence underlines the importance of the thalamus for the genesis of scalp EEG. These findings add to the evidence that both excess EEG power and Parkinsonian symptoms result from a dysfunction of thalamocortical loops (Thalamocortical Dysrhythmia) and thus have important consequences for the choice of therapeutic strategy in patients with severe forms of PD.

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# Poster Topic

## T36: Neuroimmunology

**T36-1A** Nucleoside analogues effect on glial response in Experimental Autoimmune Encephalomyelitis
*D. Stojkov, I. Lavrnja, S. Pekovic, S. Dacic, S. Jovanovic, I. Nikic, I. Bjelobaba, M. Mostarica-Stojkovic, S. Stosic-Grujicic, L. Rakic and M. Stojiljkovic, Belgrade (Serbia)*

**T36-2A** A peripheral mononeuropathy in rat produces ascending inflammatory reactions in the spinal cord.
*A. Leichsenring, M. Andriske, H. Lübbert and CC. Stichel, Bochum and Leverkusen*

**T36-3A** Schwann cells as antigen presenting cells in inflammatory neuropathies
*G. Meyer zu Hörste, HP. Hartung, H. Wiendl and BC. Kieseier, Düsseldorf and Würzburg*

**T36-1B** Multiple sclerosis-like grey matter lesions induced by immunization with a neuronal antigen and transfer of demyelinating antibodies
*A. Escher, D. Merkler, A. Rohde, R. Diem, W. Brück and C. Stadelmann, Göttingen*

**T36-2B** Role of the chemokine receptor CXCR2 during remyelination
*M. Lindner, S. Heine and M. Stangel, Hannover*

**T36-3B** Expression of APOBEC3G and related hypermutations in viral DNA is increased in primate brain during simian immunodeficency virus infection
*C. Depboylu, LE. Eiden and E. Weihe, Marburg and Bethesda (USA)*

**T36-1C** Interleukin-2 in the striatum affects behaviour in rats
*BD. Karrenbauer, J. Löhn, RKW. Schwarting and CR. Pawlak, Marburg and Mannheim*

**T36-2C** ΞSex hormones as modulators of inflammatory processes in Alzheimer's Disease
*M. Behrendt, MR. Sairam and D. Maysinger, Montreal (Canada)*

**T36-3C** Analysis of low-level motor control in tethered flying *Drosophila*
*CF. Graetzel, M. Moser and SN. Fry, Zürich (CH)*

# Nucleoside analogues effect on glial response in Experimental Autoimmune Encephalomyelitis

Danijela Stojkov[1], Irena Lavrnja[1], Sanja Pekovic[1], Sanja Dacic[2], Sasa Jovanovic[1], Ivana Nikic[2], Ivana Bjelobaba[1], Marija Mostarica-Stojkovic[3], Stanislava Stosic-Grujicic[4], Ljubisav Rakic[1] and Mirjana Stojiljkovic[1]

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Multiple sclerosis (MS) is chronic inflammatory disease of the human central nervous system (CNS) represented by repeated sensomotory disturbances. In MS and experimental autoimmune encephalomyelitis (EAE) animal model of MS, the primary insult is an inflammatory attack, which is result of an autoaggressive T-cell response against myelin. We have previously shown that combined treatment with nucleoside analogues (ribavirin – R + tiazofurin – T), inosine monophosphate dehydrogenase inhibitors, ameliorates clinical signs and histological lesions of EAE in susceptible rats, when they are given preventatively. The aim of this study was to investigate the effect of combined treatment with R + T, given with the appearance of first EAE clinical sign, on microglia and astrocytes response. These cells of the target tissue also participate in an autoimmune process. The disease was induced in Dark Agouti rats with rat spinal cord homogenate and had acute monophasic course. Ribavirin and tiazofurin were given at a dosage of 30mg/kg/day and 10mg/kg every other day, for 15 days, respectively. Control group was immunized and treated with saline. Amelioration of clinical signs and faster recovery was shown in group treated with combination of R and T in comparison to control group. Immunohistochemical analysis of the spinal cord tissue isolated after 15 days of combined therapy revealed decrease in vimentin positive cells and microglia compared to control group. Additionally, morphology of glial fibrillary acid protein (GFAP) positive cells and microglia indicated to reactive type of these cells in control group. Results of this study revealed that R and T modulate glial response and have EAE protective effects when they are given from the onset of disease.

# A peripheral mononeuropathy in rat produces ascending inflammatory reactions in the spinal cord.

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Pain is classically viewed as being mediated solely by neurons, as are other sensory phenomena. The discovery that spinal cord microglia and astrocytes amplify pain requires a change of this view. These cells are able to release proinflammatory substances, as well as proteases, which can contribute to chronic pain. Even peripheral immune cells like mast cells, leukocytes, and dendritic cells may be involved in the process that makes a pain state chronic.

We analyzed the spinal cords of rats, that underwent a modified version of the spinal nerve ligation (SNL) surgery (Kim and Chung, Pain (50): 355-363, 1992), a unilateral transection of the 5th lumbar spinal nerve (L5T). The aim of our study was to determine the longitudinal and transversal expansion and composition of the inflammatory reaction at multiple time-points after surgery.

By means of immunohistochemistry we showed an intense cellular inflammatory reaction that involved astro- and microgliosis, the appearance of macrophages, and the upregulation of the lysosomal proteases cathepsins S (CATS) and X (CATX) in L5T operated animals.

The longitudinal extension of the inflammation was strongly dependent on the time-point after surgery. In the first week after surgery the inflammatory reactions were restricted to spinal cord levels near the lesion site, whereas at later time-points they spread along the whole spinal cord.

The transversal extension of the inflammation comprised the spinal cord gray, as well as the white matter. At the lesion site (lumbar spinal cord), the gray matter of the ventral and the dorsal horn and the gracile fasciculus showed an intense gliotic reaction, whereas the extension of the inflammation at higher spinal cord levels was restricted to the dorsal nucleus and the gracile fasciculus.

The treatment with cannabinoids, which are strong pain-relieving agents, will allow us to define the role of the inflammation in the pain cascade and the chronification process.

The extensive inflammatory reactions that accompany the histopathology of neuropathic pain emphasize a perspective for antiinflammatory agents in neuropathic pain treatments.

# Schwann cells as antigen presenting cells in inflammatory neuropathies

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Schwann cells are the myelinating glia cells of the peripheral nervous system ensheathing and supporting axons. In human inflammatory neuropathies like Guillain-Barré syndrome (GBS) an autoimmune response is directed against Schwann cell components. Whether Schwann cells promote this response by presenting autoantigens to T-cells remains controversial.

Here, we show by immunohistochemistry that human Schwann cells in vitro and in vivo indeed express the intracellular machinery required for processing and presenting antigens to autoreactive T-cells. Furthermore, Schwann cells in sural nerve biopsies obtained from patients with GBS increase their expression of antigen associated transport proteins and antigen presenting MHC class I and II complexes.

In coculture experiments rodent Schwann cells activate T-cells when pre-treated with proinflammatory cytokines. These data support the ability of peripheral glia cells to present antigens and its functional relevance. Specifically targeting this pathological function of Schwann cells may in future extend our therapeutic options in GBS.

# Multiple sclerosis-like grey matter lesions induced by immunization with a neuronal antigen and transfer of demyelinating antibodies

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating CNS disease presumed to be autoimmune in nature. In the last few years demyelinated lesions of grey matter, such as cortex and spinal cord grey matter, have come into focus. In the present study, we aim to induce grey matter pathology resembling that found in MS by immunization with a neuronal antigen.

Beta-synuclein is a cytoplasmic protein located in the perikaria and presynaptic nerve terminals. Immunization of Lewis rats with beta-synuclein93-111 induces monophasic autoimmune CNS inflammation with complete recovery (Mor et al., 2003). We found inflammatory infiltrates in beta-synuclein93-111 immunized animals mainly in the grey matter of the spinal cord. No demyelination was observed. To mimick MS inflammatory-demyelinating pathology, anti-MOG antibodies were transferred into beta-synuclein peptide-immunized rats. Extensive demyelinated grey matter lesions with iNOS-positive, myelin phagocytosing macrophages could be generated. Numbers of APP-positive axons were increased in antibody transferred rats compared with animals receiving beta-synuclein93-111-immunization alone. Grey matter inflammation led to a reduction of NeuN positive neurons and an increased neuronal expression of the transcription factor c-Jun.

Our studies identify beta-synuclein93-111 induced EAE in the Lewis rat as a valuable tool to study mechanisms of grey matter pathology in MS.

# Role of the chemokine receptor CXCR2 during remyelination

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To understand the mechanisms of remyelination and the reasons for repair failure is one of the major challenges in multiple sclerosis research. The chemokine receptor CXCR2 is expressed by oligodendrocyte precursor cells (OPC) in multiple sclerosis lesions and there is evidence that CXCR2 ligands influence the functions of primary OPCs. We therefore studied the role of CXCR2 in the process of remyelination in a toxic demyelination model induced by the copper chelator cuprizone.

C57BL/6 mice were fed a 0.3 % cuprizone diet for 6 weeks. After withdrawal of cuprizone animals stayed on normal chow for another 10 weeks. At different timepoints (4, 7, 14, 21, 28, 35, 42 and 70 d after demyelination) brains were removed and paraffin embedded. The degree of de- and remyelination was quantified with Luxol fast Blue and myelin protein stainings. Expression and source of the chemokine receptor CXCR2 during remyelination was investigated immunohistochemically with double stainings for CXCR2, OLIG2 (OPC), CNPase (mature oligodendrocytes), GFAP (astrocytes) and MAC3 (microglia).

LFB and myelin stainings revealed a nearly complete demyelination of the corpus callosum after 6 weeks of cuprizone treatment. Remyelination occurred spontaneously within two weeks after withdrawal from the diet.

CXCR2 positive cells could be observed during the whole process. The number of CXCR2 positive cells exceeded the number of oligodendrocyte precursor cells. Double staining revealed that not only OPCs but also mature CNPase positive oligodendrocytes are expressing CXCR2. In addition, also microglia and astrocytes showed CXCR2 expression.

We conclude that CXCR2 signalling is involved in the remyelination process by a rather complex glial interaction.

# Expression of APOBEC3G and related hypermutations in viral DNA is increased in primate brain during simian immunodeficency virus infection

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The cytosine deaminase APOBEC3G is an innate cellular defense factor restrictng replication of retroviruses. Lymphocytes and monocytes have been proposed to produce APOBEC3G, but direct evidence in vivo is still missing. Using immunohistochemistry and in situ hybrdization we show that APOBEC3G expression in the rhesus monkey brain is induced exclusively in microglia/macrophage-derived cells and T-lymphocytes during infection with simian immunodeficiency virus (SIV) clone dB670. APOBEC3G expression is directly related to brain virus burden. Intracellularly APOBEC3G is found in the cytoplasm and/or in the nucleus. Induction of APOBEC3G is paralleled by guanosine-to-adenosine hypermutations in retroviral DNA isolated from the brains of AIDS-diseased rhesus monkeys. Although CNS-directed treatment with antiviral 6-chloro-2',3'-dideoxyguanosine suppresses brain SIV burden as well as encephalitis and reduces cerebral APOBEC3G synthesis hypermutations are still detectable. Upregulation of active APOBEC3G may restrict SIV spreading in the brain and thus limit brain damage during lentiviral infection.

# Interleukin-2 in the striatum affects behaviour in rats

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There is evidence that cytokines such as interleukin (IL)-2 can modulate motivated behaviour, and are involved in psychiatric conditions like anxiety, and depression. In previous studies, we showed that cytokine expression in specific brain tissues correlated with anxiety-relevant behaviour (open arm time) in the elevated plus-maze in male adult outbred Wistar rats. These relationships indicate that cytokines in the brain can be related to avoidance behaviour, and that this relationship is site- (striatum, frontal cortex), and cytokine-specific (IL-2 mRNA). Subsequently, we tested rats after a single striatal IL-2 injection followed by an elevated plus-maze test acutely and 24 h later. Acute tests showed no significant effects for open arm time, whereas dose-dependent differences in rearing activity, and open arm entries became apparent between IL-2 doses. A dose-dependent trend of IL-2 for more open arm time compared to control rats became apparent 24 h later, however, measures of general activity did not differ. In the present study, we also tested the effects of striatal injections, but now we used an open field paradigm, and extended the analysis to even lower doses. The results showed no differences in locmotion, and grooming between IL-2 groups and control rats. In contrast, acute analyses exhibited a trend for lower off-wall rearings in IL-2 compared to controls. Furthermore, for both test days the lowest IL-2 dose generally showed less centre time compared to control rats, with a significant dose effect 24 h later. These latter consequences may reflect proactive drug mechanisms. Alternatively, the treatments may have affected memory of the initial behavioural test (i.e. elevated plus-maze or open field). We suggest serotonergic (anxiety-related) and dopaminergic (rearings) mechanisms to be involved in this behaviour since IL-2 can influence both neurotransmitter systems in the striatum.

# ΞSex hormones as modulators of inflammatory processes in Alzheimer's Disease

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Amyloid-β (Aβ) plaque deposition is one of the hallmarks of Alzheimer's disease. It is considered a prime trigger for local inflammatory response initiated by activated microglia and the recruitment of astrocytes. Oxidative stress, an early damaging event, occuring even in the absence of detectable Aβ-deposits leads to the activation of microglia. Early degenerative and inflammatory processes in the central nervous system (CNS) are modulated by gonadotropins and sex hormones. An imbalance between estrogens and gonadotropins adversely affect the amyloid precursor protein (APP) processing. We investigated the consequences of the chronic imbalance in a new APP/PS1-FORKO hybrid mouse model. This model was generated by cross-breeding mice that lack follitropin-stimulating hormone receptor (FORKO) with mice overexpressing amyloid precursor protein (APPswe695) and presinilin 1 protein (PS1Δ9).

In this study, we assessed the role of chronic hormone-gonadotropin imbalance in enhancing the rate of plaque formation, region-specific distribution and clearance in the cortex and hippocampus. Significant changes in plaque size and density in FORKO- APP/PS1 mice were associated with a pronounced responsiveness of microglia and astrocytes. Sex and gonadotropine hormones exerted a marked effect on Aβ-induced inflammation response. These findings provide evidence for the modulatory role of sex hormones in neuronal damage and repair in the progression of neuroinflammation in the CNS. APP/PS1-FORKO mouse is a suitable new model to study mechanisms of neuroinflammation and modulatory roles of anti-inflammatory therapeutics in AD.

# Analysis of low-level motor control in tethered flying *Drosophila*

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A powerful technique to study sensory-motor control loops in fruit flies is the use of tethered flight 'simulators', in which the real-time measurement of the flight behavior is used to drive a sensory (e.g. visual) feedback loop.

We have developed novel methods based on micro-electro-mechanical systems (MEMS) and imaging technology to characterize in real time the flight forces and the kinematics, respectively, of tethered flying *Drosophila*. The high sampling rates of the two systems allow us to resolve subtle changes within single wing strokes and evaluate their effect on the resulting wing forces, providing a powerful read-out system to explore low-level flight control strategies.

The goal is to understand the functional relationship of control of wing movement and the resulting force patterns and identify the control strategies employed by the flies during flight.

In one application, we explore the dynamics and neuromotor control strategies of lift control by inducing lift reactions from vertically-moving square-grating patterns and measuring wing motions and lift forces simultaneously at 3kHz. Future applications include employing a 3 degree-of-freedom MEMS sensor for combined lift-thrust-yaw torque measurements to provide a sensitive read-out system of the neural flight control circuits in wild type and transgenic *Drosophila*.

# Poster Topic

## T37: Computational neuroscience


**T37-1A** Fundamental filter properties of spiking neurons constrain detectability of communication signals in weakly electric fish
*J. Benda, A. Longtin and L. Maler, Berlin and Ottawa (Canada)*

**T37-2A** Contribution of interneuron properties to the generation of theta oscillations in the rat hippocampal formation in vitro
*A. Boehlen, A. Kunert, A. Herz and U. Heinemann, Berlin*

**T37-3A** Memory Lifetime Depends on Synaptic Meta-Plasticity and the Size of Cell Assemblies
*C. Leibold and R. Kempter, Berlin*

**T37-4A** HOW NEURONS COULD SWITCH FROM AN INTEGRATOR TO A RESONATOR AND CHANGE THEIR SYNCHRONIZATION PROPERTIES
*U. Roehner and J. Benda, Berlin*

**T37-5A** A model for object detection in the visual system of the fly
*P. Hennig, R. Möller and M. Egelhaaf, Bielefeld*

**T37-6A** Neuronal Tension May Co-Shape V1 Orientation Maps
*C. Weber and J. Triesch, Frankfurt/Main*

**T37-7A** Seizure Prediction: Evaluation of a Combination of Prediction Methods
*H. Feldwisch genannt Drentrup, B. Schelter, J. Nawrath, J. Wohlmuth, A. Brandt, A. Schulze-Bonhage and J. Timmer, Freiburg*

**T37-8A** Learning Functional Connectivity in Neuronal Cultures
*T. Gürel, K. Kersting, S. Kandler, U. Egert, S. Rotter and L. De Raedt, Freiburg*

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**T37-12C** Implementing the Belief-Propagation Algorithm with Networks of Spiking Neurons -An approach based on Liquid-State-Machines

*A. Steimer and RJ. Douglas, Zuerich (CH)*

# Fundamental filter properties of spiking neurons constrain detectability of communication signals in weakly electric fish

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Many experimental studies demonstrate a match between the transfer function of sensory neurons and the statistics of natural stimuli. In particular it was found that the neuron's transfer function is optimal for a given stimulus statistics in terms of transmitted information. Both the neuron as well as the the statistics of stimuli, especially the ones of communication signals, may have evolved to maximize information transmission. However, neurons might not be able to adapt to any given stimulus statistics due to very basic constraints. We present data and simulations from the electroreceptors of the weakly electric fish *Apteronotus leptorhynchus* demonstrating such a constraintt. The time constant of spike-frequency adaptation in the electroreceptors sets the cutoff frequency of a highpass filter. Due to this filter property the firing rate response of electroreceptors is large for "small chirps" (a communication signal) and small for beats (arising from the superposition of the electric field of nearby fish) whose frequency is below the high-pass filter's cutoff frequency. Therefore this cutoff frequency limits the range of beat frequencies on which the chirp might be detectable. Behavioral studies on the emission of chirps supprot this finding. Why is the adaptation time constant not shorter, and thus the cutoff-frequency higher, in order to enlarge the region of beat frequencies on which the receptors respond with a high firing rate to the chirps and a low firing rate to the ongoing beat? Our simulations demonstrate that the effect of decreasing the adaptation time constant on the neuron's filter properties is counteracted by the low-pass filter properties arising from spikes and the mean firing rate. Thus, given the typical firing rates of the electrorecptors the adpatation time constant is already as short as possible and the region of beat frequencies on which small chirps can be detected as large as possible. This example demonstrates that using spikes for transmitting information imposes fundamental constraints on encoding specific stimuli.

# Contribution of interneuron properties to the generation of theta oscillations in the rat hippocampal formation in vitro

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Oscillatory neuronal activity is a phenomenon widely found in the central nervous system. In the hippocampus a particularly prominent discharge pattern with slow oscillations in the theta frequency band (4-10 Hz) has been reported. The dynamics of large-scale neuronal activity patterns depend on network and single cell properties. The reliability and temporal prescision of single neuron responses to time-structured inputs in turn depends on its membrane properties. Passive membrane properties together with a time-dependent ionic conductance may give rise to resonance and thereby membrane potential oscillations. These single neuron oscillations may contribute to the generation of an network wide theta rhythm.

Indeed, such subthreshold resonances were described in the theta-frequency band in the entorhinal cortex and in hippocampal principal cells. To investigate whether resonance phenomena exist in hippocampal interneurons, whole-cell patch-clamp experiments were performed in the current-clamp configuration from CA1 interneurons in acute rat slices.

The cellular membrane potential response to sinusoidal current injections was used to characterise the neuronal impedance as a function of the stimulation frequency. Preliminary analysis indicates that at physiological temperatures a considerable variability of the frequency-impedance relationship exists. Age of the animal, ion channel equippment and interneuron cell type seem to be major determinants.

# Memory Lifetime Depends on Synaptic Meta-Plasticity and the Size of Cell Assemblies

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Changes of synaptic states are generally thought to underly learning and memory. The storage of new memories and the associated synaptic changes necessarily impair previously acquired memory traces, and the smaller this impairment the larger is a network's memory lifetime. Memory, however, is not only defined by longevity but also by the susceptibility to new contents. Two strategies are feasible to keep old memories from being overwritten too rapidly: (1) Introducing synaptic meta levels [Fusi et al., 2005] to prolong memory lifetime by storing the history of synaptic state changes, and (2) reducing the number of cells that fire together in an assembly to decrease the interference between memory traces. Since, however, the postsynaptic depolarization depends on both the amount of presynaptic activity and the synaptic states, synapse models cannot be considered independently of the size of synchronously active cell assemblies.

We derive memory lifetimes in a randomly-coupled recurrent network [Leibold & Kempter, 2006] with synaptic meta-plasticity. Via a simultaneous optimization of assembly size and synaptic complexity, we find the maximum memory lifetime to coincide with a high level of sparseness in a simple two-state synaptic model. If the sparse-coding limit is unfeasible, synapses with a high number of meta states can be beneficial. We discuss two alternative synaptic cascade models with binary weights and find that a serial topology of synaptic state transitions gives rise to larger memory capacities as compared to a model with cross transitions. The optimal number of synaptic states for a serial model topology grows much faster as a function of the network size and connectivity than for the cross transition model. For both cascade models of synaptically stored memories, however, sparseness outweighs the virtues of meta-plasticity by orders of magnitudes. As an example, we derived memory lifetimes for parameters corresponding to the hippocampal CA3 region of rats, where assemblies have been estimated to contain few thousands of neurons. In this system a sparser code could be prohibited by requiring dynamical stability of the replay of sequential activity patterns [Lee & Wilson, 2002]. The evaluation of both meta-plasticity models in a CA3-like parameter regime yields a maximal lifetime of about 7,200 subsequent memories at an optimal cascade order n=2 for the model with cross transition, and a lifetime of 13,500 memories at n=3 for the serial topology. We thus conclude that low cascade orders are likely to be helpful to increase memory longevity in the hippocampus.

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# HOW NEURONS COULD SWITCH FROM AN INTEGRATOR TO A RESONATOR AND CHANGE THEIR SYNCHRONIZATION PROPERTIES

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Spiking neurons can be classified as integrators and resonators according to the bifurcation type of the transition from rest to periodic spiking. Integrators have a Saddle-node bifurcation while resonators a Hopf bifurcation, resulting in an S-shaped or monotonically increasing steady-state current(I)-voltage(V) curve, respectively. Further, the Phase Response Curve (PRC) of integrators is non-negative, i.e. perturbations in the input always advance the timing of the following spike. The PRC determines the synchronization properties of (weakly) coupled neurons. The bifurcation type of a neuron and thus its PRC and synchronization properties can be changed by altering the properties of the ionic currents, like the maximum conductances (set by the number of channels), the reversal potentials, the position and slope of the steady-state gating variables. We analyse for each such parameter its possible influence on a transition of an integrator to a resonator, by looking at the derivative of the steady-state I-V curve of conductance-base neuron models. The membrane capacity and the time constants of the gating variables have no influence on such a transition. Passive currents can switch an integrator to a resonator by increasing their conductance. For currents with a single gating variable we show analytically under which conditions the conductane and the reversal potential enable a transition. We treat the parameters within the gating variables numerically and show that the midpoint potential has an essential influence. For two gating variables within one current we show that the overlap of both is crucial for the transition from integrators to resonators or vice versa. We then demonstrate on a specific example model that changing the bifurcation type of a neuron also changes the synchronization behavior of coupled neurons.

# A model for object detection in the visual system of the fly

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Detecting objects is an important task when behaving in a natural environment. Flies, for example, land on small objects or avoid collisions with them. The neuronal basis of these behaviours is not clearly unravelled so far. However, the cells of the Figure Detection Cell ensemble (FD-cells) in the third optic lobe are likely to be involved in controlling these behaviours because of their preference for small objects moving relative to the background. As an underlying neuronal mechanism a lateral inhibition (center surround structure), as known from the mammalian retina, was excluded. Another inhibitory mechanism working with nonlinearities in the input circuitry of the FD-cell shows some preference for small objects [1]. However, this mechanism has never been tested against new experimental findings, such as evidence for a dendrodendritic interaction between an inhibitory neuron and the FD-cells and the dependence of the FD-cell on object and background velocity.

What mechanism can account for these experimental constraints? To answer this question, we modelled an FD-cell as a passive one-compartment model integrating retinotopic visual input with a response amplitude proportional to stimulus velocity. Two different input organisations were tested against each other.

The first takes the dendrodendritic input of the FD-cell from the inhibitory neuron into account and approximates dendritic spread of the local dendritic motion input by spatial low pass filtering. Thus, the retinotopic distribution of the inhibitory signal, although spatially blurred, is preserved until it reaches the FD-cell. In the second input organisation, the FD-cell receives inhibitory input after complete spatial pooling of the motion information. Therefore, spatial information is lost in the inhibitory signal.

Parameter optimisation revealed an advantage of the dendrodendritic structure and of the spatially distributed computation. Only with this input organisation both the sensitivity of the FD-cell for small objects and its dependence on object and background velocity could be mimicked. The model responses are within the range of variability of the experimentally determined neuronal responses.

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# Neuronal Tension May Co-Shape V1 Orientation Maps

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In recent years neuronal tension was shown to influence the gross shape of brain areas affecting for example cortical folding. This is because neurites have mechanical tension, elastic behavior, shorten slightly when not under tension, release tension under sustained stretching, and elongate actively at high tension [3]. How such forces influence structures at the microscopic scale, such as orientation maps in the primary visual cortex, is not established. In a model with realistic inter-neuronal connections as a function of orientation difference, a typical orientation map minimizes total wiring length [1].

Here we start with an unsorted set of V1 edge detectors, learnt via sparse coding from natural images. They are connected by horizontal connections, learnt as an autoassociator network [4], which are Mexican-hat shaped in feature space of orientation and topography. This associator network, aimed at noise removal, produces realistic orientation tuning curves and complex cell properties. Here we optimize the neurons' positions to minimize the lengths $d_{ij}$ of the lateral connections between neurons *i* and *j* weighted by their strengths $w_{ij}$. This yields a cost function $\sum_{i,j} |w_{ij}| d_{ij}$. An additional term $-\sum_{i,j} \ln d_{ij}$ repels the neurons from collapsing into one point.

In the Figure each little square is the receptive field of one V1 neuron. As a result of shifting the neurons along the cortical surface they arrange topographically, and group together if they code for similar orientations and frequencies. Our model predicts decreased neuron density at fractures in feature maps.

There are other physical/geometrical influences establishing a topographic LGN-V1 projection [2], and there are information theoretical accounts also for orientation maps. Wiring length minimization may coincide since the weights reflect correlations. We conjecture that small horizontal movements of cell bodies balance differential forces acting from different sides to reach equilibrium, hereby influencing the final map properties. Even though large horizontal movements of developed cortical cells are not reported and may be impossible, time lapse videos often show extensive remodelling of axons and dendrites, which might allow small shifts of a cell body over the course of days. Future work may consider incompressibility of axonal/dendritic volume and implications for rodent barrel cortex.

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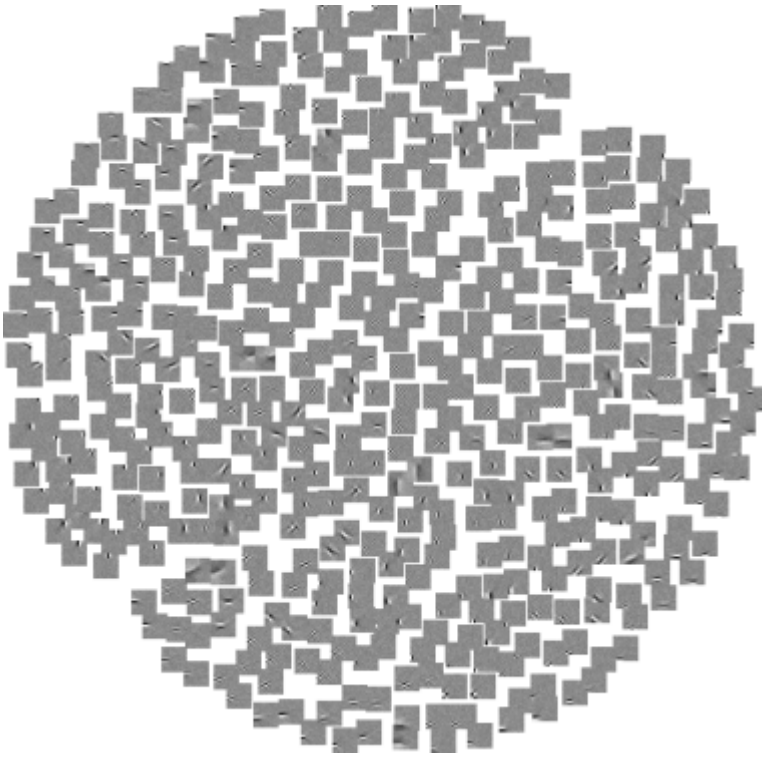

# Seizure Prediction: Evaluation of a Combination of Prediction Methods

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Concerning the assessment of the predictability of epileptic seizures, continuous and reliable analysis of neurophysiological recordings of epilepsy patients is necessary. Recent work has indicated that methods from non-linear dynamics can detect significant preseizure changes in the EEG at least for some patients. To increase seizure prediction performance we tested whether or not a combination of different seizure prediction methods yields better prediction results than each method alone.
In this study a bivariate phase synchronization index (PSI) and the univariate “Dynamical Similarity Index” (SIM) were adapted for seizure prediction (Mormann et al. Physica D 2000;144:358-369 and Le Van Quyen et al. NeuroReport 1999;10:2149-2155). The analysis of these prediction methods was based on long-term intracranial EEG data with continuous recordings of 4 patients of up to 10 days (mean 8.5) including 70 seizures. A software framework was developed for an online evaluation of these continuous data. Approaches to combine methods were applied and compared to the individual methods. All results were validated by a statistical test procedure (Schelter et al, Chaos 2006; 16: 013108).
By evaluating the EEG data continuously and without knowledge of ‘future’ events, a prediction situation analogous to an online application was possible. For a fixed set of parameters, significant prediction results could be observed for few patients by evaluating each method individually, whereas both assessed types of combinations lead to significant improvements for most patients. These findings indicate that the combination of prediction methods represents a step forward to clinical applications of seizure prediction.

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# Learning Functional Connectivity in Neuronal Cultures

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Discovering the functional connectivity and modelling the dynamics of neuronal networks is essential to understand neural information processing. In the current work, we focus on neuronal cultures, which are small living networks in a closed system. We present a machine learning approach, which constructs the functional connectivity map of a neuronal culture based on multiple spike trains of its spontaneous activity recorded by Multi-Electrode-Arrays (MEA). The spike train of an electrode is modelled as a point process, where the rate depends on the finite spike history of all electrodes. For a similar model, Chornoboy et al. presented a maximum likelihood approach for learning the parameters offline. To capture the network plasticity, however, we follow a steepest descent approach, which naturally allows for online learning. A ROC curve analysis of our experiments shows that this online approach predicts the upcoming spiking activity well.

# A model for correlation detection based on Ca2+ concentration in spines

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Understanding the mechanisms of correlation detection between pre- and postsynaptic activity at a synapse is crucial for the theory of Hebbian learning and development [3] of cortical networks. The calcium concentration in spines was experimentally shown to be a correlation sensitive signal constrained to the spine: A supralinear influx of calcium into spines occurred when presynaptic stimulation preceded a backpropagating action potential within a short time window. Its magnitude depended on the relative timing $t_{post}$-$t_{pre}$ [1,5].

There is strong evidence that NMDA receptors are responsible for the supralinear effect [1]. Previous simulation studies related the occurrence of spike time dependent plasticity to the calcium signal [2,4]. However, these simulations mainly focused on pairs and triplets of pre- and postsynaptic spikes, rather than on irregular activity.

Here, we investigate the properties of a biologically motivated [1,5] model for correlation detection based on the calcium influx through NMDA receptors under realistic conditions of irregular spike trains containing a fraction ε of events correlated in time. We identify the regime (rate, correlation coefficient, detection time) in which this mechanism can assess the correlation between pre- and postsynaptic activity. A computationally effective implementation usable for large scale network simulations is devised. We find that a simple thresholding mechanism acts as a sensitive correlation detector, that can be adjusted to work robustly at physiological firing rates.

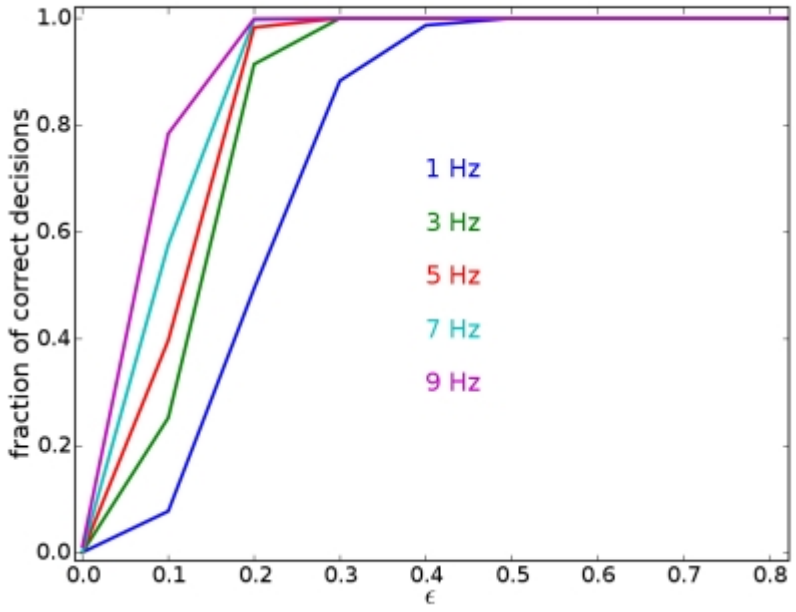


Figure: Fraction of trials where correlation is detected as a function of the correlation coefficient. Firing rates are identical for the pre- and postsynaptic Poisson spike trains. Simulation results are based on 1000 trials of 30s duration each.

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# Adding structure to *in vitro* neuronal networks

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The relation between the computational properties and the connectivity in neuronal circuits is not well understood. While discussions on such structure-function relations mostly focus on cortical tissue with its prominent layered structure, its complexity limits theoretical and experimental analyses as well as control over structural and/or functional aspects of the underlying neuronal circuits. Simpler and generalized neuronal networks would allow manipulations and matching simulations, thus enabling the verification of theoretical assumptions and the test of predictions of neuronal computation. This would facilitate a bottom-up approach to understanding the functional architecture of natural neuronal networks.

Networks in dissociated cortical cultures are such generic networks. They do not develop predefined anatomical structure or predictable connectivity in first place. Moreover, microengineering techniques enable the design of patterned cell culture substrates with cell-adhesive and cell-repellent areas. On such substrates, the adhesion of neurons can be restricted and the outgrowth of neurites can be influenced. If cultured on planar microelectrode arrays (MEA), the activity dynamics in these networks can be monitored continuously.

In our work, we use this approach to modulate the connectivity statistics of networks in culture to identify general principles of structure-function relations. Tissue from the prefrontal cortex of neonatal rats was dissociated and cultured on 60-site MEAs and glass coverslips. The substrate surfaces were patterned with the covalently linked polymers polyethylene imine (PEI; adhesion-promoting) and the polydimethyl acrylamide (repellent). In contrast to other common patterning techniques, photolithographically designed structures are stable for weeks under serum-enriched medium conditions, enabling long-term studies of the network dynamics in MEA recordings.

On these patterns, cell somata and neurite outgrowth can be restricted to the nodes of the adhesive PEI patterns; the probability for cell connections between nodes is limited to the pathways linking them. We compare the electrophysiological properties of structured and random-like networks. This approach enables the investigation of the principles of structure-function dependencies in very basic but structured neuronal circuits.

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# Eigensystems and dynamics of complex networks of excitatory and inhibitory neurons

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The coupling structure of local cortical networks and its impact on the ongoing dynamics are still to a large degree unknown. Still, modeling efforts can help to understand basic features of how underlying structure shapes network activity.

We analyzed the impact of different coupling schemes on the dynamics of networks of integrate-and-fire neurons with topologies that display random, lattice or 'small-world' characteristics. Special emphasis is laid on networks with distance dependendent coupling which may serve as models for a 2-dimensional layer of cortical tissue.

To describe those systems, we apply both graph theoretical methods as well as numerical network simulations with the goal to find critical structural parameters to describe the features of the systems on a statistical level.

In particular we found that the distribution of weights is much more important than the underlying mere topology (i.e. "who's connected to whom"). The assumption that all synapses of a neuron can only be either inhibitory or excitatory, but not both at the same time leads to synchronization phenomena and spatio-temporal pattern formation that is not captured by existing mean-field approaches. Moreover, we show that minimal models for networks of inhibitory and excitatory neurons must be 2-dimensional to describe the full spectrum of activity dynamics, which occur in the according numerical simulations.

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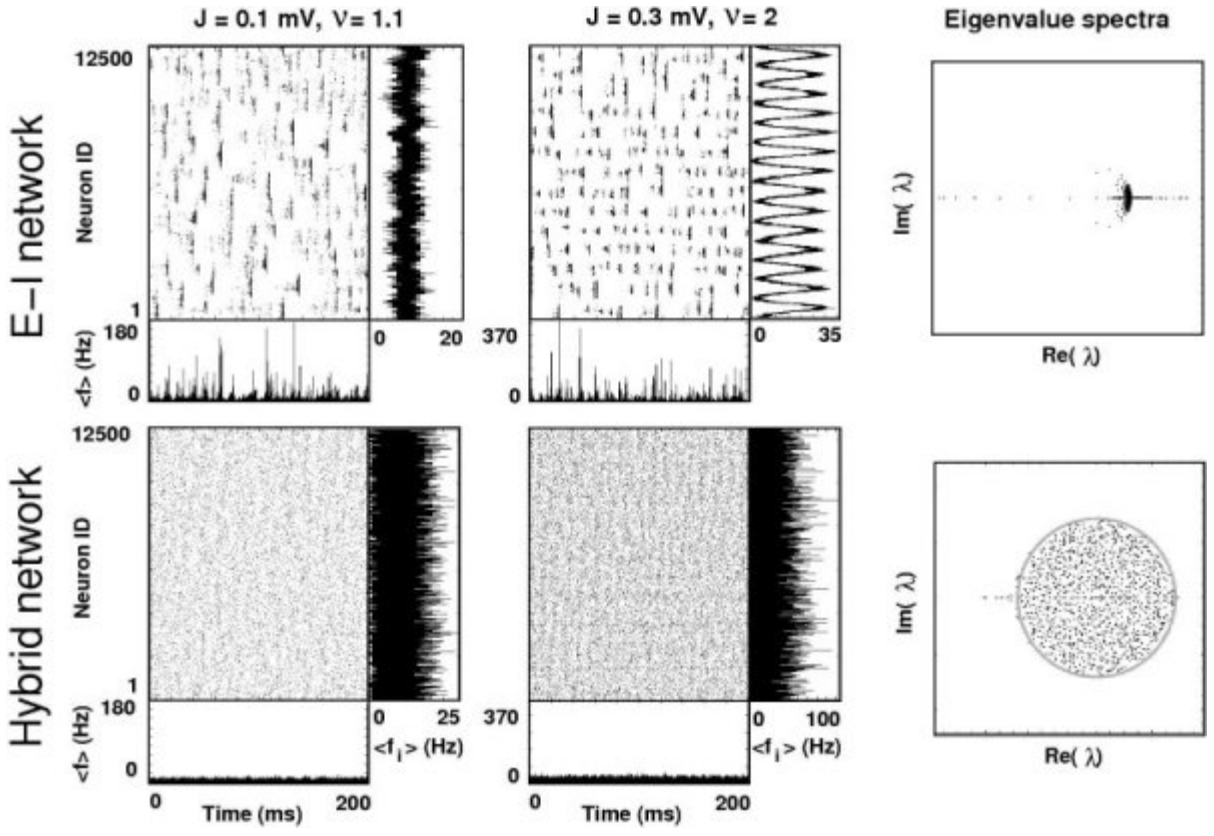
Caption: Numerical simulations of 12,500 integrate-and-fire neurons living on a ring topology, and qualitative eigenspectra of the respective network coupling matrices. The first two pictures in each row show raster plots, population rate <f> and rate per neuron <f_i> of networks where neurons are either purely hyper- or depolarizing, whereas in the hybrid case neurons can be both. This leads both to random dynamics and nearly random eigenvalue spectra.



# Modeling the dynamics of higher-order correlations in feed-forward networks

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Correlated spiking activity has been observed in various brain areas. While it is an open issue whether correlated spiking is relevant for information processing, it has been shown that correlations in the input ensemble can significantly influence the response properties of individual neurons. In this context, Kuhn et al. (2003) emphasized that in particular the higher-order correlation structure is critical. To this end, they introduced the Multiple Interaction Process (MIP) as an example model of correlated spike train ensembles. Its higher-order statistics is fully determined by only two parameters: the average firing rate and the pairwise correlation.

In this study we examined if neural network dynamics can be reproduced when MIP is used as a phenomenological model of spike patterns with higher-order correlations. We focused onto simple feed-forward networks (synfire chains) where highly synchronous spike patterns gradually emerge from an asynchronous state. We demonstrate that this synchronization process can be qualitatively reproduced if the input spike train ensembles are replaced by MIP. However, as important features of the spike patterns emerging in the chain cannot be captured by MIP and extended versions, the emulation of the dynamics exhibits several artifacts. In particular, the correlation build-up is significantly overestimated (even for small correlations) -- a fact that might limit the applicability of MIP also in other contexts.

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# Spotting higher-order spike patterns with low-order measures

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The cell assembly hypothesis (Hebb, 1949) postulates dynamically interacting groups of neurons as the building blocks of cortical information processing. Synchronized spiking across groups of neurons was later suggested as a potential signature for active assemblies. In this scenario, the assembly members would exhibit specific higher-order spike correlations. Although mathematical concepts for the detection of higher-order correlations have been suggested in the past (e.g. Nakahara & Amari, 2002), statistical estimation of the associated parameters from spike train recordings poses serious problems, mainly due to constraints induced by too small samples. As a consequence, most attempts to directly observe cell-assemblies resort to pairwise interactions. However, such approaches obviously cannot draw any conclusions about the joint operation of larger groups of neurons.

Here we present a novel procedure to spot higher-order patterns in massively parallel spike trains that circumvents the need to estimate a many higher-order parameters. Currently, our method works for correlated Poisson processes, where correlations of any order are induced by `inserting' appropriate patterns of near-synchronous spikes (see illustration below). Based on estimates of only a few low-order cumulants of certain compound signals we can devise a test for the presence of higher-order patterns in the original data, which is surprisingly sensitive. As a consequence, our method is less susceptible to limitations in sample size and can, in principle, be applied to data commonly recorded in behaving animals using multi-electrode technology. The sensitivity and reliability of the new method for data, where the Poisson assumptions are not strictly satisfied, will be critically discussed.

*Acknowledgments*
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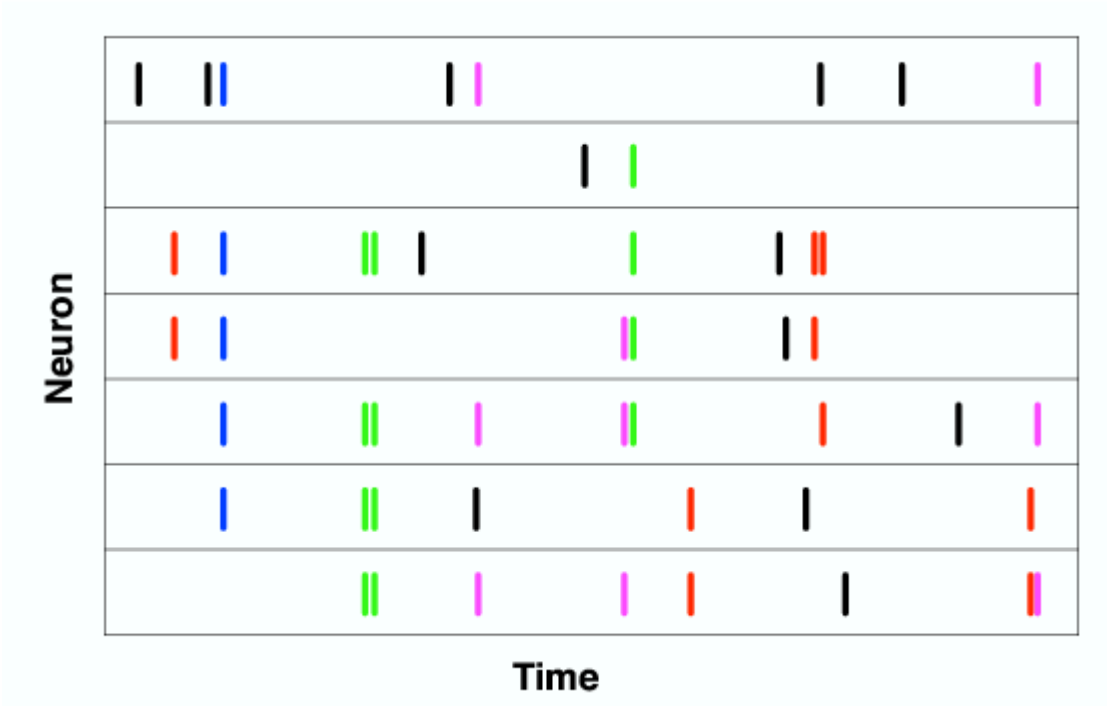

# Time scale dependence of neuronal correlations

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Neural activity is processed and measured at various signal levels. Neural systems update their states by evaluating for example synaptic conductances, synaptic currents or membrane potentials. Experimenters additionally use spike counts, local field potentials, EEG or fMRI BOLD signals to quantify the state of the system. Correlated activity has been observed at all these signal levels in different areas of the brain and has been suggested to provide valuable information about the architecture of the underlying system and the nature of neural processing. Many of these signals can be considered as filtered (superpositions of) spike trains. It is largely unknown how this filtering alters the correlation structure of the underlying spike data and what the consequences for the dynamics of the system on the one hand and for the interpretation of measured correlations on the other hand are.

In this study we focus onto signals derived from general spike signals (point processes) by linear filtering (shot-noise). As a prominent example of spike correlations we consider those caused by overlapping presynaptic neuron populations (common input correlations) in different network models. We study the effect of the marginal statistics of the presynaptic sources and of the filter kernel on the (joint) statistics of the resulting shot-noise signals both analytically and numerically.

We demonstrate that the interaction between the original spike train auto/cross-correlation structure and the filter kernel generally results in a complex dependence of second-order measures like variances, covariances and correlation coefficients a) on the statistics of the presynaptic spike trains and b) on the filter properties. Spike count correlation coefficients, for example, generally depend on the length of the counting window if the spike trains have non-Poissonian statistics. Similarly, correlations between intracellular signals like synaptic currents or membrane potentials depend on the time constants of the synapses or the membranes. Further, we show that changes in the marginal statistics of the presynaptic sources (e.g. the frequency of network oscillations) can effectively modulate the strength of correlation between postsynaptic responses. We propose that both mechanisms may be used in the brain to modulate the interaction strength between neurons or neuron populations. Finally, we show that for a large class of spike processes the high frequency components of the coherence (which does not dependent on the linear filter properties) reflects the strength of common input, irrespectively of the marginal statistics of the sources. In our network models it therefore allows a reliable estimation of the network connectivity from intracellular recordings of pairs of neurons.

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# Cortical networks with long-range patchy connections

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Most studies of cortical network dynamics assume completely random wiring, a practical but simplistic approach: The cortex has both a delicate columnar and laminar structure, e.g. featuring a combination of local and long-range connections. As the architecture of a complex network is presumably an essential determinant of its functions it is our aim to design and analyze more realistic network models of the cortex.

We embedded all neurons into a 2D space to enable distance dependent connectivity that reflects the geometry of dendrites and axons. We considered pyramidal cells with both neighborhood coupling and long-range connections, the latter arranged either in a random fashion or as clustered projections [1]. As shown the figure, three different spatial settings for the patchy projections (disks) of pyramidal cells (dots) were distinguished: Randomly and independently selected positions (left), overlapping patches for neighboring cells (middle), and a shared patches model (right).

An important difference between the various model scenarios for long-range connections is the amount of common input or output, respectively, asigned to any pair of neurons. This was quantified by a detailed input/output projection field analysis. Additionally, stochastic graph theory was used to characterize other global structural features of these networks, and to compare them to well-known types of abstract random graphs, like the small-world network.

Our approach of combining neuron geometry and network topology allows us to devise new models for cortical networks with horizontal structure, including the feature of patchy projections. We expect that our investigations will help to interpret neuroanatomical data, and thereby contribute to improve our understanding of cortical function.

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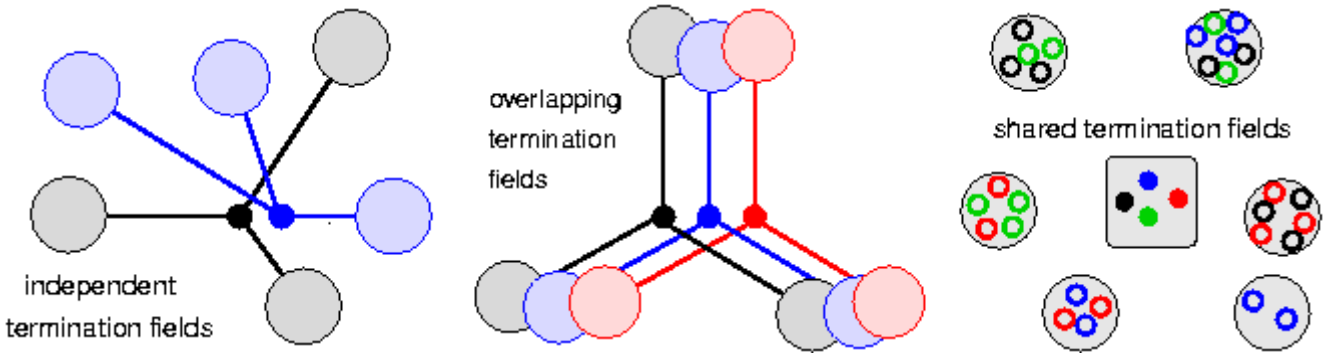

# Timing Errors in a Dynamical Model of Temporal Processing

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Processing of temporal information in the range of milliseconds to seconds is crucial for the perception of stimuli changing in time and the precise coordination of movements. Two main classes of models of these phenomena can be identified by specific experimentally provable predictions. However, a series of experiments performed at the BCCN Goettingen is not fully consistent with any of the existing models and justifies thus the formulation of a new approach.

We present a neuronal model that provides an explanation for the errors that are made in the estimation of intervals and their dependence on the length of the interval. The model consists of a population of synfire chains of equal length, but different propagation speeds, which are able to convert the temporal information about a stimulus into a quasi-spatial code. Consistent with psychophysical findings, timing errors in the model increase approximately linearly with the interval length (Weber's law) over a range of durations, while outside this range, substantial deviations from Weber's law are observed. Both phenomena result from an optimization process which selects those chains producing the minimal error for the each interval. The optimization is implemented by STDP learning of the projections onto the output layer. Furthermore, we discuss the effect of interval-selective training in the context of the presented model.

# Anticipative adaptive control of muscles using recruitment

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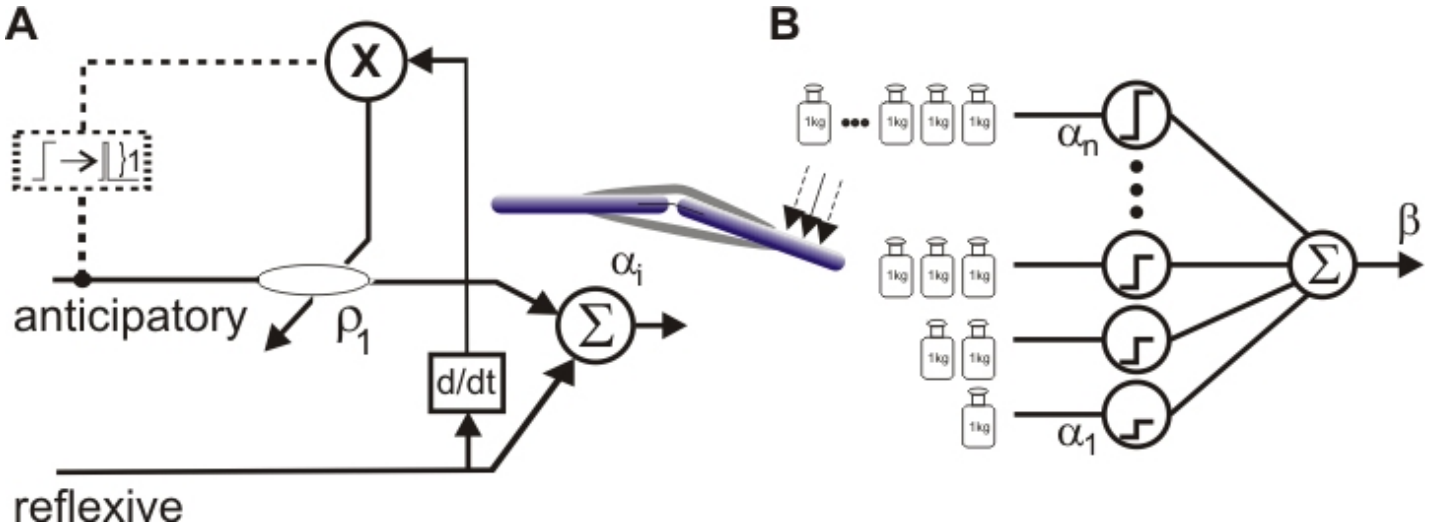
Virtually all robots control movements in a kinematic fashion using stiff joints. These controllers are insusceptible to perturbations. However, if one wants to design humanoid robots whose motions resemble those of humans one needs to find more adaptive control systems that are able to handle compliant joints.

We, as humans, use muscles. With them we can not only control movements but also maintain a fixed position. This is called homeostasis. Homeostasis is often disturbed either by external forces or by changing body postures. E.g. the moving of the forearm alters the torque of the shoulder joint. The external forces often vary and therefore the muscles have to compensate by changing the degree of contraction.

This mechanism is called recruitment and in this work we modified an adaptive neural controller (see figure part A) that bears this task-dependent change of muscle strength (see figure part B). For our simulations and for our real robotic device we use not only one actuator to control the wanted position but two antagonistically operating ones. The difference in force regulates the position and the force itself the stiffness. Imitating humans we use a simplified muscle model with an antagonist and a protagonist actuator.

During learning an anticipatory signal is correlated with a reflexive reaction. An upcoming disturbance triggers the anticipatory signal and the reflex compensates the external force and moves the arm to the desired position.

This paper shows that the muscles combined with the recruitment mechanism are able to keep perfect homeostasis within a few (about 10) learning trials and the behavior is stable even under a variety of loads.



# Image processing in the snake infra-red sensory system.

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Two groups of snakes possess an infrared detection system that is used to create a heat image of their environment. We present an explicit reconstruction model, the "virtual lens," which explains how a snake can overcome the optical limitations of a wide aperture pinhole camera, and how ensuing properties of the receptive fields on the infrared-sensitive membrane may explain the behavioral performance of this sensory system. Our model explores the optical quality of the infrared system by detailing how a functional representation of the thermal properties of the environment can be created. The model is easy to implement neuronally and agrees well with available neuronal, physiological, and behavioral data on the snake infrared system.

# Modelling the subcortical nuclei of the limbic system to perform reversal learning

Maria Adedoyin Thompson[1], Bernd Porr[1], Florentine Woergoertter[2] and Lynsey McCabe[1]

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We have developed a model of the subcortical nuclei of the limbic system. We argue that these nuclei are sufficient to perform simple reversal learning where associations between food and place can be used to model disappointment. For example a rat has to learn an association of finding food in a left compartment of a cage. After a while, the food is moved to the right compartment of the cage. The rat has to unlearn the original association of the food to the left hand corner of the cage so as to learn the new association [1]. We argue that for such a simple form of reversal learning the subcortical nuclei of the limbic system are sufficient: the nucleus accumbens (shell and core) and the ventral tegmental area (VTA).

In our model the Nacc core is implemented as a simple decision maker which is in accordance to other models of the basal ganglia. New is the circuitry around the Nacc shell which implements reversal learning. We argue that the shell associates context information from the hippocampus with food rewards [2]. Plasticity in the NAcc is believed to be controlled by the coincidence of three factors namely pre- post- and dopaminergic activity. When a reward has been encountered, the VTA releases bursts of Dopamine (DA) which enables plasticity in the NAcc core and shell. While the shell learns the association of the reward with, for exmaple, a place cell, the core performs the actual motor learning which guides the animal to the food. In the reversal condition only the inhibition from the Nacc shell to the VTA is active while no bursting is triggered because of the lack of reward. This causes a dip in DA activity and unlearning of the sensor-motor association occurs in the core.

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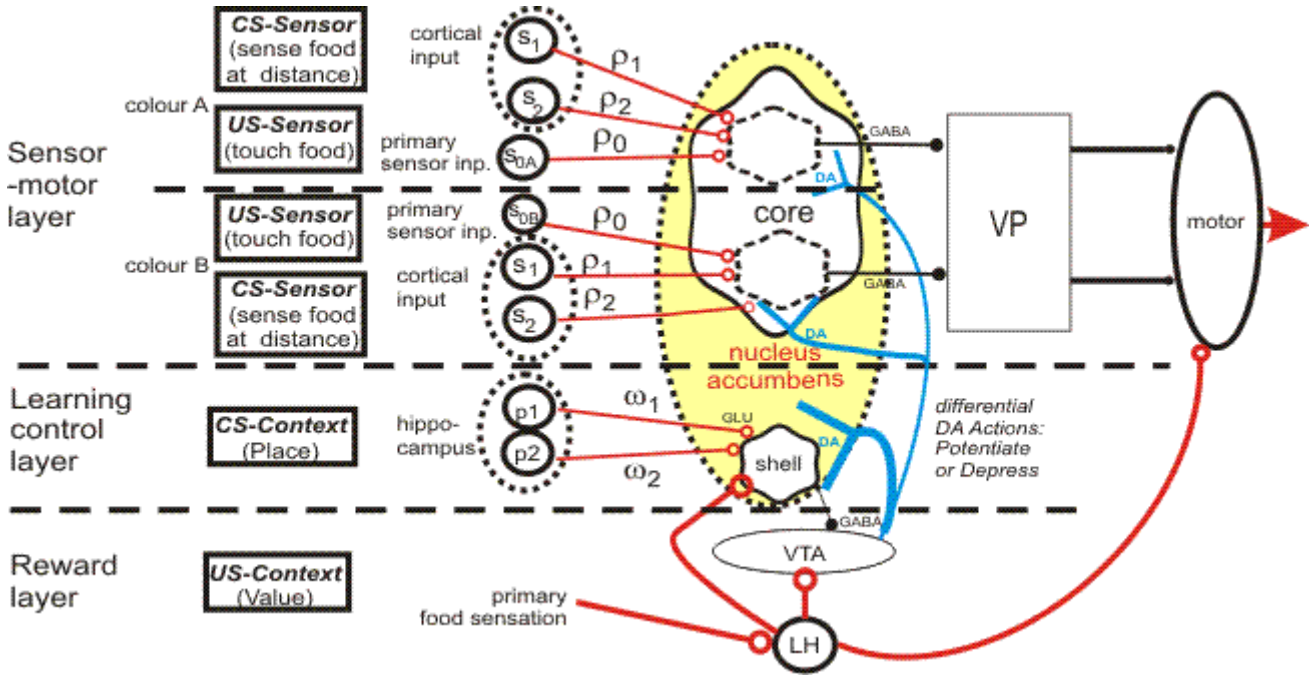

# Development of place fields and goal navigation in a closed loop scenario

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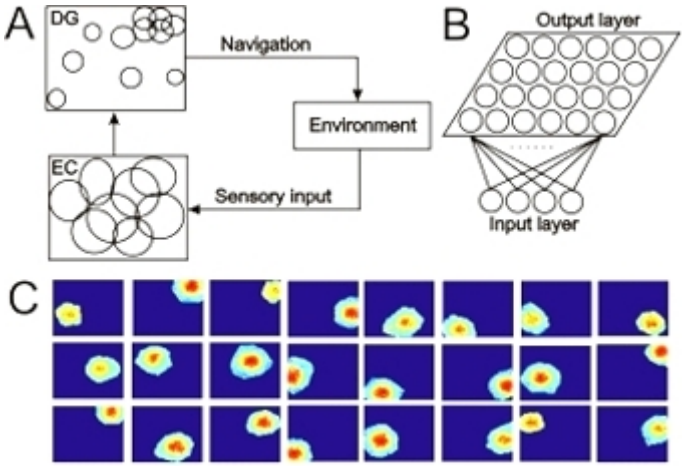
Experiments on rats show that that visual cues play an important role in the formation of place cells. Nevertheless, rats also rely on other allothetic non-visual stimuli such as auditory, olfactory and somatosensory stimuli. Most researches have seen navigation in the dark as evidence for the importance of path integration as an additional input to place cells. However, Save et all (2000) have shown that olfactory information rather than self-motion information has been used to stabilize the place fields (PF) of rats in the dark. It has also been observed that PF representation density varies in rat hippocampus depending on a goal location (Hollup at al, 2001). We address these findings by modeling olfactory information-supported varying density place cells and using them for goal navigation in a closed loop behavioral scenario.

In a model we develop PFs in the Entorhinal Cortex (EC) from external (visual and olfactory) cues as well as self generated (urine marking) cues (panel A). In the Dentate Gyrus (DG) exploration dependent PFs are obtained through Hebbian learning where denser representations are created at more frequently visited locations. Obtained PFs are used for goal navigation by ways of the Q-learning algorithm. Sensory inputs as well as place cells are affected whenever the rat navigates in the environment, thus closing the loop.

We use a fully connected feed-forward network (panel B) to create place cells where initially random connection weights W are used. Features X derived from visual and olfactory cues are fed to the input layer and the best matching unit is found at each time step according to minimal Euclidian distance: $\text{argmin}(\|X_t - W^i_t\|)$, where i=1..N, is the number of cells in the output layer. We update weights by $W^i_{t+1} = W^i_t + \mu(X_t - W^i_t)$, where $\mu$ is a learning rate, $\mu \ll 1$. Firing rate of place cell is calculated as following: $R^i_t = e^{-(\|X_t - W^i_t\|)^2 / 2\sigma^2}$, where $\sigma$=0.1. An example of PFs is shown in panel C and we observed that slightly less directional cells were obtained by using slow learning in comparison to the place fields obtained without learning. We have also obtained that use of olfactory information reduces the number of directional cells.

In this study we have shown that formation, remapping of PFs and goal navigation by ways of simple model is possible in a closed loop scenario. We also emphasize utility of olfactory cues and density of representation.

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# Neuronal Control and Learning for Adaptive, Fast Dynamic Walking of the Biped Robot "RunBot"

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The robot system "RunBot" has been developed during the last four years [1]. It is created on the basis of three different levels:

***Physical level :*** The biomechanical design of RunBot has some special features (see Fig. A), e.g. small curved feet and a properly positioned center of mass allowing it to perform dynamic walking during some stage of its gait cycles [1].

***Reflexive level :*** A low-level neural structure (see Fig. B) generates dynamically stable gaits. The reflexive network for controlling leg-motor neurons ($EM_L$, $FM_L$) is driven by signals coming from ground contact sensors ($G_{L,R}$), stretch receptors ($A_{L,R}$) and extensor as well as flexor sensors (ES, FS), while an accelerometer sensor (AS) is used to trigger the body reflex via the body-motor neurons ($EM_B$, $FM_B$). Changing some neural parameters of the network can regulate walking speed of RunBot and adapt its locomotion to different terrains [1].

***Adaptive level :*** The network can learn using mechanisms of simulated synaptic plasticity emulating the idea of learning to avoid a long-loop body-reflex. The learning goal is to finally avoid the reflex and thereby learn to also change gait parameters in an appropriate way as well as control the posture of its body (UBC) to prevent RunBot from falling during walking on different terrains (see Fig. D). This requires an adaptive network (see Fig. C) of six neurons (L1,...,6) which converge onto different neurons at the reflexive network effectively changing their activation parameters. This paper shows that Runbot is an adaptive dynamic walking machine which doesn't use any trajectory control, but instead fully relies on its two neuronal control levels.

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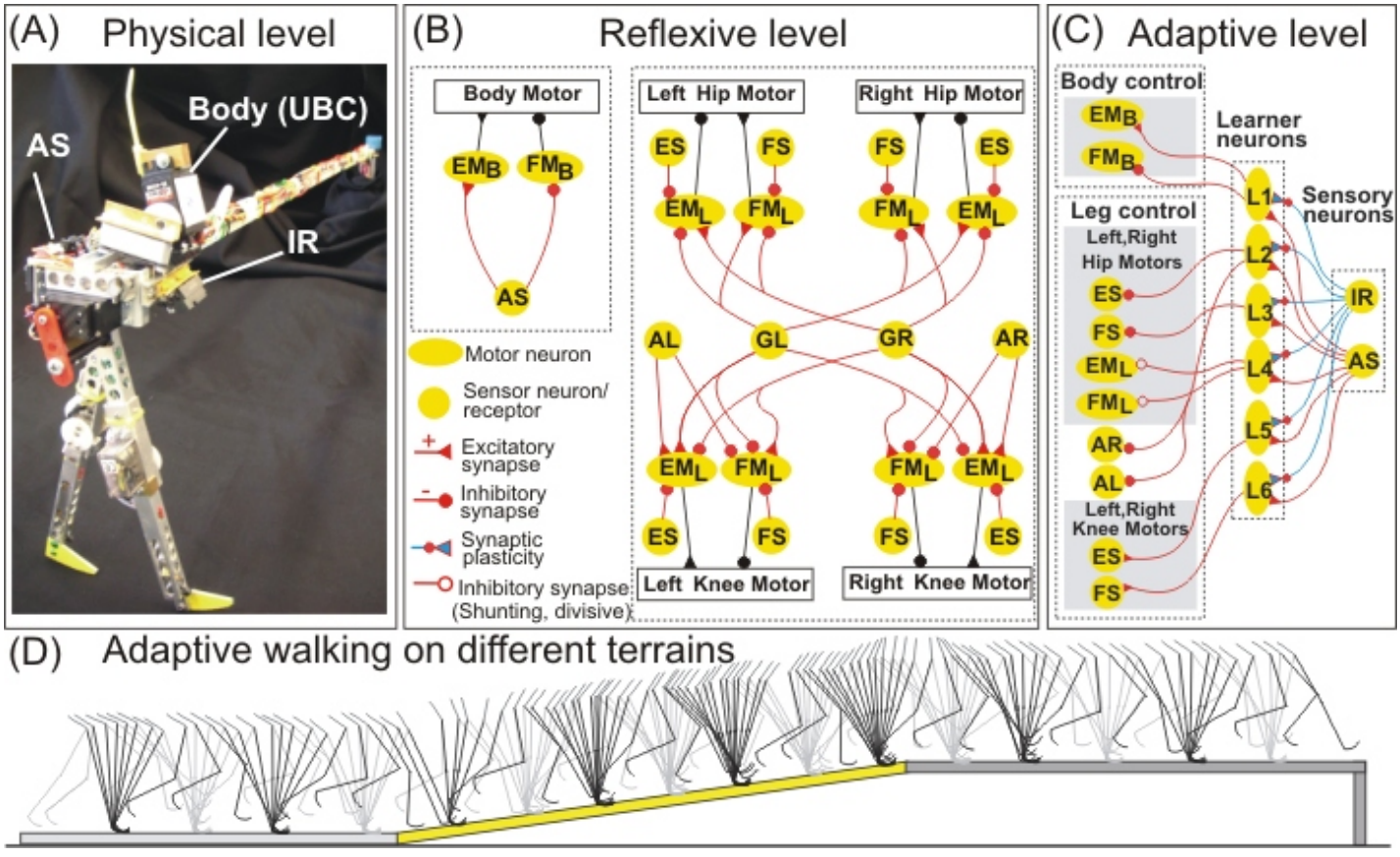

# Modelling schema generation and stochastic adaptation in goal directed arm movements

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Subjects 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. A schema may arises from a population of interacting neurons probably in the cortex.

Assuming two 2D-layers of interacting neurons, one layer consists out of excitatory neurons, with activity $A_e(x)$ in position x=[x1, x2] and one layer out of inhibitory neurons, with activity $A_i(x)$. The four synaptic weight-functions between the neurons are defined by $w_{ee}$, $w_{ei}$, $w_{ie}$ and $w_{ii}$ which represent the spatial spread of synaptic interconnections. With respect to a given distance d=|x-x'| the weights are monotonically decaying and have a Gaussian distribution of different width. The change of the activity $A_e$ and $A_i$ depend on mutual interaction between the neurons, determined by the synaptic weights and the nonlinear sigmoid activation function of each neuron, respectively. Thus we obtain a recurrent neural network eliciting complex cortical dynamics (Wilson and Cowan 1973). In terms of neural field theory this results in a partial differential equation which can be interpreted in simpler form as reaction-diffusion equation or in a more general form as integro-differential equation which may elicit static or dynamic patterns in a population of neurons.

Normalizing the excitation by global inhibition a point like stimulus causes a local bifurcation of the network by showing a stable 'bubble' (a local neural activation) of Gaussian shape. Depending on the asymmetry of the excitation spread function this 'bubble' may walk around with different speed and in different directions, respectively. Given a certain trace of activated neurons in a third layer, which only influences the asymmetry of the excitation spread function, the 'bubble' should be attracted towards this activation trace. Close to the activation trace the 'bubble' remains within a certain potential wall, like being in a 'gutter'. Assigning a certain attractiveness to a selected goal the centre of the 'bubble' may run along the activation trace, eliciting a certain sequence of joint torques and thus giving each motion primitive a certain direction.

The activation trace may represent the current movement of the hand in the workspace. However, not in terms of position but in terms of movement direction during a short time interval of a perception-action-cycle a motion primitive is generated. This is in agreement with population coding in motor cortex M1. The representation of the motion trajectory has to be mapped to the workspace. A controlled planar arm system is simulated and the resulting trajectory causes a modified or new activation trace in the neural net. Introducing a cost function repetitive arm movement modifies the activity trace and a movement schema can be learned. Corresponding with recent observations it can be shown that noisy hand trajectories become straight hitting a fixed goal. Applying different optimization criteria schemata of curved trajectories are learned which support the control of hand movements strongly.

# THE EVOLUTION OF BIOINFORMATICS AND COMPUTATIONAL BIOLOGY AS APPLIED TO SPINAL CORD INJURY RESEARCH

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Spinal cord injury can cause irreversible damage of the central nervous system leading to partial or complete loss of motor function, and is a leading cause of paralysis. Currently various cell, gene, and pharmacological therapies are being combined in order to minimize the damaging effects of secondary injury following the initial lesion.

A systems biology approach to the complexity of spinal cord injury allows for the use of interdisciplinary tools and analysis methods to understand an intricate system and its possible repair. Employing computational methods to established biological hypotheses has the potential to greatly advance research in this field and clarify existing experimental paradigms with computational models and tools.

This study will review various contributions of computational based projects as applied to spinal cord injury research from robot-assisted motor rehabilitation to data mining projects that categorize and identify possible proteins which can contribute to nerve regeneration. Additionally, this study will review how these combined studies have progressively evolved and how they have the potential to ultimately provide a standard in biomedical research and neurobiology.

# Investigating Stick Insect Extensor Muscle Properties: An Experimentally Based Muscle Simulation

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Muscles are the crucial link between nervous system activity and animal movement. To understand the neuronal basis of behaviour - in particular walking - it is important to sufficiently understand the muscle perfomance. For the stick insect extensor tibiae muscle, detailed insights in its properties have been gained recently [1]. It produces graded and relatively slow contractions ( 5mm/sec at maximum activation) and its tetanical force can add up to 200 mN. During the early part of activation, the contraction amplitude rather depends on spike number than on frequency. As a consequence of this spike number dependence variations in frequency do not alter the contraction slope in this early phase [2]. As many properties are additionally activation dependent, the complexity of the investigated muscle exceeds intuition for all but the most trivial situations. Two interesting types of questions can be addressed by implementing a computer model of the muscle: A) How do certain muscle properties effect the performance of the neuro-muscular transform? And b) : How does the nervous system cope with the complexity and ambiguity of the muscle properties?

Based on detailed experimental data, we describe the overall process of creating a muscle model, starting with experimental design through data aquisition and analysis ending with modelling and evaluation. We introduce in more detail the modelling of crucial muscle properties like: Force-length and force-velocity dependencies, series and parallel elasticity as well as activation dynamics.
At present we are working on a comparison of the model performance for artificial and physiological [3] stimulation. Model forces are evaluated using data from isometric experiments and the resulting movements, calculated by forward dynamics, are compared with isotonic and kinematic experimental data.

The presented muscle model should later work inside a real-time 3D dynamic simulation of the stick insect and provide the interface between simulated neuronal activity and the movements of the limbs [4].

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# Adaptive feedback inhibition supports learning of representations in a recurrent network of spiking neurons

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Our visual system enables us to discriminate between similar visual objects as well as to recognize objects under different viewing conditions. These capabilities require both discrimination of similar retinal inputs and generalization across different retinal inputs. Could the underlying neural mechanisms be learned during visual experience? Here we investigate the possible role of adaptive inhibitory feedback connections during learning of representations in a network of spiking neurons. Such feedback could be used to suppress the activity of non-informative neurons, thus enhancing the impact of informative neurons and the discrimination performance of the network.

The network consists of two layers of spiking neurons with connections that were adapted according to local spike-timing dependent plasticity (STDP) learning rules. Excitatory feed-forward connections from the input layer to the output layer are adapted by a Hebbian learning rule. Output neurons mutually inhibit each other via lateral inhibitory connections. In addition, we introduced inhibitory feedback connections from the output layer neurons to the input layer neurons. These connections are adapted by an Anti-Hebbian learning rule. We trained the network using simple geometric input patterns. We systematically varied the similarity of the stimuli and quantified discrimination performance of the network during and after learning.

We compared discrimination performance of the network both with and without feedback inhibition. When the stimuli were sufficiently dissimilar, the network reached a state where all stimuli could be discriminated perfectly under both conditions. However, with feedback inhibition, learning was much faster (up to 60% reduction of learning time to reach a specified level of performance). When stimuli of higher similarity were used for training, the network without feedback inhibition failed to reach perfect discrimination performance for stimuli with more than 75% overlap in input space and performance dropped gradually with increasing stimulus similarity. In contrast, with feedback inhibition the network reached perfect performance for stimulus overlap up to 88%.

We showed that inhibitory feedback connections can improve the ability of a neural network to learn selective representations even for highly similar stimuli. With additional temporal delay, this feedback inhibition could be used as mechanism for predictive coding strategies. We speculate that the microcircuitry of visual cortex, with feedback connections from layer 2/3 neurons to layer 6 neurons, which in turn project to inhibitory neurons in layer 4, could be used to implement such a feedback mechanism.

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# Synchronous activity and tonic to bursting transitions in a noisy neurons

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Synchronization of neurons is essential for many processes in the nervous system, ranging from the processing of sensory information to the onset of pathological conditions such as Parkinson disease or epilepsy. We examine phase synchronization effects in a system of two electrically coupled Hodgkin-Huxley like neurons in different dynamical states: tonic, chaos and bursting with different types of transitions between them. We use applied current as a control parameter to tune each neuron. Different types of synchronization: out of phase, chaotic and in-phase, can be observed in dependence on coupling strength, regime of spike generation and initial conditions. Out of phase and chaotic regimes always coexist with in-phase. As a remarkable result, neurons immideately in-phase synchronize in the bursting regime at a transition from chaos.
To bring our model closer to physiology we have added white Gaussian noise. In the areas where two or more synchronous regimes coexist noise induces switching between them. In the same time out of phase regime is very sensitive to noise, while chaotic and in-phase looks resistant. As a conclusion we can say that neurons (mostly in bursting regimes) can be driven from out of phase or chaotic synchronization to in-phase not only with changing of coupling strength but also with tuning of applied current. And in the presence of noise they can be easily driven back: from in-phase to chaos, using the same parameters, what is not possible in deterministic case.

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# Stimulus induced activity propagation in a layered cortical network model

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The local network of sensory cortices is reported to be organized vertically in six layers which show a high degree of heterogeneity in neuron properties, synaptic connectivity and degree of recurrence. Thalamic input, arriving in layer 4, travels through layer 2/3 down to layer 5 and 6, and closed a loop by projections from layer 6 to the thalamus. Although, anatomically well described, it is not fully understood how activity entering the system in layer 4 interacts with the ongoing recurrent activity and is eventually transferred to other parts of the network. The weak synapses (~0.1 mV) in the neocortex in vivo, further complicate the flow of activity, as any individual synapse is not able to elicit a spike in its post-synaptic neuron on its own, and it has to depend on the recurrent activity.

To understand the evolution of stimulus driven activity in the cortical layers, we simulated a layered network comprising of 200,000 neurons, mimicking about 4 $mm^3$, volume of cat cortex. The network connectivity was taken from Binzegger et al. (2004). In a previous work we described the emergent dynamical states of the layered network (Kremkow et al. 2006). Here we focused on evolution of input activity in an asynchronous-irregular (AI) type background activity state.

We stimulated the layer 4 either with AI type activity at varying frequencies, or with a synchronous volley of spikes. We found that a synchronous volley of spikes was most effective in eliciting significant responses in all subsequent cortical processing stages (layer 2/3, 5 ,6). In contrast, uncorrelated asynchronous-irregular activity with same amount of spikes, was not able to drive the whole layered network. Our results are in good agreement with recently reported in vitro data (Bruno & Sakmann 2006). We conclude therefore, that a stimulus that is composed of synchronous activity is more effective in driving the cortex. Uncorrelated inputs require a rather high input firing rate which may render the network strongly precarious.

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# 3D Reconstruction of Neural Circuits from Serial EM Images

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The neural processing of visual motion is of essential importance for course control. A basic model suggesting a possible mechanism of how such a computation could be implemented in the fly visual system is the so called "correlation-type motion detector" proposed by Reichardt and Hassenstein in the 1950s. The basic requirement to reconstruct the neural circuit underlying this computation is the availability of electron microscopic 3D data sets of whole ensembles of neurons constituting the fly visual ganglia. We apply a new technique,"Serial Block Face Scanning Electron Microscopy" (SBFSEM), that allows for an automatic sectioning and imaging of biological tissue with a scanning electron microscope [Denk, Horstman (2004) Serial block face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLOS Biology 2: 1900-1909]. Image Stacks generated with this technology have a resolution sufficient to distinguish different cellular compartments, especially synaptic structures. Consequently detailed anatomical knowledge of complete neuronal circuits can be obtained. Such an image stack contains several thousands of images and is recorded with a minimal voxel size of 25nm in x and y and 30nm in z direction. Consequently a tissue block of 1mm³ (volume of the *Calliphora vicina* brain) produces several hundreds terabyte of data.
Therefore new concepts for managing large data sets and for automated 3D reconstruction algorithms need to be developed. We developed an automated image segmentation and 3D reconstruction software, which allows a precise contour tracing of cell membranes and simultaneously displays the resulting 3D structure. In detail, the software contains two stand-alone packages: Neuron2D and Neuron3D, both offer an easy-to-operate Graphical-User-Interface.
Neuron2D software provides the following image processing functions:
• Image Viewer: Display image stacks in single or movie mode and optional calculates intensity distribution of each image.
• Image Preprocessing: Filter process of image stacks. Implemented filters are a Gaussian 2D and a Non-Linear-Diffusion Filter. The filter step enhances the contrast between contour lines and image background, leading to an enhanced signal to noise ratio which further improves detection of membrane structures.
• Image Segmentation: The implemented algorithm extracts contour lines from the preceding image and automatically traces the contour lines in the following images (z-direction), taking into account the previous image segmentation. In addition, a manual interaction is possible.
To visualize 3D structures of neuronal circuits the additional software Neuron3D was developed. The reconstruction of neuronal surfaces from contour lines, obtained in Neuron2D, is implemented as a graph theory approach. The reconstructed anatomical data can further provide a subset for computational models of neuronal circuits in the fly visual system.

# Do leech mechanosensory neurons need multiple spike initiation zones?

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The distribution of voltage-sensitive channels across the membrane of a neuron can have severe impacts on the cell's information processing qualities. In particular, the use of multiple sites of spike-initiation could enable a neuron to perform several computational tasks simultaneously based on local integration of signals.

Leech mechanosensory neurons (T-cells) were proposed (Burgin & Szczupak, J Comp Physiol A, 2003) to have two distinct spike-initiation zones. These cells send information about tactile stimulation of the skin towards the soma, which is located several millimeters away in a ganglion of the central nervous system. Based on electrophysiological experiments, Burgin & Szcuzupak proposed that a peripheral spike initiation zone signals touch stimuli, while a central one processes synaptic inputs within the ganglion.

We use a compartmental model of a T-cell (fig 1, dotted lines depict long-range processes of 5mm length) to test this hypothesis and to systematically analyze the effects of ion channel distribution on the spiking behavior. In particular, we compare a model with a homogeneous channel distribution (model A) to a model with "hot spots" of high Na+-channel density and moderately active dendrites (B) and a cell with "hot spots" and weak dendritic Na+-conductance (C). For each of these hypotheses, we determined the parameter set according to two criteria. 1) Several experimentally observed response properties had to be reproduced. 2) The parameter set had to minimize the total number of Na+-channels, because we assume this property also to minimize the energy consumption of the cell.

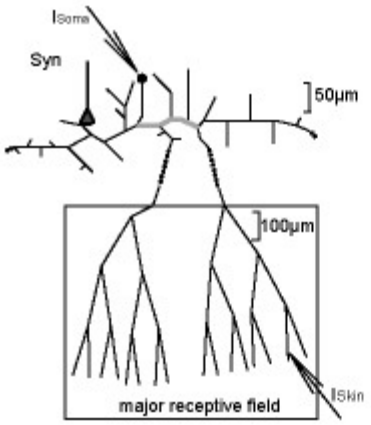


Figure 1

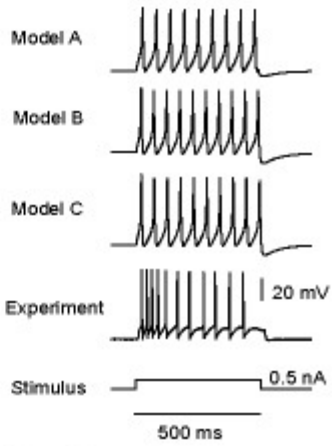


Figure 2

All three models reproduce experimentally determined responses to peripheral skin stimulation, to somatic current injection, and to central synaptic input equally well (fig 2, adaptation was not accounted for to keep the model simple). Moreover, all of them agree in their prediction, that different kinds of inputs trigger spikes at different locations. However, the models B and C, which assume a "hot spot" near the soma require a lower total number of Na+-channels. A small, highly active membrane patch allows the considerably larger rest of the membrane to have a lower Na+-conductance and, hence, can help to save metabolic energy.

In contrast, assuming "hot spots" of Na+-channels in the peripheral processes does neither improve the simulation results nor reduce the total Na+-conductance for any of the models. The reason for this finding is, that the long-range process can act as spike-initiation regions, because they have to be equipped with a high number of Na+-channels to guarantee long-distance signal propagation.

Hence, we conclude that "hot spots" of high Na+-channel density are dispensable in the periphery, while a centrally located spike-initiation zone is favorable.

# Subsymbolic planning of behavior in a four-arm-maze

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By now, many successful attempts have been made to implement sensory processing in subsymbolic systems, while planning of complex behavior is still a domain of symbolic systems. Inspired by biological example, here we try to design and implement a system handling both aspects in a homogeneous architecture (König & Krüger 2006). For the investigation of this subject we have chosen a navigation task, as the system's state can trivially be read out by determining position and orientation of the agent and a wealth of literature for comparison is available.

A Khepera robot, equipped with a camera to discriminate cues and proximity sensors to measure distances to object, navigating in a four-arm-maze environment was chosen to perform the task. For representation of the environment place fields (PF) were used, which can be learned in a similar setup (Wyss et al. (2006) PLoS). The robot learned about the environment by connecting these PFs with different actions which were randomly chosen. This experience behavior was coupled with an obstacle avoidance behavior, referenced as reflexes, redounded to the actions the robot intended to do is not necessary successful, because this actions was interrupted by bumping against the boundaries of the setting. Experiencing the setting in a manageable time 70 PF were chosen, each covering a rather large space. Navigating to a goal then could be achieved by choosing the action leading the robot with the highest probability to the goal.

While the robot was able to navigate to any goal within the setting, on average 11.4 PF were needed to pass to the goal. On average, the length of the path chosen was 76% larger than the shortest possible one. The spatial structure of the PF can account for 16% of this increase, corresponding to 8.7 PF. However, the lengthening of the robot path mainly resulted from the obstacle avoidance behavior. To account quantitatively for these effects, we distinguished between two processes, the proximal action planning and "distal reflexes", which were triggered by the obstacle avoidance behavior. Looking at the probabilities of the different actions on a state leading it to a goal, these probabilities are more spars distributed taking the discount factor of the reflexes into account, measured an entropy of 0.65% of the maximum entropy for a PF with a lot of reflexes, than not, obtained an entropy of 99%. After changing the learned environment by adding an obstacle, the system was able to adjust the best path for the robot and to pass the obstacle, on average, after 16 attempts to reach the goal. This demonstrates sustained plasticity of the architecture.

We have implemented a model capable to solve a goal-finding task in a four-arm-maze environment in a subsymbolic way. This can be achieved rapidly by learning the features of the setting and by exploiting distal reflexes to reduce the complexity of behavioral planning.

# Dynamics in the layered cortex: A comparison of experiment and model

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The formation of six distinguishable layers is a fundamental organization principle of neocortical tissue. Even though thickness and cell density of different layers may vary across brain regions and species, their cell types and basic connectivity features are highly conserved. This suggests that the six-layered structure of neocortical tissue provides a functionally versatile framework that can be optimized for a wide range of computational demands. To better understand the working principles of the layered neocortical structure it is important to study its activity dynamics. Available experimental techniques, however, do not yet allow us to observe neuronal population activity at a fine spatio-temporal scale and it is desirable to have a realistic model of a layered network, in which the vast parameter space is constrained by experimental data.

Taking advantage of the latest developments in network simulation techniques (Morrison et al. 2005) and incorporating anatomical data (Binzegger et al. 2004), we developed a layered model of the neocortex, comprising 200,000 spiking neurons (Kremkow et al. 2007). The model reproduces basic features of ongoing neuronal activity in the neocortex: stable asynchronous-irregular activity and realistic layer-specific firing rate distributions. In the present work, we tuned the network parameters to fit in vivo data from layer-specific multi-electrode recordings in the rat visual and somatosensory cortices in different activity regimes: (i) spontaneous state transitions comparable to those observed during slow wave sleep (Suchanek et al. 2007), (ii) asynchronous-irregular activity as observed during quiet wakefulness, and (iii) responses to visual stimulation (Boucsein et al. 2007). Preliminary results suggest that a certain degree of heterogeneity in passive properties of neurons and in synaptic weights helps to stabilize the activity in the network. In addition, when we restricted the mean firing rates to <1 spike/s, a large fraction of neuron remained 'silent', matching recent experimental results. We intend to enhance the biological plausibility of the model with the help of further in vivo recordings, in particular using combined intra- and extracellular recordings. In the long run, we expect to improve on our model and our understanding of cortical dynamics in vivo by such repeated iterations between modeling and experimentation.

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# Implicit Wiener Series for Estimating Nonlinear Receptive Fields

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The representation of the nonlinear response properties of a neuron by a Wiener series expansion has enjoyed a certain popularity in the past, but its application has been limited to rather low-dimensional and weakly nonlinear systems due to the exponential growth of the number of terms that have to be estimated. A recently developed estimation method [1] utilizes the kernel techniques widely used in the machine learning community to implicitly represent the Wiener series as an element of an abstract dot product space. In contrast to the classical estimation methods for the Wiener series, the estimation complexity of the implicit representation is linear in the input dimensionality and independent of the degree of nonlinearity.

From the neural system identification point of view, the proposed estimation method has several advantages:

1. Due to the linear dependence of the estimation complexity on input dimensionality, system identification can be also done for systems acting on high-dimensional inputs such as images or video sequences.

2. Compared to classical cross-correlation techniques (such as spike-triggered average or covariance estimates), similar accuracies can be achieved with a considerably smaller amount of data.

3. The new technique does not need white noise as input, but works for arbitrary classes of input signals such as, e.g., natural image patches.

4. Regularisation concepts from machine learning to identify systems with noise-contaminated output signals.

We present an application of the implicit Wiener series to find the low-dimensional stimulus subspace which accounts for most of the neuron's activity. We approximate the second-order term of a full Wiener series model with a set of parallel cascades consisting of a linear receptive field and a static nonlinearity. This type of approximation is known as reduced set technique in machine learning. We compare our results on simulated and physiological datasets to existing identification techniques in terms of prediction performance and accuracy of the obtained subspaces.

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# A neuronal model for the detection of thresholds

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A common problem in neural systems is the detection of threshold firing frequencies. For example, the responses of the nervous system to sensory stimuli are often elicited when the sensory input crosses certain threshold amplitudes. In these situations, the nervous system relies on information provided by its sensory systems, which are usually spike mediated. Known problems of accurate information readout by the second layer neurons are the limited information content of the sensory neurons and variable cellular and synaptic properties of the involved neurons, like cell input resistance and synaptic strength. While these issues usually apply to specific systems only, our investigation is concerned with a more general problem which is inherent to information processing in all neurons, namely the integration of synaptic inputs.

We used the femur-tibia (FT) joint control system of the stick insect as a model for testing the accuracy with which the nervous system is capable of eliciting transitions in motor output when sensory input crosses a certain threshold. The FT network controls the transition from stance to swing phase of the leg during locomotion. Once the leg reaches its posterior extreme position and the tibia is stretched above a certain position, the swing phase starts. Sensory information about the position and velocity of the leg movement is provided by a single sensory organ, the femoral chordotonal organ.

We developed a computer simulation based on the known network structure (Straub et al., 2004, Modelling and Simulation '2004, ESMC) in which the increase of tibia position was represented as a linear increase of sensory firing frequency (Büschges, 1994, J Exp Biol 189). We applied position stimuli with two different, but constant leg velocities to test how accurately the network is capable of predicting the correct transition point at different velocities.

We found that the proposed network was unable to maintain a specific transition position when different leg velocities were used. With higher velocities, the transition occurred at higher positions (corresponding to more extended legs). This effect was not due to a limit of the information content of the sensory neurons, but rather caused by the integration of synaptic inputs in second layer interneurons. We show that this is a general problem in postsynaptic neurons caused by the history-dependent information processing in these neurons.

Since the FT-joint control system, and most likely all nervous systems, are capable of detecting thresholds, we propose that network-based solutions exist that circumvent history-dependent effects and thus provide a more accurate readout of sensory information. Currently, we are working on the development of a neuronal model that enables the FT-joint control system to more faithfully elicit transitions from stance to swing during walking.

# A local circuit model of the primate frontal eye fields (FEF) area controls eye movements in a visual search task with saccades and anti-saccades.

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Primates can flexibly change their pattern of eye movements according to the visual input. For example, in a visual selection task a monkeys can select one of four targets that differs from the others in color. Depending on the shape of that target they made either a saccade towards it or an anti-saccade away from it. Hence, the pattern of the eye movements depends not only on the visual saliency of the stimulus which was highest for the target. An abstract rule of how to move the eyes was associated with the shape of the stimulus. Responses of single cells in the frontal eye fields (FEF) of awake behaving monkeys are correlated to the visual selection of the singleton as well as the selection of the endpoint of the saccade or anti-saccade [1].
Little is known about how the FEF transforms the input it gets from visual areas into a motor signal in such a flexible way. The local structure of neurons and their connections provides the anatomical substrate for this computation. This 'canonical' cortical microcircuit was extensively studied only in cat visual cortex [2,3]. Nevertheless, the uniformity of neocortex makes it possible to infer the local connectivity pattern of the FEF [4].
Here we present a local circuit model that extends the idea of a cortical microcircuit to the function of area FEF. The network of integrate and fire neurons has a layered structure that was inferred from known anatomical results. The strength of connections was then tuned in order to achieve the functionality of the FEF as observed in many experiments. The network is able to make single saccades to a visual target. It can also scan a visual scene and it is able to perform more complicated tasks as the saccade vs. anti-saccade task in which the type of eye movement depends on the feature of the singleton in an array of targets.
The dynamics of the model reproduce well the dynamics of neurons observed in the FEF and the behavioral results match those observed in psychophysical experiments. In particular, the responses of the neurons in different layers are similar to different types of cells described in [1]. By being biologically realistic, the model allows us to compare the simulation results to single cell electrophysiology as well as to the behavioral results. In addition, the detailed layered structure of the model predicts the likely structure of the anatomical microcircuit of the FEF.

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# The microstructure of patchy lateral connectivity

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Bulk injection of anterograde and retrograde tracers into the superficial layers of the neocortex produces a distinctive labelling pattern of both neuronal somata and axons. This pattern, which can be observed in many areas of cortex, involves regularly spaced patches of label radiating from the injection site. When examined in tangential section, the rosette structure of this pattern is visually evocative of the petals of a flower, prompting this system to be named the "Daisy Architecture" [Douglas & Martin, 2004]. It is commonly assumed that this system relates to a like-connects-to-like functional constraint on the long range lateral connections of pyramidal neurons [e.g. Mitchison & Crick, 1982]. However, evidence for this constraint is rather weak even in the spatially periodic functionality of visual cortex.

In order to understand the neuronal origin of the large-scale patch structures, described in a way that is relevant to any cortical area, we have simulated the transport of label in a field of neurons, subject to various assumptions on the spatial patterns of connection of the neurons. We define geometric models for individual axonal arbors, and then generate a "sheet" of simulated cortex that represents a tangential slice through the superficial layers. We simulate injections of tracers, and compare the resulting labelling patterns with those observed in vivo.

On the assumption that transport is not trans-synaptic, and is independent of neuronal activity, we find that bulk injections into a sheet of neurons with an homogenous overall distribution of connectivity (as implied by a like-connects-to-like constraint and a spatially periodic functional map) should not give rise to periodic patches of label, even if individual neurons have varying and anisotropic axonal arbors that form only a few periodic clusters of boutons. This is because the bulk injections label individual neurons in every possible phase of spatial connectivity, so that the clustered contributions of their terminal boutons sum to a uniform labelling at the larger spatial scale of the injection.

Alternatively, when we assume that the neurons form overlapping periodic populations with two different patterns of connection (periodic clusters of boutons, and isotropic projections as observed near "pin-wheel" singularities [Yousef et al., 2001]), then our model predicts patches of label patterns similar to those observed in vivo following both focal and large injections of tracer. This result implies that locations across cortex in which neurons make isotropic, non-clustered projections may be a general feature of cortical areas in which the daisy architecture has been observed.

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# MECHANICAL PROPERTIES OF NEURITES FOR SIMULATIONS OF CORTICAL DEVELOPMENT

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We are developing a computer simulation of corticogenesis. At present, the simulation starts at the stage when the neuroblasts of the ventricular layer begin mitotic division and continues until their neuronal offspring have migrated centrifugally to form the cortical laminae. In future, we plan to extend the simulation to provide the neuronal morphologies and average connectivities observed in the visual cortex of the cat. We simulate the development quite literally: The neurons divide, migrate, and grow their axons and dendrites in a 3D space. They also secrete and respond to morphogens that diffuse through the space. Beside the implementation of these biological processes, it is also necessary to take into account the physical properties and interactions of the neurons. Using an approach similar to Finite Element Analysis, we represent a neurite as a series of springs joining point masses. This representation is justified because neurites do exhibit viscoelastic properties - with a spring constant of between 10 and 100 microdyn/micrometer (Denerll et al. 1988). The tension induced by this elastic property also plays important roles in development, because it is a stimulus for active elongation or retraction in neurites (Denerll et al. 1989), and it also determines their differentiation into axons (Zheng et al. 2002). We consider a branch of dendrite or axon to be a piecewise linear structure composed of cylindrical segments that enclose each spring. A similar wrapping, spherical or ellipsoidal, represents the cell soma. These cylindrical or spherical envelops are used to define a repulsive force when two objects attempt to inter-penetrate. The force is proportional to the volume of the overlap, and prevents two structures from inter-penetrating. The tension of a stretched neurite propagates along its spring chain. The total force acting on a segment then moves the local point mass according to the classical mechanics of a medium with strong friction. In addition, the increase (or reduction) of tension provides a signal for cell elements to evoke metabolic lengthening (or shortening) of the segment.

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# Implementing the Belief-Propagation Algorithm with Networks of Spiking Neurons -An approach based on Liquid-State-Machines

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In many real world situations living beings have to deal with incomplete knowledge about their environment and still have to be able to act reasonably. For example, think of a herbivore who sees just parts of a predator hiding in high grass. Based on this incomplete visual information, the animal has to infer the 'true' stimulus (the predator) in order to initiate an appropriate behavioral response (to flee).
A large variety of such problems, e.g. in the general framework of object recognition, can be described by so called 'graphical models' like Bayesian Networks or Markov Random Fields [Löliger(2004), Weiss&Freeman(2001)]. These models describe statistical relationships between a set of variables and give rise to algorithms computing probabilities about states of unknown variables based on the observed information. 'Belief-Propagation' is an efficient method for this task [Kschischang et al.(2001), Rao(2006)] and is also a potential candidate for a biological implementation in the brain, because it is entirely based on local information processing [Rao(2006)]. Computational units (the nodes of a graphical model) communicate with each other by distributing so called 'messages' exclusively to their neighbors in the graph.
Within this contextual framework, our working hypothesis is that the experimentally observed patches of synaptic boutons, prominent in layer 2/3 all over the cortex [Angelucci et al.(2002)], are a physical representation of nodes in a graphical model. At the same time we assume that a patch constitutes a canonical microcircuit of neurons which is able to calculate the Belief-Propagation message update equations.
For that, each patch is interpreted as a collection of 'Liquid State Machines' [Maass et al.(2002)] consisting of a common liquid-pool of recurrently connected neurons, and several readout units. Each combination of the liquid-pool and any readout, realizes a particular message signal transmitted from a node to one of its neighbors. Messages arriving at a node are all fed into the common liquid-pool, whose main task is to implement nonlinear projections of the low-dimensional input into a high-dimensional space [Maass et al.(2002)]. This operation is crucial for the Belief-Propagation algorithm which utilizes highly nonlinear message update rules [Löliger(2004)]. 'pDelta' learning [Auer et al.(2005)] is used for the supervised training of the readouts. It is based on populations of perceptron units, however it can also be applied to spiking neurons
when message signals are represented in space rate code [Maass et al.(2002)]. Therefore, our aim is to formulate microcircuits with a modular character, i.e. with the same input and output coding of spikes.

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# Poster Topic

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# Capturing Electrophysiological Waveforms with a Soundcard

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Modern soundcards are highly sophisticated hardware components that are designed to add sound capability to a computer. Apart from its capability to produce or record sounds, a soundcard can also be regarded more generally as a digital-to-analog (DA) and analog-to-digital (AD) input/output (IO) interface. Usually, soundcards are designed to cover the human audible frequency range from 20Hz to 20000Hz. The frequency content of extracellular recordings fits well in the same frequency range. Apart from this basic requirement soundcards show also advantages compared to commercial data acquisition boards. Since the main purpose of data acquisition boards is focused on data acquisition, output channels are sometimes neglected or offer only what is absolutely necessary. Modern soundcards usually offer high sampling rates up to 96 kHz and high resolutions of up to 24 Bit in input and output channels, which make them very attractive for electrophysiological research. Standard soundcards with two input channels are sufficient for most types of recordings. Soundcards with eight input channels are also available below 300€ which could make high quality multi-channel recording extremely well-priced. If more than eight channels are required, it is possible to cascade soundcards to achieve up to 32 channels.

Despite the fact that it is possible technically to use soundcards as electrophysiological recording device, there is no software available for the use of soundcards to my best knowledge. The lack of software is a big obstacle in using soundcards in electrophysiology. Although Software Development Kits (SDK) for addressing soundcards are available (e.g. DirectSound SDK as part of DirectX SDK), building a software application from the beginning can be very time consuming.

Here I present a software solution, which makes electrophysiological recordings with soundcards possible. Since it uses DirectSound as abstraction layer it provides another advantage. The abstraction layer allows the exchange of soundcards. Therefore this software is not restricted to a special kind of soundcard, which makes it very flexible and scaleable. The only requirement is that the soundcard has to support DirectSound. Beside the basic requirements of electrophysiological multi-channel recording the software offers a stimulus interface for the most common auditory stimuli. Additional auditory stimuli, not included in the stimulus interface, can be applied as wav-files. Software filter makes it possible to record and store original signals and apply the filter afterwards. Instead of using all input channels for electrophysiology one channel can also be used to monitor the heart beat of the experimental animal. Electrophysiological recordings can be stored binary or in ASCII file format which makes them available for offline analysis tools. A stimulus interface for visual stimuli will be provided in future versions of this software.

# Topographic extracellular multichannel recording in freely flying Barn owls on the basis of a new designed intelligent telemetric system

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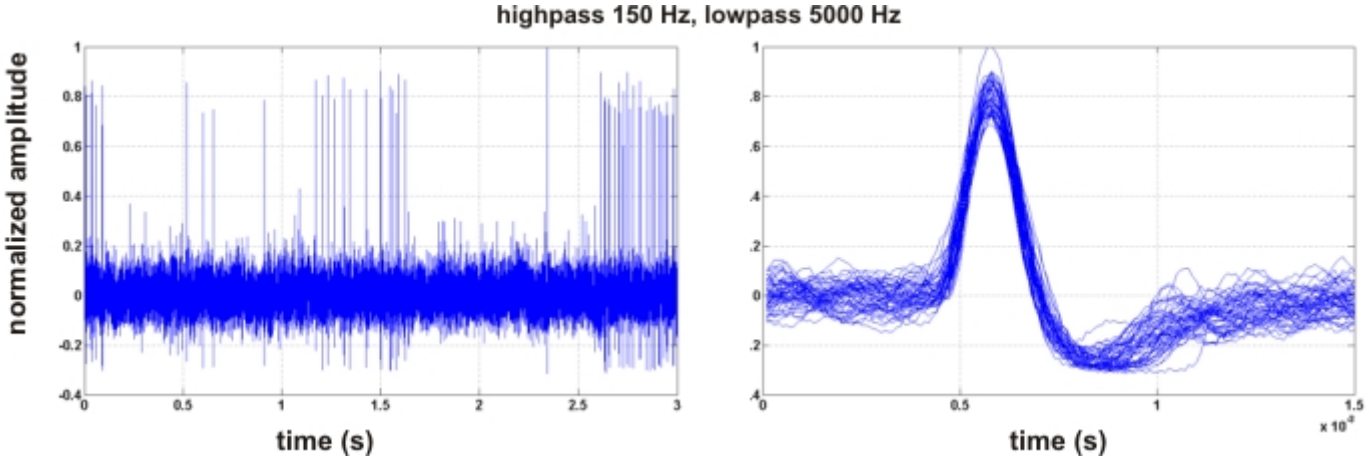
Barn owls are specialists in several aspects. Their sensory systems seem perfectly adapted to successfully hunt in almost complete darkness. Research has long since focused on their passive 3D sound localization capability. Recording from stationary birds and observing freely flying barn owls under experimental conditions have yielded valuable data. However, little to none is known on how the afferent auditory information is processed while the species is approaching a target. This work starts a first approach to combine electrophysiological and behavioral aspects and learn more about how this sensory information is processed while the owl "plans", "decides" and "executes" its homing behavior. For this type of experiment we develop a new extracellular recording technique. It is based on an intelligent telemetric system, which collects and preprocesses multichannel single unit recordings from chronic implants, while simultaneously recording position and head orientation data from the mobile owl in real-time. The aim for the final systems dimension is 1.5x1.5x1.5 cm@ 30 g of max. weight. The figure below illustrates first recordings of rat auditory midbrain unit by the analog sub-core of the device.



# Optimizing accuracy of dynamic clamp systems based on real-time Linux

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Optimizing accuracy of dynamic clamp systems based on real-time Linux

Dynamic clamp allows for inserting artificial conductances during intracellular recordings by controlling the amount of injected current in dependence of the recorded membrane potential. This technique can be used to study the role of ionic currents by either manipulating their properties or by adding new currents to the cell. Further, by simulating synaptic currents the recorded neuron can be placed within an virtual network of other neurons.

Existing approaches implement the dynamic clamp technique as a loop running on real-time Linux. Often, a set of ordinary differential equations modeling a single voltage-gated ionic current, a complete neuron, or even a network of neurons, is integrated during this loop. Following the standard design of periodic real-time tasks, the number of steps used for integrating the differential equation in each cycle of the loop is fixed. Thus, this approach does not make use of computing time that might be still available during a cycle in order to improve the accuracy of the computed result.

The aim of this work is to use this wasted computing time in dynamic clamp loops for improving the accuracy of the signal that is injected back into the neuron. This concept is known from real-time computing as IRIS (*increased reward with increased service*) task. We implemented this concept by adapting commonly used algorithms (Euler, Runge-Kutta) for integrating differential equations with increasing accuracy during each cycle as long as computing time is available.

Furthermore, the IRIS concept requires an adapted interaction between the dynamic clamp loop and the data acquisition hardware (DAQ). Former implementations perform the output of the signal inside the loop straight after finishing signal's calculation in time, i.e. before the succeeding recording operation is scheduled. In our approach, the described process of improving signal's accuracy has no discrete ending and thus has to be interrupted externally according to the deadline for the succeeding recording command. In order to still allow an output to be performed after this cut-off, we programmed the DAQ to take over time control over both, input and output. The dynamic clamp loop's task is now to provide the hardware with data being refreshed at every cycle. At output time, the DAQ chooses the most recent and accurate value for the signal to be injected into the neuron.

The achievements of this new approach have been evaluated by various benchmarks, showing the reached accuracy and performance.

# Synchrotron radiation based imaging of rodent and insect brains in 2D and 3D

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Metals and metalloids are involved in the production of free radicals, the causing factor for oxidative stress. However, metals and metalloids are also necessary components of specific antioxidant enzymes. The identification of metalloproteins and the determination of their cellular and subcellular distribution will provide valuable hints with regard to the functions and the biological role of the metals and metalloids in question. Metalloproteomics, i.e. proteomics focused on the metalloproteins, requires specific bioanalytical tools to identify these compounds and to study their distribution and biological functions.

A micro-synchrotron radiation X-ray fluorescence procedure (μ-SRXRF) was developed as such a bioanalytical tool. The procedure allows fast scanning of histological tissue sections with a focused X-ray beam and determination of the trace elements distributed among the brain areas by means of their characteristic X-ray emission. With new X-ray optics, a spatial resolution down to the micrometer range is achievable. The shown analyses were carried out at the SRXRF beamline at HASYLAB in Hamburg, Germany. At HASYLAB, synchrotron radiation emitted from positrons in large storage rings is used for fundamental and applied research. We show results from investigations of different neurodegenerative disorders of the central nervous system.

Synchrotron radiation based microtomography allows us to visualize the internal microscopic structure of small brains like the honeybee brain. While classical tomography provides a spatial resolution in the millimeter range, microtomography is expanding the spatial resolution down to the micrometer range. Beside other tomographic techniques based on absorption, phase-contrast, or X-ray scattering, X-ray fluorescence computed tomography achieves multielement capability by recording characteristic X-ray emission. The head as well as isolated brains of worker honeybees were investigated by synchrotron radiation-based computered microtomography at HASYLAB. First results of measurements in absorption contrast mode are shown.

Our poster and presentation demonstrate the application of these synchrotron radiation based imaging techniques.

# Novel strategies and structures for open access publishing of neuroscientific media

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New media are rapidly changing the scientific work. Methods and tools, communication and collaboration and even publishing in science and science education move to the digital world and allow a more dynamic and intense development. However, traditional publications, in print or digital form, are not capable of representing this influence of new media in an adequate way. New editorial and technical approaches to publishing should help to overcome traditional restrictions and to establish a new publishing culture that includes a stronger influence of those who generate the scientific content: the scientists.

One of today's main issues for modern publishing is open access, which means that publications are freely accessible on the web, anytime, from anywhere by anybody. Currently, more than 2000 open access journals are available at the market, and the free author's copies of restricted journal publications deposited in institutional or disciplinary repositories are getting more and more each day. Even traditional high impact journals are, due to market pressure, increasingly open for this new publishing strategy, since it is supposed to dramatically increase the number of readers and, therewith, the chance to be cited.

However, open access is only the prerequisite for adding value in modern publishing. Advanced innovation comes from integrating new media techniques and opportunities into publishing processes as well as into the published content itself. Many neuroscientific findings today are only adequately represented if traditional text-image layouts are combined with supplementary materials such as data, films and simulations. If all this can be easily shared, reused and recombined a new quality of scientific communication is achieved.

An example for breaking new ground in scientific publishing within the neurosciences is provided by the non-profit open access e-journal *Brains, Minds & Media* (http://www.brains-minds-media.org). This international, peer-reviewed journal combines both, new editorial and technical structures, to support a publishing infrastructure for new media, based on scientific self-organisation in close cooperation with university librarians. *Brains, Minds & Media* is specialized to the publication of new media for neuroscientific content and educational material. The main focus lies on articles providing supplementary material, being dynamic or interactive visualizations or tools, tutorials or educational simulations and the like. Any kind of data or media may be published with an article and, thus, is made accessible to a broad audience. In addition, each contribution can be discussed or commented online. The publication process is scheduled to three month from submission to publication. Due to online opportunities, each article can be discussed and refined. Each supplementary material is freely accessible and may be used and distributed by others due to the Digital Peer Publishing Licence (DPPL - see: http://www.dipp-nrw.de).

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# Exogenous stimulation of muscles and neurons in *C. elegans* via photoactivation of Channelrhodopsin-2

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Except for sensory neurons responding to specific stimuli, there were no satisfying methods available to precisely stimulate certain neurons in *C. elegans*, neither *in vivo*, nor in dissected animals. We developed a method that allows to stimulate distinct neurons (or other excitable cells, i.e. muscle cells) in *C. elegans* simply by illuminating the animals with blue light. To this end, we heterologously expressed the light-gated cation channel Channelrhodopsin-2 (ChR2) from the green alga *Chlamydomonas reinhardtii*, using specific promoters and cultivated the transgenic animals in the presence of the chromophore *all-trans* Retinal.

This noninvasive method enables the precise and repeated stimulation of certain neurons by light, thereby triggering specific behaviours. For example expression of ChR2 preferentially in the backward command interneurons AVA and AVD (using the nmr-1 promoter) led to rapid reversal behaviours during illumination. In principle, this method should allow to stimulate any neuron in *C. elegans* and to study the behavioural output of this cell *in vivo*, provided a specific promoter is available.

Besides the characterisation of neuronal circuits we also want to apply our method to study synaptic transmission mechanisms at the neuromuscular junction of *C. elegans* using a light-induced neurotransmitter release. Therefor we expressed ChR2 in different classes of motoneurons. For a light-induced acetylcholine release we expressed ChR2 in cholinergic motoneurons from the *unc-17* promoter. We were able to trigger strong muscle contractions during illumination in these animals. Light-induced GABA release was achieved by the expression of ChR2 in GABAergic motoneurons driven by the *unc-47* promoter. This led to a strong paralysis of the nematodes during illumination. We now want to use these transgenic animals for the characterisation of new synaptic vesicle proteins in electrophysiological as well as behavioural experiments.

Nagel, Brauner, Liewald, Adeishvili, Bamberg, Gottschalk (2005) Curr Biol 15: 2279

# *FIND* - Finding Information in Neuronal Data
# An open-source analysis toolbox for multiple-neuron recordings and network simulations

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In parallel to the tremendous technical progress in data acquisition (e.g. large number of simultaneous electrode recordings), there is a growing need for new computational tools to analyze and interpret the resulting large data flow from experiments and simulations. While there is undeniable progress in novel analysis methods, implementations are difficult to reproduce based on literature or are hidden in (ill-documented, in-house) software collections.

We are developing *"FIND"* (www.find.bccn.uni-freiburg.de) to address the urgent need of an unified, well-documented interface, to various analysis tools.

*FIND* - stands for Finding Information in Neuronal Data and will be shared to the community as an open-source analysis toolbox for electrophysiological recordings and network simulation environments.
This platform-independent toolbox can be used to analyze neurophysiological data from single- and multiple-electrode recordings by providing a set of standard and more advanced analysis and visualization methods.
We are building on experience in design and application of such methods [1] (www.meatools.brainworks.uni-freiburg.de) and furthermore, we will also incorporate other open source toolboxes (e.g. neuroanalysis.org/toolkit, an information theory based toolbox).
Currently the *FIND-Toolbox* accommodates import of multiple proprietary data formats, based on the Neuroshare Project (www.neuroshare.org). Physiological data from different acquisition systems (up to now: Alpha Omega, Cambridge Electronic Design, Multi Channel Systems GmbH, NeuroExplorer, Plexon Inc., R.C. Electronics Inc., Tucker-Davis Technologies, Cyberkinetics Inc.) and data from Network simulations Environments (e.g. NEST, www.nest-initiative.org [2]) can now be compared using identical analysis methods. This allows verifying of both results across experiments and laboratories as well as direct comparison of simulation results and electrophysiological recordings.
To enable the incorporation of new algorithms - a weakness of most commercial toolboxes - FIND will be open source, providing the possibility to extend the collection of algorithms and data formats with new ones. We expect that this will facilitate the development and distribution of new techniques among the scientific community.

Please visit www.find.bccn.uni-freiburg.de to see announcements for new features, release versions, tutorials and training workshops.

*Acknowledgements:*
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# Imaging of respiratory population activity in the rat pre-Bötzinger Complex with single cell resolution

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The pre-Bötzinger Complex in the rostral ventrolateral medulla contains a kernel involved in respiratory rhythm generation. Its respiratory activity has been predominantly analyzed by electrophysiological approaches. Recent advances in fluorescence imaging now allow for the visualization of neuronal population activity in rhythmogenic networks. In the respiratory network mainly voltage-sensitive dyes have been used, but their low sensitivity prevents an analysis of activity patterns of single neurons during rhythmogenesis. We now succeeded using more sensitive $Ca^{2+}$ imaging to study respiratory neurons in rhythmically active brainstem slices of neonatal rats. For the visualization of neuronal activity fluo-3 was suited best in terms of neuronal specificity, response magnitude, and minimized background fluorescence. The tissue penetration of fluo-3 was clearly improved by hyperosmolar treatment during dye loading (100 mM mannitol). Rhythmic population activity was imaged with single cell resolution using a sensitive CCD-camera and a high NA 20x objective and it was correlated with extracellularly recorded mass activity of the contralateral pre-Bötzinger Complex. Correlated optical neuronal activity was obvious online in 29% of slices. Deeper located rhythmic neurons became detectable during offline image processing. Based on their activity patterns, 74% of rhythmic neurons were classified as inspiratory-like and 26% as expiratory-like. Our approach is well suited to visualize and correlate the activity of several single cells with respiratory network activity. We demonstrate that neuronal synchronization and possibly even network configurations and failure can be analyzed in a non-invasive approach with single cell resolution and at frame rates currently not reached by most scanning-based imaging techniques.

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# A MRI-compatible, radio-controlled lightweight ultrasonic actuator for neuroethologic research in primates

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Introduction. Neuroethological research aims at correlating electrophysiological events to behavior. The attempt to record neuronal activities in socially interacting, freely moving animals raises a series of methodological difficulties, e.g. to feed microelectrodes without manipulating an animal or to visualize recording sites in vivo without sacrificing it. The use of small non-human primates adds further problems, not only with respect to their complicating three-dimensional lifestyle, but also because of ethical restrictions. In order to facilitate studies on social vocal behavior in squirrel monkeys, we developed a MRI-compatible, wirelessly controlled lightweight microfeed which can be mounted on a temporally implanted synthetic platform.

Materials and methods. For the actuator, based upon a low-cost ultrasonic resonant actuator, type X15G (Elliptec®), a carrying frame from PEEK (Polyetheretherketon) was constructed, the steel spring of the original design was replaced by dental rubber bands and the steel balls of the ball-bearing of the slider were substituted for ceramic globes. Pro/Engineer® Wildfire 2.0 was used to design size and shape of a customized protective shell processed by rapid prototyping (3D Systems GmbH) from DuraFormPA. To prove the compatibility of the actuator, high resolution T1-weighted 3D MRI (3D FLASH, TR/TE = 22.3/7.6 ms, isotropic resolution = 500 µm, 1 averages, 6 min) was carried out at 2.35 T with a MRBR 4.7/400 mm magnet (Magnex Scientific, Abingdon, UK) equipped with B-GA20 gradients (i.d. 200 mm, 100 mT m-1) and driven by a DBX system (Bruker BioSpin MRI GmbH) to prove the compatibility of the actuator.

Results. The actuator has a maximum size of 25 x 25 x 8 mm3, a weight of 10.5 g including electronics and a step-width resolution of 12 µm (± 6.5 µm) with an absolute stroke of 5 mm and a minimum force of 50 mN. The actuator is powered by a rechargeable battery (Varta® LPP402025CE, 150 mAh, 3.7 V power, weight 3.7g) for operational time of 3 days without stand-by or sleep mode. The device is wirelessly controlled via infrared (IR) signals (RC5 Coding) transmitted by a commercially available HiFi remote control. A IR-receiver (Vishay TSOP6236), placed on the front side of the protection cover (42x36x38 mm3), feeds a microcontroller (Atmel Tiny26I) also gating ultrasonic electric power impulses to drive the actuator. Feedback for the operator is signaled by an illuminating diode on top of the shell. MR-images lacked visible magnetic susceptibility distortions on a water phantom mounted with the actuator bare of electronic components.

Summary. A MRI-compatible, radio-controlled, automated lightweight actuator for feeding recording electrodes is described. The device is intended to drive chronically implanted microelectrodes and to allow in vivo demonstration of recording sites by MRI during neuroethological research in small non-human primates.

# Neural circuit reconstruction using serial block-face scanning electron microscopy

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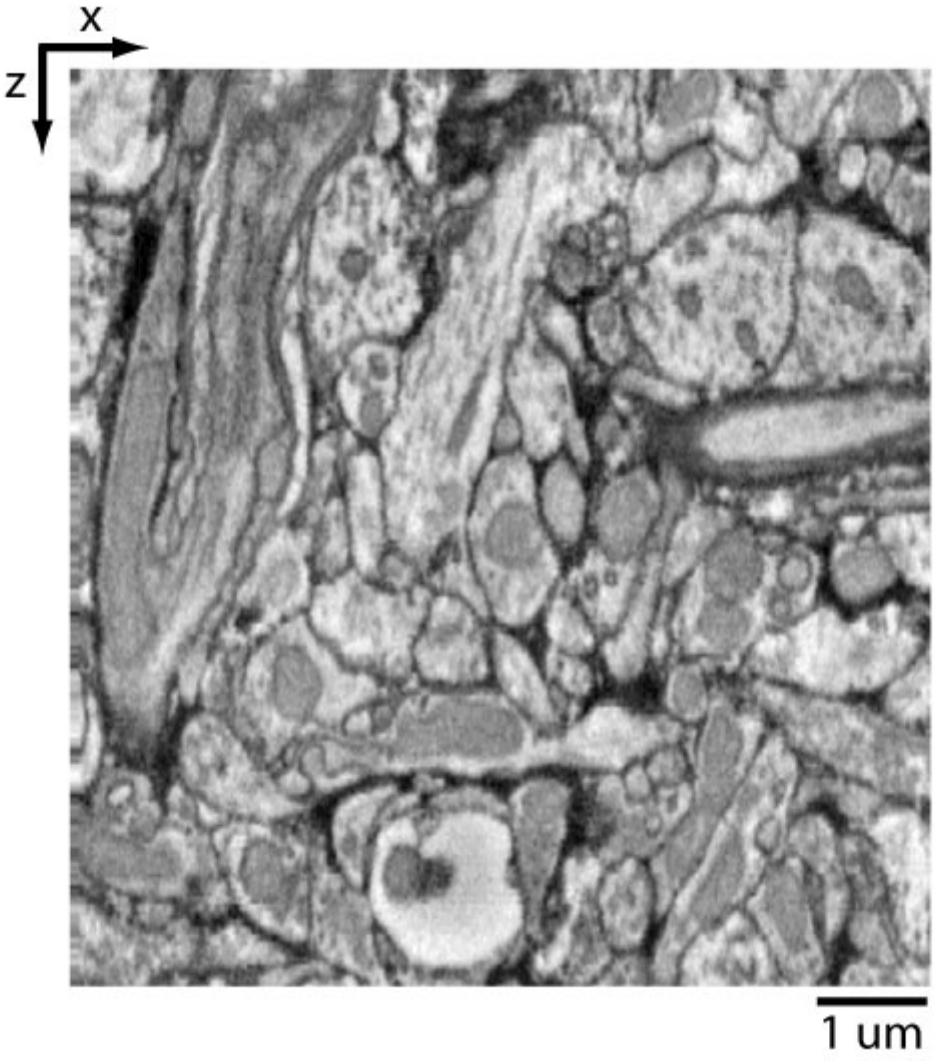
A fundamental limitation to understanding the function of neural circuits is the lack of complete connection, or 'wiring', diagrams. The accurate anatomical reconstruction of neural circuits requires a resolution sufficient to follow all neural processes through dense neuropil. Some of the smallest neuronal processes in the cerebral cortex are axons (~100nm in diameter) and the necks of dendritic spines (~50nm in diameter), well below the resolution of current light microscopy techniques. Serial block-face scanning electron microscopy (SBFSEM) automates serial EM sectioning by sectioning blocks of tissue within a scanning electron microscope, eliminating the difficult processes of manually cutting, mounting, and imaging sections, as well as the need to align and de-distort the resulting images. Blocks are mounted on a high-repeatability XY translation stage allowing large areas of the block face to be 'tiled' at high magnification (15 nm/pixel).

The ability to follow processes at arbitrary orientation is limited by the least resolved direction. In the lateral, XY, direction a resolution of <30 nm is easily achievable. The resolution along the Z direction is, however, primarily limited by the ability to cut ultrathin sections from the block face. Sectioning is dependent on the mechanical properties of the embedding material, parameters of the knife (such as angle, speed, and oscillation frequency), and the electron dose that the block face was exposed to prior to the cut. By optimizing these parameters, we are able to reliably cut 30 nm sections from the block face while imaging large areas (250x250 um) at high magnification. We present results obtained from sectioning large volumes of the mammalian retina and neocortex.



# Patterning of neurite guiding peptides to create neuronal networks with long term stability

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The use of field effect transistors (FETs) in neuronal research has been in rapid progression in recent years. Due to the development of a wide field of applications that range from bioanalytical technique to studying networks properties the control of neuronal cell position and guiding neurites is of fundamental interest. The microcontact printing technique provides the possibility to produce patterns of cell adhesion molecules on silicon oxide of glass and microelectronic devices to guide neurons and the outgrowth of their processes. Cell adhesion proteins are ideal candidates for microcontact printing to control neurons' growth. Extracellular matrix proteins and biopolymers eg. poly-D-lysine were previously applied for patterning biomolecules on the FET silicone oxide surface by local physisorption. In order to achieve long term stability of the protein- and neurons patterns on microelectronic devices and to improve the coupling of the neurons on the sensor arrays we established a modified micocontact-printing protocol. Thereby, the laminin peptide PA22-2, known to guide neurites was used by microcontact printing to covalent attach cell adhesion molecules on silicone oxide.

For guiding neurons to the FET sensor sites, we developed a stamp design, exposing nodes with a diameter of 12µm and lines (4µm) interconnecting the nodes, which correspond to the recording sites of the FETs. The master for the stamps was made by photolithography [1,2]. We achieved accurate alignment of the stamp pattern to the device by using a fineplacer which enables optical control of both surfaces. The stamps which consist of the polymer material polydimethylsiloxane (PDMS) were cleaned and incubated in PA22-2 solution. After gently drying the stamp it was printed on the silicone oxide surface which was previously functionalized. Thereby, the silanol-groups of the substrate were coated by APTES (3-amino-propyltriethoxysilane) for functionalization resulting in amine-terminated surface layer. The heterobifunctional crosslinker (sulfo-GMBS) which is reactive to sulfhydryl groups was attached. Finally, the SH-group of the cysteine-terminated peptide PA22-2, covalent bond the free reactive group of the crosslinker leaving a carpet of the peptide on the surface. The pattern was tested in cell culture and electrophysiological experiments. Cortical neurons from embryonic rat (E18) grown on this PA22-2 in the defined geometry. The electrophysiological activity of the neurons was verified by patch clamp recording. The stability of the neurons networks patterns is under current investigation.

The introduction of covalent bonds between groups of the silicone surface and the cell adhesive peptides would provide a lot of advantages in comparison to physisorptionally attached patterns by enhancing stability of neuronal networks for long time measurements. Further, the electrical coupling of single neurons on micro electronic devices would be further increased.

[1] L Lauer et al. (2001): Aligned microcontact printing of biomolecules on microelectronic device surfaces Biomaterial Engieering, IEEE Transactions on, 48, 7

# Proteome Analysis of Brain Plasma Membranes

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Cell-cell signaling in the brain involves the cooperation of various plasma membrane proteins such as neurotransmitter release proteins, neurotransmitter receptors, primary and secondary active transporters, and ion channels. Proteomic technologies provide powerful tools for broad insight into the protein repertoire of a brain region. One drawback is that large amounts of starting material are required. To circumvent this drawback and the need to kill many animals, we set out to establish a protocol which is based on the differential partitioning of membranes in aqueous polymer two-phase systems. The aim was to purify plasma membranes from small amounts of brain tissue, e.g. a single cerebellum or less. We tested our protocol in the cerebellum, which is anatomically and physiologically well characterized, yet only little is known about the molecular machinery that determines its structure and function. The purity of the isolated membranes was analyzed by immunoblots. Our protocol enabled the enrichment of plasma membrane proteins and proteins from synaptic vesicles, whereas proteins from the endoplasmic reticulum and mitochondrial proteins were reduced. The results were corroborated by the determination of marker enzyme activities. We recovered 15% of the initial plasma membrane marker activity, yet only 0.2% of the mitochondrial marker activity and 1.1% of the marker activity for the endoplasmic reticulum. Yield of plasma membranes was nearly doubled when 4.7% mitochondria and 2.9% of the endoplasmic reticulum were accepted as contamination. Mass spectrometric analyses of the purified membranes identified a total of 889 different proteins. 281 of those (32%) could be allocated to the plasma membrane. Among these were various proteins involved in neurotransmitter release, such as several syntaxins, SNAP23 and SNAP25. Within the group of neurotransmitter receptors, different ionotropic and metabotropic glutamate receptors and different subunits of GABA receptors were identified. Among those we found GABRA6 which, according to literature, is restricted to the cerebellum. Additionally, purinergic receptors were detected. Proteins involved in neurotransmitter re-uptake and various primary and secondary active transporters were identified as well as proteins involved in cell-cell communication. Further, various ion channels (Ca2+, K+, and Na+) were found. We also recovered TRPV6, a member of the transient receptor potential cation channel subfamily. To our knowledge, our data provide the first evidence of the presence of TRPV6 in the cerebellum. We performed in situ hybridization to confirm this finding. Preliminary data indicate the occurrence of TRPV6 in the Purkinje cell layer. Taken together, we have established a sensitive, simple and low-priced protocol that allows the proteomic analysis of plasma membranes from small subregions of the brain. Our approach is suitable to identify hitherto unknown proteins in distinct brain regions and thus contributes to an enhanced understanding of the protein profiles that determine the structural and functional characteristics of these regions.

# Improved Performance of a New Generation of Genetically Encoded Calcium Indicators *in vivo*.

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Genetically Encoded Calcium Indicators (GECIs) based on green fluorescent protein (GFP) offer great potential for the analysis of concerted action of functionally related neurons in intact brains. However, compared to synthetic Ca2+ sensors GECI performance still bears weaknesses in signal-to-noise ratio (SNR), dynamic range and kinetics of the fluorescence signals in response to neural activity. Recent work has sought to overcome these problems by genetic engineering of GFP and Ca2+ binding proteins, leading to the new GECIs Yellow Cameleon (YC) 3.60, YC 2.60, TN-XL and GCaMP 2. Using 2P microscopy we investigated YC 3.60 and TN-XL in transgenic *Drosophila* at presynaptic boutons of the larval neuromuscular junction. Currently we generate transgenic flies expressing GCaMP 2 and YC2.60. Fluorescence changes from these new GECIs are compared to signals from previous variants (Yellow Cameleon 3.3, TN-L15 and GCaMP1.6). For reference we injected the synthetic Ca sensor Oregon Green Bapta-1 into NMJs and measured Ca responses to the same set of stimuli. We find that the new GECIs show unprecedented properties in SNR and signal kinetics.

# Systematic Co-localization Errors between Acridine Orange and EGFP in Astrocyte Vesicular Organelles

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Previous studies used dual-color imaging of acridine orange (AO) labeling and EGFP expression to visualize the exocytosis of a presumingly well-defined sub-population of EGFP-tagged secretory vesicles. In this letter, using spectral fluorescence detection and lifetime imaging, we demonstrate that green fluorescent AO monomers inevitably coexist in astroglial secretory vesicles with red fluorescing AO dimers. The green emission overlaps with that of EGFP and produces a false co-localization. On AO spectral images, the EGFP signal is obscured by the fluorescence from AO monomers, precluding a reliable EGFP detection.

# Differential and compartmental proteome analysis of the adult rat brain

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The scope of our study is to differentially analyse the healthy brain structures caudate-putamen complex (CPU), substantia nigra, olfactory bulb and the cerebellum in comparison to 6-OHDA leasioned brains (Parkinsonian rat model). Before performing comparative proteomics we aim to optimize the 2D-PAGE with regard to the rather fatty brain tissue. Furthermore, the whole IEF and SDS electrophoresis were efficiently improved in terms of reproducibility. In a preliminary study the above mentioned compartments of the adult rat brain have been processed. Here, we performed a transcardial perfusion (0.9% NaCl) to wash out the blood of the vascular brain system followed by an exact dissection of the structures. Directly, after dissection the storage and most steps of processing the probes have been performed under frozen conditions. Major steps which have been adapted to the belongings of brain tissue were: 1) Homogenisation step, 2) first dimension (desalting and IEF) including an appropriate rehydration buffer and prolongation of the focusing time, 3) equilibration step of the second dimension. Image registration was followed by quantitative comparison of 2D-gels. Firstly, we observed many spots in different areas within the same control brain which are constant in terms of optical density and 2D-distribution. However, beside transregional regular spot patterns specific distinctions of single spots can be discerned. We will present results of differential spot expression of the above mentioned brain compartments. The data analysed by mass spectrometry of differential spot expressions will be shown. Overall, our approach gives rise to a reliable differential proteome analysis of brain areas with great significance for investigating conditions for cell transplantation in Parkinsonian models.

# *In vivo* brain connectivity: optimization of manganese enhanced MRI for neuronal tract tracing.

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One of the main problems in systems biology is to obtain information on signal processing between interconnected groups of neurons in highly distributed networks. The recently introduced technique of manganese ($Mn^{2+}$) enhanced MRI (MEMRI) to study neuronal connectivity *in vivo* opens the possibility to these studies. However, several drawbacks exist that challenge its applicability. High $Mn^{2+}$ concentrations produce cytotoxic effects that can perturb the circuits under study. In the other hand, the MR signal is proportional to the $Mn^{2+}$ concentration in tissue and thus, significant amounts of $Mn^{2+}$ are required to produce detectable contrast and reliable connectivity maps.

Here we attempt to optimize the MEMRI technique by preventing toxicity and improving the quality and extension of the obtained connectivity maps.

The somatosensory cortex of male SD rats was stereotaxically injected with different $Mn^{2+}$-containing solutions. Total amount of injected $Mn^{2+}$ ranged between 1 and 16 nmol and the injected volumes between 10 and 80 nL. Osmolarity and pH effects were investigated injecting pH buffered solutions of $Mn^{2+}$ (pH 7.3 in Tris-HCl buffer vs. 5.5 in $H_2O$) at different concentration (0.05, 0.1 and 0.8 M $MnCl_2$). Same amounts of $Mn^{2+}$ (8nmol) delivered to the tissue at different infusion rates were also compared. Following the injection, $T_1$-weighted MR imaging (250 mm isotropic resolution) was performed in a 7T scanner at different time points. Fifteen days after the injection animals were sacrificed and brains processed for histology. Nissl staining as well as GFAP and NeuN immunohistochemistry (selective staining for astrocytes and neurons, respectively) were performed in the brain sections to examine cellular toxicity.

All injections produced connectivity maps consistent with the known anterograde projections of SI cortex based on classical neuronal tract-tracing techniques. Our results show that pH buffered solution improve the effectiveness of MEMRI, increasing $T_1$ contrast in the projection sites. In addition, injections of pH buffered and isotonic solutions of 50 and 100 mM $MnCl_2$ yielded more extensive connectivity maps, in particular, ipsi- and contra-lateral corticocortical connections were evident in all animal injected with those solutions but not with the more usual MEMRI protocol (0.8M $MnCl_2$ in $H_2O$). Hypertonic and non-buffered solutions containing 8nmol $Mn^{2+}$ resulted in neuronal death and astrogliosis in extensive areas around the injection point. In sharp contrast, no neuronal toxicity was observed with injections containing up to 8nmol of $Mn^{2+}$ in isotonic solutions of up to 100 mM $MnCl_2$ and pH 7.3. Slow infusion rates demonstrated also to be advantageous and permitted application of larger amounts of $Mn^{2+}$ without toxic effects, resulting in better $T_1$ contrast in the low density projection fields. Any sign of toxicity was observed in any condition in the projection fields.

We conclude that refined protocols for MEMRI improve the quality and extension of connectivity maps and preserves tissue viability, assuring the application of this technique in longitudinal experiments.

# A Data Management System for Electrophysiological Data Analysis

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Recent advances in both electrophysiological recording techniques and hardware capabilities have enabled researchers to simultaneously record from a large number of neurons in different areas of the brain. This opens the door for a wide range of complex analyses potentially leading to a better understanding of the principles underlying neural network computations. At the same time, due to the increasing amount of data with increasing complexity, significantly more emphasis has to be put on the data analysis task. Although high-level scripting languages such as Matlab can speed up the development of analysis tools, in our experience, a too large amount of time is still spent on (re)structuring and (re)organizing data for specific analyses.

Therefore, our goal was to develop a system which enables experimental neuroscientists to spend less time on organizing their data and more on data collection and creative analysis. We developed an object oriented Matlab toolbox which supplies the user with basic data types and functions to organize and structure various types of electrophysiological data. By using an object oriented, hierarchical layout, basic functionality, such as integration of metadata, or storage and retrieval of data and results, is implemented independent of specific data formats or experimental designs. This provides maximal flexibility and compatibility with future experiments and new data formats. All data and experimental results are stored in a database, so the experimenter can choose which data to keep in memory for faster access and which to save to disk to save resources. Additionally, we have created an extensive library of basic analysis and visualization tools that can be used to get an overview of the data.

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