Picture: Marina Matyash, Berlin

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Plenary Lectures

Chronic traumatic encephalopathy (CTE): an update including the problem with football (soccer)

Ann C McKee[1]

[1]Boston University School of Medicine/ VA Boston Healthcare System, Boston University CTE Center, 150 S Huntington Avenue, 02130 Boston, USA

Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative disease associated with exposure to repetitive mild traumatic brain injury including concussion, subconcussion and blast injury. CTE was first recognized in boxers nearly a century ago as “dementia pugilistica” but has been more recently identified in athletes who played a variety of contact sports, including American football, soccer, ice hockey, baseball, rugby, boxing and wrestling as well as in military veterans. Clinical features of CTE consist of abnormalities in behavior, including explosivity, impulsivity, and suicidality; mood, including depression and hopelessness; cognition, including executive dysfunction, memory loss, and dementia; and movement including parkinsonism. In advanced disease, memory loss, executive dysfunction, cognitive impairment and dementia are common. Symptoms of CTE may begin in young adulthood, but often appear after age 50, decades after the exposure to trauma, and may be mistaken for symptoms of Alzheimer’s disease. Like many other neurodegenerative diseases, CTE is diagnosed with certainty only by neuropathological examination of brain tissue. Significant advances have been made over the past decade in classifying and characterizing the neuropathological and immunohistochemical features of CTE. In 2013, McKee and colleagues proposed criteria for the pathological diagnosis and staging of CTE. The McKee criteria for CTE delineated the accumulation of hyperphosphorylated tau (p-tau) as neurofibrillary tangles (NFTs), astrocytic inclusions and dotlike neurites in a distinctive pattern around small blood vessels in the cortex, with a striking tendency to occur in clusters at the sulcal depths as unique features of CTE. They also stratified CTE p-tau pathology into four stages of increasing severity, stages I-IV, that, in American football players, were significantly associated with the age of the subject at death and the number of years of exposure to football. In the mildest forms of CTE, a few perivascular CTE lesions are found in the cortex; in advanced CTE, p-tau NFTs and neurites are widely distributed in other cortical regions as well as the hypothalamus, thalamus, and brainstem. In 2015, a NINDS consensus conference of expert neuropathologists, using the preliminary McKee criteria, defined the pathological diagnosis of CTE as a unique tauopathy with a pathognomonic perivascular p-tau lesion. In 2017, the largest and most methodologically rigorous case series of individuals diagnosed with CTE was reported in American football players. The study showed that of 202 American football players whose brains were donated for research, 177 were diagnosed with CTE, including 110 of 111 former National Football League players. There have been scattered reports of CTE in soccer players, including several young soccer players, one of whom was also diagnosed with motor neuron disease. Accumulating evidence in active soccer players indicates that heading and head impacts in soccer are associated with risk of cognitive changes, neuropsychological alterations, and microstructural changes in the brain. Current research is focused on preventative measures to reduce the risk for CTE, early detection of CTE pathological lesions in living subjects using fluid and neuroimaging biomarkers, and identification of unique components of the pathology that can be targeted therapeutically.

Consciousness: From Theory to Practice

Giulio Tononi[1]

[1]Department of Psychiatry, University of Wisconsin-Madison, 6001 Research Park Blvd, 53719 Madison, Wisconsin, USA
[2]Wisconsin Institute for Sleep and Consciousness, University of Wisconsin-Madison, 6001 Research Park Blvd, 53719 Madison, Wisconsin, USA

What is consciousness, and what is its neural substrate in the brain? Why are certain parts of the brain important for consciousness, but not others that have even more brain cells and are just as complicated? Why does consciousness fade with dreamless sleep even though the brain remains active? Does consciousness always fade when patients become unresponsive after brain damage, during generalized seizures, during general anesthesia, or even in deep sleep? And are newborns, animals, and intelligent computers conscious? Integrated information theory (IIT) is an attempt to answer these and other questions in a principled manner. IIT starts not from the brain, but from consciousness itself - the world of experience – and derives from it what it takes for a system to be conscious. The results of this exploration can account for many empirical findings, lead to counterintuitive predictions, and has motivated the development of promising new tests for the practical assessment of consciousness in non-communicative subjects.

Molecular disease mechanisms and therapeutic approaches in Parkinson's disease

Friederike Zunke[1], Joseph R. Mazzulli[2], Paul Saftig[1], Stefan Rose-John[1]

[1]Christian-Albrechts-Universität zu Kiel, Department of Biochemistry, Rudolf-Hoeber Str.1, 24118 Kiel, Germany

[2]Northwestern University, Department of Neurology, Chicago, IL, USA

The conversion of a-synuclein into insoluble amyloidogenic aggregates is considered a key event in the development of Parkinson's disease (PD) and related synucleinopathies. Mutations in glycosphingolipid (GSL)-degrading glucocerebrosidase (GCase) are strong genetic risk factors for PD, indicating that GSL-clearance is involved in a-synuclein aggregation.
Recent studies indicate that a-synuclein exists as both natively unfolded monomers and folded multimers under physiological conditions, however the relationship of these species to pathological aggregates is not known. Surprisingly, mechanisms of a-synuclein aggregation were not well understood until recently. We were now able to show that lysosomal GSLs seem to play an important role in -synuclein aggregation pathways and can cause a reversible structural change in a-synuclein, promoting its aggregation and toxicity [1]. Moreover, we could show that reduction in the glycolipid restores physiological a-synuclein and diminishes pathology in induced pluripotent stem cell (iPS)-derived midbrain neurons of PD patients [1-3]. This pharmacological decrease, which could be obtained either by boosting GCase function or inhibiting GSL-synthase function, may be applicable to synucleinopathies as well as other diseases involving the conversion of folded proteins into amyloid fibrils.

1. Zunke F, Moise AC, Belur NR, Gelyana E, Stojkovska I, Dzaferbegovic H, Toker NJ, Jeon S, Fredriksen K, Mazzulli JR (2017) Reversible Conformational Conversion of alpha-Synuclein into Toxic Assemblies by Glucosylceramide. Neuron
2. Mazzulli JR, Zunke F, Isacson O, Studer L, Krainc D (2016) alpha-Synuclein-induced lysosomal dysfunction occurs through disruptions in protein trafficking in human midbrain synucleinopathy models. Proc Natl Acad Sci U S A 113, 1931-1936
3. Mazzulli JR, Zunke F, Tsunemi T, Toker NJ, Jeon S, Burbulla LF, Patnaik S, Sidransky E, Marugan JJ, Sue CM, Krainc D (2016) Activation of beta-Glucocerebrosidase Reduces Pathological alpha-Synuclein and Restores Lysosomal Function in Parkinson's Patient Midbrain Neurons. J Neurosci 36, 7693-7706

Encounters in anion channelrhodopsin research - a personal perspective on the development of inhibitory optogenetic tools

Jonas Wietek[1]

[1]Experimental Biophysics, Humboldt Universität zu Berlin

Jonas studied biophysics at the Humboldt-University Berlin, where he received his doctoral degree in experimental biophysics in 2018. During his PhD he focused on the development and investigation of inhibitory anion-conducting channelrhodopsins (ACRs), which opened up new possibilities of neuronal silencing experiments in recent years. His work covers the design of the first light activated ACRs, creation of improved variants with altered kinetics and spectral properties, and the identification of naturally occurring ACRs.
In the TSF technology award lecture, Jonas will summarize the history of ACRs, which covers only half a decade but has already developed enormously. As with every section someone looks back to, developing and discovering ACRs does not just involve pure scientific and hard data. In addition, encounters, coincidences, and other curiosities, play an important role of which some might be revealed.

Cognition without a Cortex

Onur Güntürkün[1]

[1]Biopsychology, Faculty of Psychology, Universitätsstr. 150, 44780 Bochum, Germany

Studies of the last two decades revealed that especially corvids and parrots are cognitively on par with apes. Indeed, there is no cognitive ability demonstrated in chimps that these birds with their smaller, non-cortical brains can't also achieve. How is this possible? I will argue that a) the avian pallium is partly homologous to cortex, b) that the overall circuitry of the avian telencephalon is highly similar to that of mammals, c) that sensory parts of the bird non-cortical forebrain contain a "hidden" laminated organization that may spark similar computational properties as the mammalian cortex, d) that birds independently developed a "prefrontal cortex"-like are for executive functions, and that e) brain size and pallial neuron numbers correlate with cognitive capacity within but not between members of vertebrate classes. Overall, these data show that birds and mammals display a mosaic of radically different and astonishingly similar forebrain components. It is very likely that most of the similarities between avian and mammalian telencephala evolved in convergent manner. But why should evolution repeat itself by inventing the same wheels twice? It seems that we have to propose the concept of "constrained evolution" to understand these findings. Evolution seems to face limited degrees of freedom when crafting neural circuitries for sensory and cognitive operations. As a result, comparable telencephalic areas and circuitries evolved in two vertebrate classes that are separated by more than 300 million years. Despite all these similarities, there is but one especially glaring difference between birds and mammals: Birds achieve similar behavioral/cognitive abilities with far less neurons and far smaller brains. I would love to know why this is and how birds achieve it.

Neural codes for natural navigation in the hippocampal formation of bats

Nachum Ulanovsky[1]

[1]Weizmann Institute of Science, Department of Neurobiology, Herzl str. 234, 76100 Rehovot, Israel

The work in our lab focuses on understanding the neural basis of spatial memory and spatial cognition – using bats as our animal model. In my talk I will present some of our recent studies, which explored the following questions: (i) How does the brain represent positions and directions in 3D ? A set of studies revealed 3D place cells, 3D head-directions cells, and 3D grid cells in the bat hippocampal formation. (ii) How are navigational goals represented in the brain ? We discovered a new kind of vectorial representation of spatial goals – whereby hippocampal neurons encode the direction and distance to a spatial goal. (iii) I will describe our recent discovery of “social place-cells” in the bat hippocampus – neurons that represent the position of other bats (conspecifics). (iv) Finally, I will describe ongoing work towards elucidating hippocampal neural codes in realistic, kilometer-scale environments – where we discovered an unexpected multi-scale coding of space. Our long-term vision is to develop a “Natural Neuroscience“ approach for studying the neural basis of behavior – tapping into the animal's natural behaviors in complex, large-scale, naturalistic settings.

Mechanisms of presynapse function and assembly

Volker Haucke[1,2,3], Tolga Soykan[1], Anela Vukoja[1], Ulises Rey[2], Astrid G. Petzoldt[2], Dymtro Puchkov[1], Christoph Ott[1], Stephan J. Sigrist[2,3]

[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie im Forschungsverbund Berlin e.V., Molekular Pharmacology and Cell Biology, Robert-Rössle-Str. 10, 13125 Berlin, Germany

[2]Freie Universität Berlin, Faculty of Biology, Chemistry, Pharmacy, 14195 Berlin, Germany.

[3]NeuroCure Cluster of Excellence, Charité Universitätsmedizin Berlin, 10117 Berlin, Germany.

Nervous system function relies on the polarized architecture of neurons, established by directional transport of pre- and postsynaptic cargoes. While delivery of postsynaptic components depends on the secretory pathway, the identity of the membrane compartments that supply presynaptic active zone (AZ) and synaptic vesicle (SV) proteins is unknown.

In my talk I will first present our recent studies on presynaptic biogenesis. Using combined live imaging in *Drosophila* larvae and mouse hippocampal neurons we found that presynaptic biogenesis is mediated by axonal co-transport of AZ and SV proteins in presynaptic lysosome-related vesicles (PLVs). Loss of the lysosomal kinesin adaptor Arl8 results in the accumulation of AZ and SV protein-containing vesicles in neuronal cell bodies and a corresponding depletion of AZ and SV components from presynaptic sites leading to impaired neurotransmission. Conversely, axonal transport of AZ proteins and presynaptic function are facilitated by genetic upregulation of PLV transport. Our data reveal an unexpected function for a lysosome-related organelle as the basic building block for presynaptic biogenesis.

In the second part of my lecture I will focus on how SVs cycle once a functional presynaptic compartment has been assembled. Specifically, I will describe our identification of a clathrin-independent endocytosis (CIE) pathway that appears to be the primary route for compensatory membrane uptake following action potential induced exocytic SV fusion. This pathway depends on formin-mediated actin assembly as well as on the orchestrated activity of BAR domain proteins that drive the formation and dynamin-mediated scission of membrane invaginations from which SVs reform by clathrin/ AP-2-mediated budding. These studies bear important implications for the ability of neurons to respond to a vast range of stimulation frequencies to process and store information.

The Synapse: Memory in a Fluid Membrane

Antoine Triller[1]

[1]Ecole Normale Supérieure, Institut de Biologie de l'ENS, 46, rue d'Ulm, 75005 Paris, France

The efficiency and accuracy of neurotransmission strongly depends on two apparently antagonist properties of synaptic membrane: the stability of its organization and its ability to adapt to plasticity events. In addition, the structural stability of synapses has to be reconciled with the notion that cell membranes are fluid. Membrane molecules are compelled to move within the membrane surface due to thermal Brownian agitation, which favors the homogeneous distribution of the molecules. As a result, neurons spend energy to stop or reduce these movements, and maintain molecules in certain locations via mechanisms that decrease this fluidity. Combination of single particle tracking and super-resolution methods, open access to molecular counting and energy involved in receptor-scaffold interactions as well as on and off rate of molecular interactions. Thus beyond super-resolution methods is chemistry “in cellulo” accounting for the regulation of receptor number and consecutively that of synaptic strength. The dynamic regulations of receptor-scaffold and scaffold–scaffold interactions appear as a central tenet for the maintenance and plasticity-related changes of receptor numbers at synapses.
These processes are likely to be deregulated in pathological situations such as in neurodegenerative diseases. We reported that the Alzheimer beta-amyloid oligomers interact with the neuronal membrane and form an extracellular scaffold that impairs the diffusion of metabotropic glutamate receptors. This induces deleterious events that can be antagonized by specific mGluR antagonists. This patho-mechanism involving misfolded proteins applies also to Parkinson diseases via interactions of α-synuclein with NaK-ATPase, it can be generalized to other neurodegenerative diseases. The tuning of these deleterious processes by microglia open new therapeutic routes.

The brain as a timer: day, season and moon phase coordination in the sea

Kristin Tessmar-Raible[1]

[1]MFPL/ University of Vienna, Research Platform Rhythms of Life, Dr. Bohrgasse 9/4, 1030 Vienna, Austria

Right timing is crucial in life. Thus, many organisms use an interplay of multiple timing systems to control physiology and behavior. While the basic molecular and cellular mechanisms of the circadian (period length about 24hrs) clock and its interaction with environmental stimuli are well understood, very little is known about endogenous oscillators with other periods, such as monthly (circalunar) clocks.

The marine bristle worm Platynereis dumerilii harbors a light-entrained circadian, as well as a circalunar clock. Our studies suggest that the circalunar clock persists even when circadian clock oscillations are disrupted. The circadian clock disruption is suggested by the complete absence of molecular and behavioral circadian oscillatory patterns.
However, the circalunar clock impacts on the circadian clock on two levels:
a) It regulates the level of a subset of core circadian clock genes.
b) In addition to its molecular input, we furthermore find that the circalunar clock changes the period and power of circadian behavior, although the period length of the daily transcriptional oscillations remains unaltered.
In order to study the molecular and cellular nature of the circalunar clock, as well as its interaction with the circadian clock, we have established transient and stable transgenesis, inducible specific cell ablations, chemical inibitors, as well as TALEN-mediated genome engineering. We have been investigating the extent of transcript and proteome changes in the brain caused by the circalunar clock and compare these changes to other major conditions (sex determination, maturation) occurring during the life of the worm.

We are furthermore investigating the phenotypes of worms, which are mutant for cOpsin1 and Lcry light receptors. Mutations in these light receptors lead to distinct changes in rhythmic behavior and physiology.

Symposia

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S21 Behavioral decisions based on multimodal information

S22 The neuronal basis of tinnitus

S23 Early information selection for robust vision

S24 Form follows function? Rules and consequences of structural synaptic plasticity

S25 Go with the flow? Processing of sensory flows across modalitie

S26 Neural mechanisms of social decision-making

S27 Neurodegenerative diseases: shaping neuronal circuits by membrane trafficking

S28 Modulatory circuits of central pain processing

S29 Orexin beyond sleep

S30 Inhibitory synapse diversity in health and disease

S31 The tripartite synapse in health and disease

S32 Hearing system adaptation for diverse lifestyles across the animal kingdom

S33 Pro-survival versus toxic NMDA receptor signaling and the fight against neurodegenerative disorders

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Symposium

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S1-6 Hoverfly vision in naturalistic surrounds
Karin Nordstrom

Adaptive responses and population dynamics in the olfactory system of *Drosophila*

Carlotta Martelli[1]

[1]University of Konstanz, Department of Biology, Neurobiology, Universitätsstraße 10, 78457 Konstanz, Germany

Stimulus driven adaptation is a common mechanism of sensory system to adjust neural coding to the concurrent stimulus statistics. I use *in vivo* electrophysiology, optical imaging and modeling to understand the mechanisms and functions of adaptation in the olfactory system of *Drosophila*. I will show that adaptation occurs on different timescales in different neuron types. The firing rate of Olfactory Receptor Neurons (ORNs) adapts on timescales of hundreds of milliseconds. This change in response shifts and decreases the dynamics range of the response to odor pulses. However calcium imaging at the ORN axon terminals in the Antennal Lobe (AL) reveals that sustained stimuli induce little decrease in presynaptic calcium and similarly little adaptation to an odor background. On the contrary calcium responses in the ORNs postsynaptic neurons, the Projection Neurons (PNs), adapt on multiple timescales. We demonstrate that this adaptation is mostly a result of depression at the ORN-PN synapses and discuss the mechanisms that mediate these transformations. Finally I will describe how it affects the odor representation at the population level.

A computational logic for olfaction

Barani Raman[1]

[1]Washington University in St. Louis, Biomedical Engineering, One Brookings Drive Campus Box 1097, 63130 Saint Louis, USA

Sensory stimuli evoke spiking activities patterned across neurons and time that encode information about its identity. Since the same stimulus can be encountered in a multitude of ways, how stable or flexible are these stimulus-evoked responses? I will examine this issue in the locust olfactory system. I will reveal how spatial and temporal features of odor-evoked responses can vary significantly with stimulus-history. Next, I will show how these variations allow the antennal lobe circuit to enhance the contrast of the stimulus with respect to the cues previously encountered, but as a result confound the information about odorant identity. I will go on to reveal drawbacks of using conventional decoding schemes based on combinatorial and temporal properties of odor-evoked responses. Instead a linear coding scheme involving a flexible subset of neurons to robustly recognize the odorant identity will be presented. I will conclude my talk with a brief discussion of how tradeoff between stability vs. flexibility can be achieved in sensory coding.

Subgroups of femoral chordotonal organ neurons differentially affect leg movements and coordination in *Drosophila melanogaster*

Alexander S. Chockley

[1]University of Cologne, Department of Animal Physiology, Zülpicher Str. 47b, 50674 Köln, Germany
[2]Geisel School of Medicine, Dartmouth University, 1 Rope Ferry Rd., Hanover, NH, USA

Animal behavior, specifically locomotor behavior, is continuously modulated by sensory systems. This modulation introduces a degree of adaptability to general motor output variability, unforeseen obstacles, and variations in the walking surface; this adaptability is needed to produce successful locomotor behavior. Among the sensory structures in the insect leg that contribute to this, the femoral chordotonal organ (fCO), an internal proprioceptor in the proximal femur, contains dozens to hundreds of neurons, depending on the species. Studies have shown that, in addition to detecting substrate vibrations, fCO primary sensory neurons provide information about the leg's state during walking by measuring the position, velocity, and acceleration of the tibia relative to the femur[1]. We characterized the morphology and function of groups of fCO neurons in Drosophila melanogaster to investigate how leg position information is encoded and how this information is used to influence locomotor behavior. As functional groups of neurons have been found in the fCO of other insects[2] that respond to similar kinematic parameters of tibial movement, we first investigated whether fCO neurons can be genetically grouped and if these groups have distinct effects on leg movement. Using the resources available at the Bloomington Drosophila Stock Center and the FlyLight database (Janelia Research Campus), we have tested Gal4 lines that label subgroups of fCO neurons and have characterized their morphology within the legs as well as their projection patterns in the ventral nerve cord (VNC). The distributions of these neurons within the fCO as well as their central projection patterns vary between Gal4 lines, but seem to reflect the categorization into club, claw, and hook groups[3]. Activation of these subsets using Chrimson[4] produced leg movements in some lines (tibial extension or flexion), with some lines showing no obvious effects. Inhibiting these fCO neurons optogenetically (GtACR1[5]) in a free-walking paradigm showed mostly mild effects on walking, with clear qualitative differences between the various Gal4 lines, suggesting that these subsets of neurons encode different movement parameters of the femorotibial joint. For example, one line that showed tibial extension during activation demonstrated deficits in tibial flexion during inhibition. This was seen as increased stance duration in the hind legs and increased swing duration in the front legs. Finally, we present data from calcium imaging using a genetically encoded calcium indicator expressed in fCO neuron subsets with precise movements of the tibia, demonstrating different response properties of these groups of neurons. Our findings add to the understanding of the functional structure of the dipteran fCO and the role of proprioceptive feedback encoding various movement parameters in leg movements and coordination during walking.

[1]Field and Matheson 1998, Adv in Insect Phys 27; [2]Hofmann et al. 1985, J Exp Biol 114; [3]Mamiya et al. 2018, BioRxiv; [4]Klapoetke et al. 2014, Nat Methods 11(3); [5]Mohammad et al. 2017, Nat Methods 14(3)

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Unraveling a Delay-Line and Coincidence Detector Circuit for Auditory Pattern Recognition

Berthold Hedwig[1], Edith Julieta Sarmiento Ponce[1], Stefan Schoeneich[2], Konstantinos Kostarakos[3], Xinyang Zhang[1], Matthias Hennig[4], Jan Clemens[5]

[1]University of Cambridge[2]University of Leipzig
[3]University of Graz[4]Humbolt University Berlin[5] ENI Goettingen

Detecting and analysing species-specific communication signals is a fundamental task of auditory pathways in vertebrates and invertebrates. In case of acoustically communicating crickets, simple neuronal networks may be expected to underlie the processing of the males song pattern, consisting of stereotypical sequences of sound pulses, grouped into chirps of 3-4 pulses.
As the basis of auditory pattern recognition a delay-line coincidence detector circuit has been revealed in the brain of female crickets. It is composed by the axonal output structures of an ascending auditory interneuron (AN1) and 4 local neurons (LN2, LN3, LN4, LN5). In short: A key feature of the system is a non-spiking interneuron (LN5), which in response to a sound pulse is inhibited. It subsequently generates a delayed postinhibitory rebound and forwards this excitation to a coincidence detector neuron (LN3). The coincidence detector receives a direct input coupled to acoustic stimuli via AN1 and a delayed input via the non-spiking neuron. It responds strongest, when the pulse period of sound stimuli matches the internal delay of the non-spiking interneuron, and thus creates the basis for species-specific pulse pattern recognition (Schöneich et al. 2015).
The function of the circuitry implies that for each pulse within a chirp different combinations of direct and delayed activity are processed at the level of the coincidence detector, e.g. the first pulse of a chirp will not coincide with a delayed signal and delayed response to the last chirp, will not match a subsequent direct signal. When the duration of single pulses in a chirp is systematically varied, this leads to different behavioural responses. The animals tolerate long pulses at the end of a chirp, and accept very short pulses at the beginning of a chirp. As a consequence attractive and non-attractive chirp patterns can be generated: e.g. a short pulse at the beginning and a long pulse at the end of a chirp are attractive, but when played in reverse order the same pulse pattern is not attractive (Hedwig and Sarmiento-Ponce 2017).
At the neuronal level the pattern recognition brain neurons respond very differently to the attractive and non-attractive pulse patterns. For example, in the coincidence detector neuron (LN3) and the feature detector neuron (LN4) a non-attractive chirp elicits a response only to the last sound pulse, whereas an attractive chirp elicits a response to at least two pulses. The response to the attractive pattern is similar to that evoked by a normal chirp pattern. Also the post-inhibitory rebound of the delay-line neuron (LN5) in response to the non-attractive pulse sequence is altered (Zhang and Hedwig, in prep).
Interestingly the different behavioural responses are also reflected in a modelling approach, aimed to rebuild the functional properties of the network components with filters and non-linearities. Designed to test the pattern recognition properties to “standard” song patterns, the model provides pattern recognition tuning curves, matching female phonotaxis. Moreover when challenged with the attractive and non-attractive sound patterns as novel stimuli, the model system gives very similar responses as the behavioural data. This demonstrates the inherent response properties to novel stimuli and also the robustness of the chosen computational approach to model pattern recognition as a delay-line and coincidence detector system (Clemens et al. in prep).

Cellular and circuit mechanisms that separate luminance and contrast sensitivity in peripheral visual processing

Katja Sporar, Madhura Ketkar[1], Burak Gür[1], Marvin Seifert[2], Marion Silies[1]

[1]European Neuroscience Institute, RG Visual Processing, Grisebachstr. 5, 37073 Göttingen, Germany
[2]School of Life Sciences, University of Sussex, RG Vision and Visual Ecology, Brighton

The interpretation of the visual world by the brain is vital. Especially changes in the patterns of incoming light provide important information to the nervous system, for example to detect visual motion cues that are important for many visually guided behaviors. At the same time, motion computation has to be robust to changes in the environment, such as changing luminance. In the *Drosophila* visual system, the core motion-detecting circuits have recently been characterized. Two distinct pathways are specialized to detect moving contrast increments and decrements, or ON and OFF motion cues, respectively. These parallel pathways split postsynaptic to photoreceptors, where the first order interneuron L1 provides input to the ON pathway, and the two first order interneurons L2 and L3 are the major inputs to circuitry that detects OFF edge motion. We aimed to understand how and why these parallel OFF pathways obtain distinct functions.

Using *in vivo* two photon imaging to record calcium signals in L2 or L3 axon terminals in response to prolonged light stimuli, we could show that L2 responses were transient and sensitive to contrast, providing downstream circuits with information about recent changes in light intensity. L3 responses instead were sustained and sensitive to luminance, responding strongest in the dark. Thus, the two cells in the OFF pathway responded to fundamentally different features of the visual scene. To understand these early differences in visual processing, we tested the contribution of different photoreceptors inputs, the effect of lateral circuit inputs, as well as cell-autonomous differences. We showed that the same photoreceptor inputs shape L2 and L3 responses, and that calcium signals in photoreceptor cells showed a sustained component. Genetically isolating L2 and L3 from their circuit environment revealed that the initial response kinetics were independent of circuit interactions, whereas the loss of a luminance sensitive baseline in L2 was circuit dependent. In contrast, dark-sensitivity in L3 appeared to be fully cell-autonomous. Recent data suggests that an L3-specific transcription factor, *dFezf*, mediates the differences between L2 and L3 function, and promotes luminance- or suppresses contrast-sensitivity. We are currently working towards understanding where these distinct visual features are combined in downstream circuits, as well as probing the specific behavioral roles that these two OFF pathways play in motion detection. Taken together, our work is linking the molecular specialization and circuit interactions that shape physiological properties of identified cell types to the implementation of neural computations and visually-guided behaviors.

Hoverfly vision in naturalistic surrounds

Karin Nordstrom[1]

[1]Flinders University, College of Medicine and Public Health, GPO Box 2100, SA 5001 Adelaide, Australia

Despite being equipped with low-resolution eyes and tiny brains, many insects show exquisite abilities to detect and use visual information. For example, the optic flow generated by a flying insect can be used to maintain a straight flight course, or to avoid obstacles. Many insects, such as killer flies, dragonflies and hoverflies, are also amazingly good at pursuing small moving targets, such as prey or conspecifics, even in highly complex surrounds. Subsequently, animals whose survival depends on early target detection are often equipped with a sharply tuned visual system, yielding robust performance in challenging conditions. Indeed, in the insect brain we find some neurons tuned to the detection of optic flow, and others tuned to the visualization of target motion. We have found that the target neurons found in the optic lobes respond robustly to the motion of small moving objects, even when displayed against syn-directional background clutter.

Importantly, in diptera, the encoding of visual information by the descending neurons, which are more directly involved in generating the behavioral output, has received less attention. To redress this deficiency we have characterized target selective neurons and optic flow sensitive neurons in their ventral nerve cord. We have characterized dipteran target-selective descending neurons (dTSDNs) that only respond to target motion if the background is stationary or moving slowly, moves in the opposite direction, or has un-naturalistic spatial characteristics. We could manipulate the backgrounds by using the fact that natural scenes are not as random as they might appear, but are constrained in both space and time. The 2-dimensional spatial constraints can be described by quantifying the image statistics of photographs. One common parameter to extract from such natural scenes is the slope constant of the rotationally averaged amplitude spectrum. Indeed, the spatial statistics are correlated with the behavior of hoverflies in the field, higher-order neurons in the hoverfly brain are tuned to naturalistic amplitude spectra, as are descending optic flow sensitive neurons.

In the dTSDNs, the response to target motion is suppressed when the naturalistic background and the target move at similar velocities, which is strikingly different to the response of target neurons in the optic lobes. As the descending neurons are pre-motor, these findings affect our interpretation of the neurophysiology underlying target-tracking behaviors.

Symposium

S2: Optogenetics - tool development and application in neuroscience

Structural mechanisms and applications of channel-type optogenetics tools

Yoon Seok Kim[1]

[1]Stanford University, Deisseroth / Kobilka lab, 318 Campus drive west, 94305 Stanford, USA

Within the broad family of light-gated ion channels, anion selectivity was initially created by crystal structure guided re-design of natural cation-conducting channelrhodopsins (CCRs); anion selectivity was subsequently found to have naturally evolved in certain cryptophyte algae. Both designed and natural anion-conducting channelrhodopsins (dACRs and nACRs) have since been applied as optogenetic tools (enabling selective inhibition of targeted-cell activity during behavior in many vertebrate and invertebrate animals), but each also exhibits performance limitations, underscoring key tradeoffs in channel structure/function relationships. For example, befitting their provenance from CCRs that achieved versatile applicability in part through engineered gating properties spanning ~6 orders-of-magnitude from several milliseconds to tens of minutes), dACRs offer a much wider range of kinetics relevant to neuroscience than do nACRs; on the other hand, nACRs exhibit larger photocurrents (despite high anion selectivity). Therefore, molecular and structural insight, jointly into both dACRs and nACRs, will be critical not only to understand the fundamental mechanisms of light-gated anion-channel function, but also to enable creation of next-generation optogenetic tools. Here we report the first high-resolution crystal structures of an nACR (the most widely used variant GtACR1, at 2.9 Å), and a dACR (multiple structures of iC++ at pH 8.5 and 6.5, at 2.9 Å and 3.2 Å resolution respectively). The resulting series of structural, spectroscopic, electrophysiological, and computational analyses provided unexpected insights into ACR pH-dependence, substrate recognition, channel gating, and ion-selectivity. Finally, synthesis of insights from the structures of both iC++ and GtACR1 enabled design, verification, and practical application of the first ACR integrating all the key functional features of large photocurrent magnitude and 20-fold faster kinetics alongside exclusive anion selectivity.

Interrogation of neuronal circuit function using customized optogenetic actuators and silencers

Silvia Rodriguez-Rozada

[1]Center for Molecular Neurobiology Hamburg, Research Group Synaptic Wiring and Information Processing, Hamburg, Germany

[2]Humboldt-Universität zu Berlin, Institute for Biology-Experimental Biophysics, Berlin, Germany

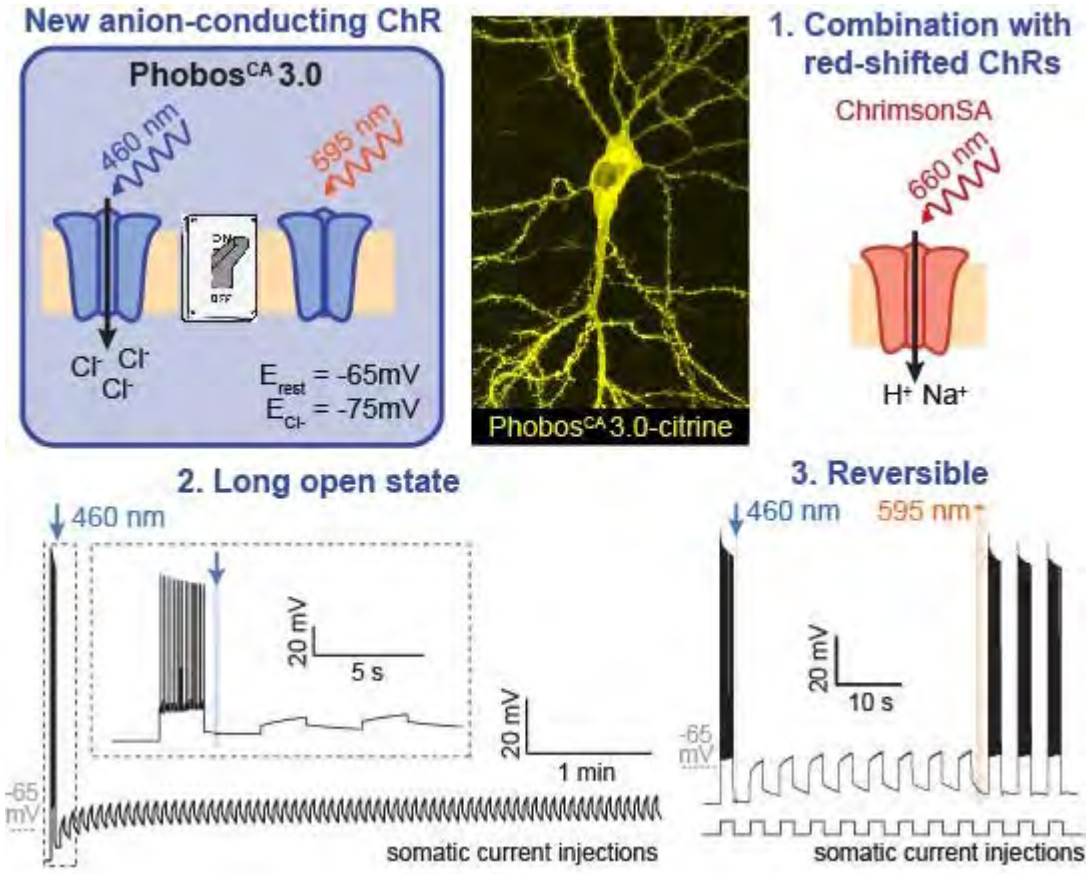
[3]Center for Molecular Neurobiology Hamburg, Research Group Neuronal Patterning and Connectivity, Hamburg, Germany

[4]Center for Molecular Neurobiology Hamburg, Institute for Synaptic Physiology, Hamburg, Germany

Perturbation of neural activity by optogenetic means is a powerful approach to assess the function of defined neuronal populations from the physiological to the behavioral level. Compared to activation, light-induced inhibition of neurons has proven to be technically more challenging. The development of anion conducting channelrhodopsins (ACRs) by targeted mutagenesis of cation conducting ChRs and the discovery of natural ACRs introduced a new class of inhibitory optogenetic tools that overcome some of the limitations of the commonly used ion pumps Halorhodopsin and Archaeorhodopsin. Nonetheless, reversible and temporally precise silencing of neuronal activity for extended periods of time remains a challenge. Our lab has recently developed ACRs with color-tuned action spectra and modified kinetics that efficiently inhibit neuronal activity in hippocampal slice cultures. The introduction of a point mutation (C128A) greatly enhanced the light sensitivity of the engineered ACRs due to a slowed-down photocycle, yielding effective inhibition with reduced light power. The functionality of these ACRs was validated *in vivo* in *Drosophila* larvae, where they showed robust and specific light-dependent inhibition of locomotion and nociception.

Here, we present two new blue-shifted step-function ACRs, termed Phobos^{CA}2.0 and 3.0, with enhanced photocurrents and longer open states, which allow long-lasting silencing of neurons in the absence of light. In order to achieve fine control of neuronal activity, a tool with temporally precise on- and offset is required. Notably, these ACRs can be reversibly toggled between open and closed states using light of different wavelengths, granting termination of silencing with high temporal precision. In addition, due to their blue-shifted activation spectra, Phobos^{CA}2.0 and 3.0 can be combined with red-shifted optogenetic actuators to implement a dual-color excitation/inhibition system, providing an efficient way to dissect neuronal circuits. Combination of the newly developed ACRs and spectrally different excitatory ChRs with genetically defined expression and local illumination allows selective and independent up- and down-regulation of distinct neuronal populations. For instance, driving excitation in CA3 principal neurons, while transiently silencing CA1 interneurons in hippocampal slices, provides an ideal experimental setup to investigate the temporal aspects of feed-forward inhibition during synaptic plasticity of the Schaffer collateral pathway.

In summary, the new color-tuned ACRs with long-lasting, reversible activity broaden the available toolkit of optogenetic silencers in the spectral and temporal domain, thus expanding the possibilities for optical manipulation of neuronal networks.


New anion-conducting ChR
Phobos^CA 3.0
460 nm
595 nm
ON
OFF
Cl- Cl-
Cl-
E_rest = -65mV
E_Cl- = -75mV
Phobos^CA 3.0-citrine
1. Combination with red-shifted ChRs
ChrimsonSA
660 nm
H+ Na+
2. Long open state
460 nm
20 mV
5 s
20 mV
1 min
-65 mV
somatic current injections
3. Reversible
460 nm
595 nm
20 mV
10 s
-65 mV
somatic current injections

Optogenetic tools for neuroscience beyond the classical application of microbial rhodopsins.

Benjamin R. Rost[1]

[1]German Center for Neurodegenerative Diseases (DZNE) Berlin, Neuroscience Research Center, Charité-Universitätsmedizin Berlin, Schmitz lab, Charitéplatz 1, 10117 Berlin, Germany

Microbial rhodopsins are widely applied to depolarize or hyperpolarize the plasma membrane and thereby alter neuronal firing. Our research aims at expanding the neuroscientist's toolbox beyond these classical optogenetic applications. We develop novel optogenetic tools targeted to specific subcellular compartments, and utilize flavin-binding light-sensitive molecules such as photoactivated adenylyl cyclases in order to control second-messenger sensitive proteins such as ion channels and kinases. Given the large variety of natural and engineered light-sensitive proteins, our work aims to open up new perspectives for optogenetic applications in neuroscience.

Optogenetic manipulation of the stress response in larval zebrafish

Soojin Ryu[1], Jatin Nagpal[1], Min K Choi[1], Holger Beckmann[1]

[1]Universitätsmedizin Mainz, German Resilience Center, Duesbergweg 6, 55128 Mainz, Germany

The stress response is controlled by the cells of the hypothalamo-pituitary-adrenal (HPA) axis, which produce highly conserved set of stress hormones with a wide range of target cells and varying function in different time domains. To understand how stress hormones affect physiology and behavior, therefore, it is critical to have a precise temporal control of their production and action. We use optogenetics to manipulate the activity of the HPA axis in larval zebrafish non-invasively with a high temporal precision to investigate its hitherto undiscovered roles. By targeting corticotroph cells in the pituitary with optogenetic proteins and performing different behavioral analysis, we could demonstrate rapid modulatory roles of these cells in regulating locomotion, avoidance behavior and sensory responsiveness at the onset of stress. In another example, we used optogenetics to achieve over exposure to Glucocorticoids (GCs) in early life, which has been implicated in the HPA axis dysregulation leading to stress-induced disorders. To accomplish this, we employed a transgenic fish expressing the optogenetic tool, Beggiatoa photoactivatable adenylyl cyclase (bPAC) in adrenal gland driven by Steroidogenic actue regulatory protein promoter. In this fish, blue light exposure leads to enhanced cAMP level that leads to increased cortisol production. Strikingly when reared under ambient light conditions, 6 and 12 old transgenic larvae exhibited elevated basal cortisol level and a completely compromised stress response. Next, in order to identify the effects of elevated cortisol during early life on hypothalamic cells, another transgenic fish was used in which hypothalamic stress controlling region is labeled with GFP. The double transgenic fish with bPAC expression in adrenal gland and GFP expression in the hypothalamus was then used in FACS and RNA-seq experiments to identify comprehensively targets of the elevated GC in the hypothalamus during development. The insights that we have gained using these approaches will be discussed.

Near physiological spectral resolution and dynamic range of cochlear optogenetics

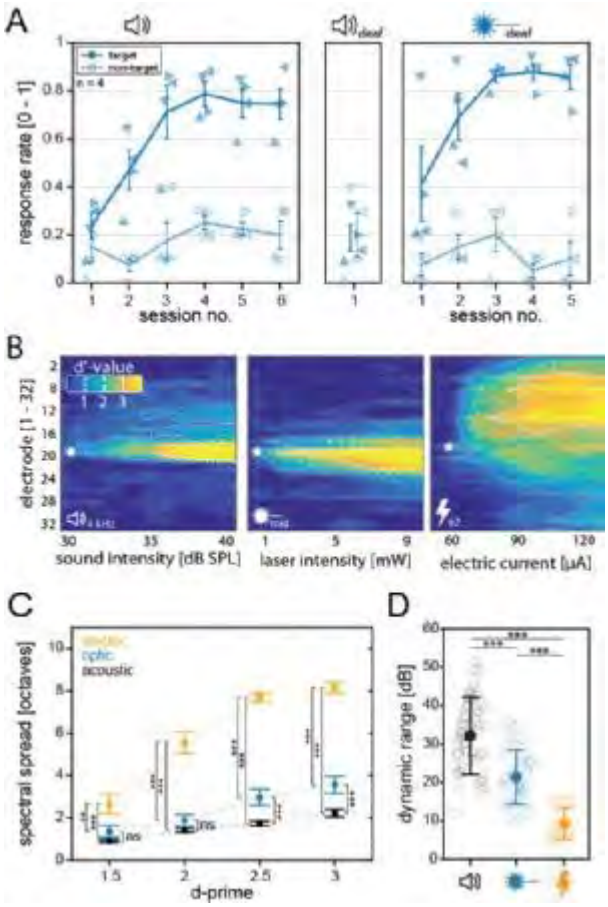
Alexander Dieter, Marcus Jeschke[1,3,4], Tobias Moser[1,2,3]

[1]University Medical Center Göttingen, Institute for Auditory Neuroscience, Robert-Koch-Straße 40, 37075 Göttingen, Germany
[2]Göttingen Graduate School for Neurosciences and Molecular Biosciences, University of Göttingen, 37075 Göttingen
[3]Auditory Neuroscience and Optogenetics Laboratory, German Primate Center, 37077 Göttingen
[4]Cognitive Hearing in Primates Group, German Primate Center, 37077 Göttingen

Cochlear implants (CIs) – the state of the art treatment for profound sensorineural hearing loss – electrically stimulate the auditory nerve and partially restore hearing in half a million users. However, wide spread of electric current from each electrode in the cochlea leads to channel crosstalk and limits the number of independent stimulation channels to less than ten. Consequently, both frequency and intensity resolution of artificial sound encoding are limited, resulting in compromised signal detection, especially in noisy environments. Optogenetic stimulation, in which genetically modified photosensitive SGNs are stimulated with light, might overcome this inherent limitation of electrical CIs. Since light can conveniently be confined in space, cochlear optogenetics holds the potential to increase the number of independent stimulation channels and thus frequency and intensity resolution of artificial sound encoding.

In this study, AAVs carrying the channelrhodopsin-2 variant CatCh were injected into the spiral ganglion of adult Mongolian gerbils. Using the shuttle box paradigm, animals were trained on an avoidance task in order to indicate the perception of an acoustic stimulus via locomotion. Gerbils were subsequently deafened by intracochlear kanamycin injections and deafness was confirmed physiologically and behaviorally. We then implanted a fiber-based optical cochlear implant in order to stimulate the SGNs optogenetically. Our results show that cochlear optogenetics could restore auditory function in the deafened cochlea and provide a percept strong enough to cue avoidance behavior (fig. 1A). In the second part of this study, we performed 32-channel electrophysiological recordings of multi-unit activity in the tonotopically organized inferior colliculus of anesthetized gerbils while stimulating the auditory nerve acoustically, optogenetically or electrically. We then constructed spatial tuning curves and performed an activity-based analysis in order to compare the spread of excitation and output dynamic range of these different stimulation modalities (fig. 1B). Our results demonstrate increased frequency resolution and output dynamic range of cochlear optogenetics as compared to monopolar electric stimulation (fig. 1C-D).

In conclusion, we proof the presence of an optogenetically evoked percept in a gerbil model of sensorineural hearing loss, a prerequisite for considering cochlear optogenetics for hearing restoration. Furthermore, we showed increased coding capabilities of optogenetic over electric stimulation of the auditory system, suggesting a potential way to overcome the major limitations of the most successful neuroprothesis.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session no.
B
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electrode [1 - 32]
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laser intensity [mW]
electric current [µA]
C
spectral spread [octaves]
d-prime
D
dynamic range [dB]

Optogenetic dissection of prefrontal circuits for cognitive control

Ofer Yizhar[1]

[1]Weizmann Institute of Science, Department of Neurobiology, 234 Herzl st., 76100 Rehovot, Israel

The capacity to withhold a response when confronted with negative consequences is crucial for cognitive function, and is a hallmark of goal-directed behavior. Frontostriatal circuits, linking the medial prefrontal cortex (mPFC) and the striatum, have been implicated in the regulation of impulsive behavior. However, the roles of distinct mPFC neuron populations in coordinating such behavioral responses remains unclear. I will describe experiments in which we examined the contribution of mPFC neurons to behavioral performance in mice trained on the 5-choice serial reaction time task. Silencing of the ventral mPFC caused an impairment in the ability of mice to adapt to changes in task structure, consistent with the crucial role of the mPFC in flexible control of goal-directed behavior. To determine the contribution of nucleus accumbens-projecting mPFC neurons to behavioral performance in this task, we developed and validated a novel somatically-targeted anion-conducting channelrhodopsin (stGtACR2). We showed that stGtACR2 allows robust and highly light-sensitive silencing of sparsely labeled neuronal populations in vivo. Inhibition of accumbens-projecting neurons in the mPFC led to reduced impulsivity, without altering performance or motivation. Finally, electrophysiological recordings of mPFC neurons in mice performing the task revealed diverse, yet stable, responses to defined behavioral events. Our results establish the role of the mPFC and of frontostriatal neurons in regulation of cognitive control.

Symposium

S3: Keeping neurons alive - erythropoietin, its variants and its receptors

S3-1 How erythropoietin mediates its neuroprotective effects
Daniela Ostrowski

S3-2 EV-3, an endogenous human erythropoietin isoform with distinct functional relevance.
Christel Bonnas, Liane Wüstefeld, Daniela Winkler, Romy Kronstein-Wiedemann, Ekrem Dere, Katja Specht, Melanie Boxberg, Torsten Tonn, Hannelore Ehrenreich, Herbert Stadler, Inge Sillaber

S3-3 Epo-Induced Neuroprotection: Crucial Role for Orthologues of the Orphan Cytokine Receptor CRLF3
Nina Hahn

S3-4 Erythropoietin signaling in Mouse Angio-Oligo-Neurogenesis
Edith Marianne Schneider Gasser, Kasifa Khalid, Christina Köster-Hegmann, Paola Muttathukunnel, Fátima Sanchís Calleja, Michael Wälti, Max Gassmann, Jean-Marc Fritschy

S3-5 Erythropoietin regulates anti-apoptotic TMBIM family members after ischemic stroke
Pardes Habib, Jörg B. Schulz, Arno Reich

How erythropoietin mediates its neuroprotective effects

Daniela Ostrowski[1]

[1]Truman State University, Biology Department, 100 E. Normal Ave., 63501 Kirksville, USA

Erythropoietin (Epo) has initially been described as the main regulator of red blood cell production in vertebrates. Predominantly released by the adult kidney, circulating hormonal Epo stimulates the homodimeric Epo receptor (EpoR, type I cytokine receptor) on erythroid progenitor cells in the bone marrow. Besides the kidney Epo is synthesized and released by several other mammalian tissues, including the nervous system, mediating adaptive cellular responses to injury in a paracrine fashion. In the nervous tissue Epo production is enhanced in neurons, glial, and endothelial cells following hypoxic stimuli and other insults. Administration of Epo increases cell survival following hypoxic/ischemic injury, suppresses neuroinflammation, and supports regeneration after axonal damage. EpoR is widely expressed in vertebrate nervous tissues; however Epo-mediated neuroprotection during ischemia persists in mice following conditional knock-down of EpoR expression. Furthermore, the identification of neuroprotective but non-erythropoietic Epo splice variants and Epo derivatives indicated the existence of other types of neuroprotective Epo receptors. Various candidate receptors that may be involved in the neuroprotective effect of Epo are currently discussed. Evidence shows that EpoR forms a heteromeric complex with common beta receptor chain (βcR) and Epo-mediated neuroprotection through EpoR/βcR has been demonstrated in various studies. However, various brain regions and cell types that exhibit Epo-mediated protection do not co-express EpoR and βcR, indicating that additional protective receptors exist in the mammalian nervous system. Another type of receptor that may be involved in neuroprotective Epo signaling includes the Ephrin B4 receptor (EphB4). EphB4 is expressed in the nervous system and stimulates proliferation of neural stem cells. The potential of Epo binding to EphB4 has been demonstrated in cultured kidney cells, but the direct involvement of Epo-EphB4 signaling in neuroprotection needs to be still investigated. The neuroprotective and regenerative functions of Epo have been described in the nervous systems of both vertebrates and invertebrates, indicating that tissue-protective Epo-like signaling has evolved prior to its erythropoietic function in the vertebrate lineage. A recent study demonstrated that the Cytokine receptor-like factor 3 (CRLF3) represents a functional neuroprotective receptor for Epo in primary brain cell cultures from the beetle *Tribolium castaneum*. Knock-down of CRLF3 expression abolished Epo's protective effects following serum deprivation and hypoxic injury. Whether CRLF3 functions as a neuroprotective receptor for Epo in mammals including humans remains to be investigated. It is widely accepted that Epo mediates various beneficial effects on the development, maintenance and regeneration of nervous systems but the molecular nature of the involved Epo receptors has not been completely characterized.

EV-3, an endogenous human erythropoietin isoform with distinct functional relevance.

Christel Bonnas[1], Liane Wüstefeld[2], Daniela Winkler[2], Romy Kronstein-Wiedemann[3], Ekrem Dere[2], Katja Specht[4], Melanie Boxberg[4], Torsten Tonn[3,5], Hannelore Ehrenreich[2], Herbert Stadler[1], Inge Sillaber[1]

[1]Epomedics GmbH, Göttingen, Germany.

[2]Clinical Neuroscience, Max Planck Institute of Experimental Medicine and DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany.

[3]German Red Cross Blood Donor Service North-East, Institute of Transfusion Medicine, Dresden, Germany.

[4]Institute of Pathology, Technische Universität München, Munich, Germany.

[5] Department of Experimental Transfusion Medicine, Medical Faculty Carl Gustav Carus, Technische Universität Desden, Dresden, Germany.

Generation of multiple mRNAs by alternative splicing is well known in the group of cytokines and has recently been reported for the human erythropoietin (EPO) gene. Here, we focus on the alternatively spliced EPO transcript characterized by deletion of exon 3 (hEPOΔ3). We show co-regulation of EPO and hEPOΔ3 in human diseased tissue. The expression of hEPOΔ3 in various human samples was low under normal conditions, and distinctly increased in pathological states. Concomitant up-regulation of hEPOΔ3 and EPO in response to hypoxic conditions was also observed in HepG2 cell cultures. Using LC-ESI-MS/MS, we provide first evidence for the existence of hEPOΔ3 derived protein EV-3 in human serum from healthy donors. Contrary to EPO, recombinant EV-3 did not promote early erythroid progenitors in cultures of human CD34+ haematopoietic stem cells. Repeated intraperitoneal administration of EV-3 in mice did not affect the haematocrit. Similar to EPO, EV-3 acted anti-apoptotic in rat hippocampal neurons exposed to oxygen-glucose deprivation. Employing the touch-screen paradigm of long-term visual discrimination learning, we obtained first *in vivo* evidence of beneficial effects of EV-3 on cognition. This is the first report on the presence of a naturally occurring EPO protein isoform in human serum sharing non-erythropoietic functions with EPO.

Epo-Induced Neuroprotection: Crucial Role for Orthologues of the Orphan Cytokine Receptor CRLF3

Nina Hahn

[1] Department of Cellular Neurobiology, Georg-August-University Goettingen, Goettingen, Germany

The cytokine Erythropoietin (Epo) is mainly known for its function in erythropoiesis. However, Epo plays additional roles in cell protection in various tissues including the nervous system. While the signaling pathway of its erythropoietic function is understood in great detail, the receptor mediating neuroprotection remains enigmatic. Erythropoiesis is stimulated by circulating Epo binding to the homodimeric classical Epo receptor (EpoR) on erythroid progenitors. The nature of the “alternative” Epo receptors involved in neuroprotection is currently under discussion. Knowledge about the signaling pathway of Epo’s neuroprotective effect is essential in order to judge its potential therapeutical benefits against neurodegenerative diseases. In this study, we provide evidence the orphan cytokine receptor-like factor 3 (CRLF3) is involved in Epo-mediated neuroprotection.

Although insects seem to lack orthologues of Epo and EpoR, we demonstrated a neuroprotective effect of recombinant human Epo on primary brain cells of the beetle *Tribolium castaneum* and the locust *Locusta migratoria* under challenging conditions (e. g. hypoxia or serum-deprivation). In order to knock down the orthologues of the type I cytokine receptor CRLF3 in these cells, we established soaking RNAi as a convenient method for loss-of-function studies in insect primary brain cell cultures. Knock down of CRLF3 abolished the neuroprotective effect of Epo and its non-erythropoietic variants demonstrating its necessity in Epo-induced neuroprotection *in vitro*.

In addition, we currently broaden our research to mammalian cell lines from various tissues investigating the importance of CRLF3 in cellprotection and its expression in response to harmful stimuli. Our studies aim to support the development of Epo derivatives that specifically activate neuroprotective mechanisms.

Erythropoietin signaling in Mouse Angio-Oligo-Neurogenesis

Edith Marianne Schneider Gasser[1,2,3], Kasifa Khalid[1,2], Christina Köster-Hegmann[1], Paola Muttathukunnel[1,2], Fátima Sanchís Calleja[1], Michael Wälti[1,2], Max Gassmann[3], Jean-Marc Fritschy[1,2]

[1]Institute of Pharmacology and Toxicology, Group Neuroprotection , Winterthurerstrasse 190, 8050 Zürich, Switzerland

[2]Center for Neuroscience Zurich (ZNZ), Zürich, Switzerland

[3]Institute of Veterinary Physiology, Vetsuisse Faculty and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Switzerland

Epo and is highly expressed in the central nervous system during embryogenesis, and its expression decays but remains until adulthood. Here we explored in healthy mice constitutively overexpressing Epo in the brain (Tg21), whether Epo may affect angio-oligo-neurogesis. We showed that Epo overexpression augmented all three processes, whereas removal of the receptor from neural precursor (Nestin-cre,EpoR$^{f/f}$) and oligodendrocyte precursors (Sox10-cre,EpoR$^{f/f}$) reduced differentiation, without altering apoptosis. EpoR was detected in endothelial cells and undifferentiated cells being highly expressed during postnatal ages (P1-P14). In cultured neurons, EPO application stimulated differentiation, and blocking EpoRs decreased it, without increasing apoptosis. Thus we could demonstrate that EPO in the central nervous system acts through its EpoR as a pro differentiating agent.

Erythropoietin regulates anti-apoptotic TMBIM family members after ischemic stroke

Pardes Habib[1], Jörg B. Schulz[1], Arno Reich[1]

[1]Medical School, RWTH Aachen University, Department of Neurology, Pauwelsstraße 30, 52074 Aachen, Germany

Ischemic stroke is a leading cause of mortality and is first listed for severe disability worldwide. Besides tissue plasminogen activators, many experimental studies to mitigate stroke related brain injury have failed in clinical trials. While the infarct core is irreversibly damaged by necrotic cell death, in the adjacent penumbra cell death is mainly apoptotic which is potentially amenable for therapeutical approaches.
Transmembrane BAX Inhibitor-1 Motif-containing (TMBIM) family members exert inhibitory activities in apoptosis and necroptosis. Recently, the neuroprotective potency of FAIM2 (TMBIM2) and its regulation by erythropoietin (EPO) via PI3K/Akt signaling after murine focal ischemia were demonstrated. Analog to FAIM2, GRINA (TMBIM3) is predominantly expressed in the brain.
To assess the role of GRINA in transient brain ischemia, its potential synergistic effects with FAIM2 and its regulation by EPO treatment, we subjected GRINA deficient (GRINA-/-), FAIM2 deficient (FAIM2-/-), double deficient (FAIM2-/-GRINA-/-) and wild-type littermates (WT) mice to transient (30 minutes) middle cerebral artery occlusion (tMCAo) followed by 72 hours of reperfusion. EPO or saline was administered 0, 24 and 48 hours after tMCAo. In addition, primary murine cortical neurons (pMCN) of all mouse strains were subjected to oxygen–glucose deprivation (OGD) after GRINA and/or FAIM2 gene transfection followed by vitality assays as well as gene and protein expression analyses.
Similar to FAIM2-/-, infarct volumes of GRINA-/- mice were increased compared to littermate controls ($p<0.01$). Highest neurological deficits and largest infarct sizes were seen in double deficient mice. EPO administration upregulated GRINA and FAIM2 mRNA levels. It decreased infarct sizes and abrogated neurological impairments significantly in wild-type controls only. GRINA and/or FAIM2 deficiencies showed increased expression levels of caspase 3 and of pro-apoptotic Bax. Further, upstream caspases were differentially upregulated: caspase 8 in FAIM2-/- and caspase 9 in GRINA-/- mice. Overexpression of GRINA and FAIM2 in wild-type and in double deficient pMCN significantly decreased cell death rate after OGD.
In conclusion, TMBIM family members GRINA and FAIM2 are highly expressed in the brain and mediate neuroprotection after cerebral ischemia-reperfusion involving different caspases. EPO regulates TMBIM family members, which might be a key for successful strategies in post-ischemic neuroprotection.

Symposium

S4: Neurological autoimmunity: the role of pathogenic autoantibodies against neuron and glia proteins

S4-1 The clinical spectrum and diagnosis of AQP4-IgG-associated and MOG-IgG-associated disorders
Brigitte Theresia Wildemann

S4-2 Autoantibodies against myelin oligodendrocytes glycoprotein (MOG)
Edgar Meinl

S4-3 Autoantibodies in peripheral neuropathies
Claudia Sommer

S4-4 Development of autoantibody test systems against neural proteins
Dominik Jaeger, W Stoecker, C Probst, S Saschenbrecker, L Komorowski

S4-5 Anti-FGFR3 antibody : a biomarker of sensory neuronopathies or an active player of neuron degeneration?
Yara Nasser, Christian Moritz, Evelyne Reynaud-Federspiel, Jean Philippe Camdessanche, Jean Christophe Antoine, Nadia Boutahar

The clinical spectrum and diagnosis of AQP4-IgG-associated and MOG-IgG-associated disorders

Brigitte Theresia Wildemann[1]

[1]University of Heidelberg, Department of Neurology, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany

IgG antibodies directed against conformational epitopes of aquaporin-4 (AQP4) - an astrocytic water channel protein - and myelin oligodendrocyte glycoprotein (MOG) are detectable with high specificity in the serum of patients with neuromyelitis optica spectrum disorders (NMOSD) and MOG-encephalomyelitis (MOG-EM) respectively. Both NMOSD and, according to current evidence, also MOG-EM are distinct entities, but are important mimics of multiple sclerosis (MS), as the clinical and radiologic presentation may overlap and the course of disease is relapsing in many patients. When left untreated, both disorders carry a high risk of future attacks and - in particular in the presence of AQP4-IgG - of permanent neurologic disability. Treatment, however, differs from MS, and therapeutic strategies must take into account that AQO4-IgG and MOG-IgG are pathogenic antibodies and directly involved in tissue damage affecting the optic nerves, brain and spinal cord. Prompt diagnosis and institution of antibody-eliminating and appropriate preventive therapies are of utmost importance. Cell-based assays are currently considered as gold standard for the detection of both antibodies with optimal sensitivity and specificity.

Autoantibodies against myelin oligodendrocytes glycoprotein (MOG)

Edgar Meinl[1]

[1]LMU Munich, Insitute of Clinical Neuroimmunology, Großhaderner Str. 9, 82152 Martinsried, Germany

- Detection of autoantibodies against MOG in patients
- Histopathology of anti-MOG associated encephalitis
- Clinical spectrum of patients with MOG Abs
- Pathogenic potential of patient-derived antibodies to MOG

Autoantibodies in peripheral neuropathies

Claudia Sommer[1]

[1]Universitätsklinikum Würzburg, Neurologische Klinik, Josef-Schneider-Straße 11, 97080 Würzburg, Germany

The node of Ranvier with its paranodal proteins is an important target of autoimmunity, potentially defining a next entity of neuropathies. Characteristic neuropathies have been discovered with IgG antibodies to the paranodal proteins contactin-1, neurofascin, and contactin-associated protein 1 (Caspr1). Patients may suffer from diseases that with a Guillain-Barré (GBS) or, more often, a chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) like phenotype. Nerve pathology is characteristic with disruption of the paranodal architecture, which can also be seen in skin biopsy specimens. Passive transfer has shown the pathogenicity of some of these antibodies. While patients in the acute phase respond to standard CIDP treatment like intravenous immunoglobulins (IVIG), they may need plasmapheresis or B-cell directed therapies later on.

Development of autoantibody test systems against neural proteins

Dominik Jaeger[1], W Stoecker[1], C Probst[1], S Saschenbrecker[1], L Komorowski[1]

[1]EUROIMMUN AG, Product Management, Seekamp 31, 23560 Luebeck, Germany

The spectrum of neurological autoimmune diseases has expanded substantially in the last 15 years due to the discovery of new anti-neuronal antibodies. There are at present numerous technical challenges for developing and improving standardized serological test systems for the detection of these autoantibodies, some of which occur very rarely. In particular, the determination of autoantibodies against complex cell surface structures generally requires authentically presented target antigens. Furthermore, multiparameter testing might be favoured over selective or sequential analysis to avoid diagnostic gaps. In laboratory practice this strategy enhances the serological hit rate compared to targeted analysis and often provides a fast and reliable, sometimes even life-saving diagnosis.

Anti-FGFR3 antibody : a biomarker of sensory neuronopathies or an active player of neuron degeneration?

Yara Nasser[1,2,3], Christian Moritz[1,3], Evelyne Reynaud-Federspiel[1,3], Jean Philippe Camdessanche[1,2,3], Jean Christophe Antoine[1,2,3], Nadia Boutahar[1,2,3]

[1]INSTITUT NEUROMYOGÈNE, INSERM U1217/ CNRS UMR 5310 - Université de Lyon - Université Claude Bernard Lyon 1.
[2]UNIVERSITY HOSPITAL OF SAINT ETIENNE, FRANCE.
[3]JEAN MONNET UNIVERSITY, SAINT ETIENNE, FRANCE.

Sensory neuronopathies (SNN) are multifactorial diseases characterized by neuron cell death in the dorsal root ganglia. In 2015, Antoine and al. have identified an autoantibody, directed toward the fibroblast growth factor receptor 3 (anti-FGFR3), as a serum biomarker of a subgroup of SNN. Some of those patients also presented a dysimmune background. Preliminary results in our lab on neuron cultures have shown that a rabbit anti-FGFR3 antibody recognizing the intracellular domain of FGFR3 induced neuron cell death while the control rabbit IgGs did not. Therefore, we decided to study the role of this rabbit anti-FGFR3 antibody as well as the molecular mechanism involved in neuronal cytotoxicity. The two main signaling pathways associated with FGFRs are MAPK-ERK1/2 and MAPK-p38. Our analysis revealed an increased expression of FGFR3 receptor genes and glutamatergic AMPA and NMDA receptor subunits in anti-FGFR3 antibody-treated neuronal cultures, suggesting a potential excitotoxic neuronal death. This overexpression is prevented when adding U0126 or SB230580, inhibitors of the two MAPK pathways. The inhibition of FGFR3 activation with Dovinitib, a tyrosine kinase inhibitor and a drug used in cancer treatment, showed similar results to those previously obtained with anti-FGFR3 antibody. This corroborates the hypothesis that neuron degeneration may be due to the inhibition of the FGFR3 tyrosine kinase domains by anti-FGFR3 antibodies. We also tested the potential activation of autophagy after the internalization of antibodies by neurons. The mRNA expression of Optineurin and p62 genes, two key markers of autophagy, were increased in the presence of anti-FGFR3 antibodies meaning that autophagy activation may also play a role in neuron degeneration.

Symposium

S5: Serotonin and its developmental role in shaping brain plasticity and neuropsychological phenotypes

S5-1 Serotonin and development: the role of the peripheral serotonergic system
Natalia Alenina

S5-2 Role of serotonin in maternal behaviour
Sophie Scotto-Lomassese, Aude Muzerelle, Pierre-Louis Ruet, Cornelia Hainer, Michael Bader, Natalia Alenina, Patricia Gaspar

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Serotonin and development: the role of the peripheral serotonergic system

Natalia Alenina[1]

[1]MDC, Molecular biology of peptide hormones, Robert-Roessle-Str.10, 13125 Berlin, Germany

Serotonin, is a monoamine working as an autacoid in the periphery and as a neurotransmitter in the central nervous system. Tryptophan hydroxylase (TPH) is a rate limiting enzyme of serotonin synthesis. It converts tryptophan (Trp) to 5-hydroxytryptophan (5-HTP) and belongs to the family of pterin-dependent hydroxylases, that also comprises tyrosine and phenylalanine hydroxylases (PAH). In mammals TPH has 2 isoforms: TPH1, responsible for serotonin synthesis in periphery, and TPH2, which is restricted to serotonergic neurons in the raphe nuclei in the brain and in the enteric nervous system. Since in adult mammals serotonin cannot cross the blood-brain barrier, these two enzymes define two serotonin systems with independent regulation and different functions. During development, besides its own production by TPH1 starting embryonic day E14, and TPH2 starting E12, there are other sources of serotonin, including maternal 5-HT, that is actively transported through the placenta via the serotonin transporter (SERT). In the early phases of embryonic and postnatal life, 5-HT is a trophic factor that modulates not only cell proliferation, migration and differentiation in the brain and in peripheral tissues but also cell survival and synaptogenesis, through its role in the connective organization of the CNS.
In my talk I will give an overview of possible sources of serotonin during prenatal development, based on the data obtained in mice lacking different components of serotonergic system such as SERT, TPH1, and TPH2. Moreover, I will show our recent data providing evidence that PAH represents an alternative enzyme for serotonin synthesis and is the major source of serotonin in animals lacking TPH enzymes.

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Role of serotonin in maternal behaviour

Sophie Scotto-Lomassese[1], Aude Muzerelle[1], Pierre-Louis Ruet[1], Cornelia Hainer[2], Michael Bader[2], Natalia Alenina[2], Patricia Gaspar[1]

[1]INSERM U839 - Institut du Fer à Moulin, Paris, France
[2]Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin-Buch, Germany

In mammals, maternal care has an essential role to ensure offspring survival. Triggering the repertoire of behavioural responses for adequate maternal care implies functional changes in brain circuits linked to numerous physiological, cellular and molecular modifications. Serotonin (5-HT) neurotransmission is involved in this plasticity since genetic models with constitutive reduction (Pet1 KO) (1) or absence (TPH2 KO) (2, 3) of brain 5-HT, show perturbed maternal behaviour and compromised litter survival. However, the underlying mechanisms are not known since 5-HT could have different effects on the maternal brain, either to impact the development and plasticity of pup-oriented brain circuits, or to control the hormonal changes linked with parturition. To gain better understanding on these points, we analyzed maternal behaviour and pup survival in different conditions: 1) virgin and primiparous Pet1 KO and TPH2 KO mice, 2) secondiparous Pet1 KO, 3) secondiparous mice lacking 5-HT in the dorsal (B7) or the median (B8) raphe due to the AAV-mediated delivery of Cre recombinase to brain of adult Vmat2$^{fl/fl}$ mice (B7$^{Vmat2-/-}$; B8$^{Vmat2-/-}$). Surprisingly, in all 5-HT deficient mothers, except TPH2 KO, pup retrieval in home cage, nesting behaviours and time spent in the nest with pups, were similar to controls. Still, pup survival was strongly reduced in litters of primiparous Pet1 KO and TPH2 KO, as well as in secondiparous Pet1 KO and B7$^{Vmat2-/-}$. All mothers also showed a significant reduction in nursing time. Finally, preliminary experiments in virgin Pet1 KO mice showed a deficiency in initiating parental care. Altogether, these data indicate that motivated maternal response is globally maintained in dams with partial (B7$^{Vmat2-/-}$; B8$^{Vmat2-/-}$) or strong (Pet1 KO) brain 5-HT reduction, but not in mice entirely lacking brain 5-HT (TPH2 KO), suggesting some compensatory mechanisms in the former models. Additionally our observations indicate that 5-HT transmission from the dorsal raphe nucleus contributes to the synergistic interaction between dams and pups to promote optimal lactation onset at the time of parturition, even in experienced mothers.

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Increased maternal extracellular serotonin levels beneficially influences offspring's anxiety- and anhedonia-like behaviour

Judith Homberg[1], Sabrina Hanswijk[1], Marcia Spoelder[1], Michel Verheij[1], Weizhou Li[2], Chunqing Liu[2], Jan Buitelaar[1]

[1]Donders Institute for Brain, Cognition, and behaviour, Radboud University Medical Centre, Cognitive Neuroscience, Kapittelweg 29, 6525 EN Nijmegen, Netherlands

[2]College of Medical Laboratory, Dalian Medical University, Dalian, Liaoning 116044, China

Serotonin is a critical player in brain development whereby serotonin neurotrophic actions can be regulated through maternal-foetal interactions. Hence, maternal rather than offspring's serotonergic genotype may determine variation in serotonin levels in the early foetal brain, which might result in downstream effects on the development of the brain and potentially influencing behaviour. Indeed, serotonin has been shown to be involved in psychiatric disorders such as autism, anxiety, and depression, but the nature of its etiology so far is unclear.

In our first study, we investigated whether changes in extracellular serotonin levels due to serotonin transporter (SERT) availability (SERT rat model) in the mother influenced the maternal care. Maternal care is a major constituent of early life environment and seems to be related to offspring's behaviour and serotonin levels. We observed that one of the most prominent forms, licking-grooming their offspring, is significantly less often performed by SERT knockout (KO) dams then SERT wildtype (WT) dams. Thus, variation in licking-grooming behaviour seems to be determined by maternal serotonergic genotype.

To delineate whether maternal serotonergic genotype influences offspring's development through changes in foetal serotonin levels and/or through changes in licking-grooming behaviour, we set up a breeding such that both these two questions could be answered. In this study, the offspring was subjected to several behavioural assessments. Our data showed that potential alterations in foetal serotonin levels (KO mother) and a decrease in licking-grooming behaviour (KO care) synergistically strengthen their impact on behaviour. More specifically, we observed diminished anxiety (elevated plus maze test) and diminished anhedonia (sucrose consumption test) in adult offspring from SERT KO mothers which received SERT KO care.

These findings indicate that genetically-induced increases in maternal extracellular serotonin levels has a beneficial effect on offspring's behaviour due to both potentially alterations in foetal serotonin levels and decreased maternal licking-grooming behaviour. For this reason, maternal SERT genotype seems to be involved in the development of psychiatric disorders.

To understand in which direction the maternal SERT genotype alters foetal serotonin levels we are currently investigating serotonin metabolism in the placenta, and foetal forebrain and hindbrain, by high performance liquid chromatography.

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High and low serotonin: implications for neuropsychiatric disorders.

Agnieszka Nikiforuk[1]

[1]Institute of Pharmacology, Polish Academy of Sciences, Department of Behavioural Neuroscience and Drug Development, Smetna 12, 31-343 Kraków, Poland

An increasing amount of data indicate that while central serotonin (5-HT) functions are important for normal brain development, an altered 5-HT signalling may be associated with neurodevelopmental disorders, including autism spectrum disorders (ASD). It has been suggested that either lowered or enhanced 5-HT tone during development may lead to autism-related deficits. The ASD symptoms are grouped in two main diagnostic criteria: social/communicative deficits and restricted, repetitive patterns of behaviours. These symptoms manifest themselves at early childhood, persist into adulthood and limit or impair everyday life. ASD is also accompanied by diversity of comorbid features, as for example, anxiety, hyperactivity, impulsivity, inattention, irritability, sensory abnormalities and cognitive deficits. Key roles in the regulation of 5-HT tone play tryptophan hydroxylase 2 (TPH2, a rate-limiting enzyme of serotonin synthesis) and the serotonin reuptake transporter (SERT). Therefore, SERT- and TPH2 - deficient rats represent valuable models to study the consequences of neurodevelopmental central 5-HT enhancement or depletion, respectively. To further evaluate the role of central 5-HT in the manifestation of autistic-like phenotype, SERT-KO and TPH2-KO rats were subjected to a series of tests that are known to reflect core and comorbid ASD symptoms. The results revealed a complex patter of changes, suggesting that either increased or decreased 5-HT levels may lead to certain aspects of autistic-like phenotype.

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The Impact of Serotonergic Signaling in Astrocytes

Franziska E. Müller, Sophie K. Schade[1], Volodymyr Cherkas[1], Andre Zeug[1], Evgeni Ponimaskin[1]

[1]Medizinische Hochschule Hannover, Zelluläre Neurophysiologie, Carl-Neuberg Str.1, 30625 Hannover, Germany

Serotonin is an important neurotransmitter regulating various brain functions via activation of specific serotonin receptors (5-HTRs), known to be expressed by neurons but interestingly also by astrocytes. The unique morphology of these glia cells allows single astrocytes to modulate thousands of synapses over distinct anatomical regions. It is also expected that astrocytes' Ca^{2+} signaling is implicated in these functions. Therefore, it is important to understand which signaling cascades are involved in controlling astrocyte morphology.

In neurons, 5-HTRs can modulate multiple signaling pathways including activation of small GTPases of the Rho family, which determine cell morphology. We investigate molecular mechanisms by which 5-HTRs regulate small GTPases of the Rho family to control astrocyte morphology and astrocyte Ca^{2+} signaling.

We show that astrocytes express the 5-HT_4R *in vivo* and in an *in vitro*-model of primary mouse hippocampal astrocyte cultures. Using FRET-based biosensors, we show that 5-HT_4R activation results in increased RhoA activity as well as elevated cAMP levels, indicating a functional coupling to Gα13 and GαS, respectively. Furthermore, transient expression of constitutively active variants of the small GTPase RhoA results in drastic morphological changes with decreased size and perimeter of the astrocytes. Sholl analysis also reveals an impact of RhoA on the arborization of mouse hippocampal astrocytes. 5-HT_4R stimulation leads to a reorganization of the actin cytoskeleton, presumably via the Gα13-RhoA signaling pathway, therewith influencing astrocyte morphology and function.

Moreover, our data suggest that astrocyte morphology correlates with their Ca^{2+} dynamics. Together, these data indicate that 5 HTRs are critically involved in the regulation of astrocyte morphology and Ca^{2+} signaling and thus 5-HTR activation in astrocytes can have substantial impact on neuronal networks.

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Symposium

S6: Novel insights into the regulation of hypothalamic neurocircuits and functions

S6-1 Central amygdala circuits controlling appetitive behavior
Ruediger Klein

S6-2 Temporal separation of neuronal ensembles in hypothalamus regulates innate behaviours
Alexey Ponomarenko, Marta Carus-Cadavieco, Maria Gorbati, Franziska Bender, Suzanne van der Veldt, Changwan Chen, Mihaela Anca Corbu, Christoph Börgers, Soo Yeun Lee, Charu Ramakrishnan, Natalia Denisova, Franziska Ramm, Karl Deisseroth, Tatiana Korotkova

S6-3 TRP ion channels – internal/hypothalamic temperature sensors and guardians of homeostasis?
Jan Siemens, Gretel Kamm, Juan Boffi, Kun Song, Hong Wang

S6-4 UCP2 in astrocytes regulates the activation of NPY neurons to control feeding behavior
Cristina Garcia Caceres

S6-5 Remodeling of the hypothalamic vasculature upon hypercaloric feeding depends on astroglial HIF1α and VEGF
Tim Gruber, Chenchen Pan, Beata Legutko, Ali Ertürk, Cristina García-Cacéres, Tamas L. Horvath, Matthias H. Tschöp

S6-6 Applying unsupervised machine learning to study the lateral hypothalamic circuitry underlying motivated behaviour in freely moving mice
Hanna Elin van den Munkhof

Central amygdala circuits controlling appetitive behavior

Ruediger Klein[1]

[1]Max-Planck Institute of Neurobiology, Molecules - Signaling - Development, Am Klopferspitz 18, 82152 Martinsried, Germany

The complex behaviors underlying reward seeking and consumption are integral to organism survival. The hypothalamus and mesolimbic dopamine system are key mediators of these behaviors, yet regulation of appetitive and consummatory behaviors outside of these regions is poorly understood. The central nucleus of the amygdala (CeA) has been implicated in feeding and reward, but the neurons and circuit mechanisms that positively regulate these behaviors have remained unclear until recently. We have defined the neuronal mechanisms by which CeA neurons promote food consumption. Using in vivo activity manipulations and Ca2+ imaging in mice, we found that GABAergic serotonin receptor 2a (Htr2a)-expressing CeA neurons modulate food consumption, promote positive reinforcement and are active in vivo during eating. We have demonstrated electrophysiologically, anatomically and behaviorally that intra-CeA and long-range circuit mechanisms underlie these behaviors. We have also shown that CeA-Htr2a neurons receive inputs from feeding-relevant brain regions. These results have illustrated how defined CeA neural circuits positively regulate food consumption (Douglas, Kucukdereli, Ponserre, et al., Nat. Neurosci., 2017). More recently, we have begun to anatomically and functionally map the inputs and outputs of the two major central amygdala subpopulations, the appetite-stimulating CeA-Htr2a neurons and anorexigenic CeA-PKCä neurons, to learn how the central amygdala participates in learning processes that link environmental cues with food availability or food quality.

Temporal separation of neuronal ensembles in hypothalamus regulates innate behaviours

Alexey Ponomarenko[1], Marta Carus-Cadavieco[1,2], Maria Gorbati[2], Franziska Bender[1], Suzanne van der Veldt[3], Changwan Chen[2], Mihaela Anca Corbu[2], Christoph Börgers[4], Soo Yeun Lee[5], Charu Ramakrishnan[5], Natalia Denisova[1], Franziska Ramm[1], Karl Deisseroth[5], Tatiana Korotkova[2]

[1]Institute of Clinical Neurosciences and Medical Psychology, Medical Faculty, Heinrich Heine University, Universitaetsstr. 1, 40225, Dusseldorf

[2]Max Planck Research Group "Neuronal circuits and behavior", Max Planck Institute for Metabolism Research, Cologne

[3]Integrated Program in Neuroscience (IPN), Montreal Neurological Institute, Montreal, Quebec, Canada

[4]Department of Mathematics, Tufts University, Medford, MA 02155, USA

[5]Department of Bioengineering and Department of Psychiatry and Behavioral Sciences, W080 Clark Center, 318 Campus Drive West, Stanford University, Stanford, CA 94305, USA

During network oscillations, neuronal populations typically fire at times when local inhibition is overridden or followed by an excitatory input or due to rebound spiking upon cessation of inhibition. These temporal control mechanisms have been well studied in the cerebral cortex and hippocampus in relation to perception and spatial representations. It has been demonstrated that intra and extra-hippocampal (medial entorhinal) inputs to pyramidal cells convey distinct information modalities at different phases of network oscillations. We aimed at revealing significance of these mechanisms for the generation of vital for survival innate behaviours including food intake and exploratory locomotion. Using electrophysiological recordings of multiple isolated neurons in combination with optogenetics in behaving mice we have investigated timing of neuronal discharge in the hypothalamus and it's forebrain inputs, signalled via the lateral septum (LS), a region crucial for initiation of adaptively appropriate innate behaviours. We found that the timing of neuronal discharge in the LS is modulated by theta (5-10 Hz) and gamma (30-90 Hz) oscillations, coordinated with hypothalamic network oscillations. Being a virtually entirely inhibitory nucleus, LS provides oscillatory inhibition to the hypothalamus, including the lateral hypothalamic area, as indicated by correlations of LH firing times with phases of LS gamma oscillations. Surprisingly, LS inputs preferentially influenced activity of LH neurons, active at the food location, associated with a prominent inhibition of these „food-zone match cells" at times of the maximal rhythmic activity of the LS population. This mechanism enabled a temporal separation of LH cells' activity depending on their functional identity and, furthermore, increased firing rate of „food-zone mismatch cells". Optogenetic facilitation of gamma oscillations in the LS-LH pathway promoted food-seeking behaviour without increasing food intake. In contrast, fast stimulation of Vgat – cells in the LH, known to evoke food intake, increased firing of „food-zone match cells". These results demonstrate a novel feed-forward inhibitory projection-specific mechanism, which enable forebrain inputs to influence mutual timing and firing rates of hypothalamic populations and regulate innate behaviours.

TRP ion channels – internal/hypothalamic temperature sensors and guardians of homeostasis?

Jan Siemens[1], Gretel Kamm[1], Juan Boffi[1], Kun Song[1], Hong Wang[1]

[1]University of Heidelberg, Department of Pharmacology, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany

Transient receptor potential (TRP) ion channels have been identified as versatile, multimodal molecular sensors. Particularly, several members of the extended TRP ion channel family detect temperature changes in the somatosensory nervous system. Pharmacology and genetic deletion experiments have shown that TRP channels are necessary for mediating responses to painfully hot or cold temperatures and they become sensitized under inflammatory conditions leading to exacerbated nociceptive signals. TRPs have therefore emerged as targets for analgesic therapy.

Besides constituting a warning system alerting us about noxious thermal conditions, temperature detection in the innocuous range serves another important feat: Mammalian organisms possess the remarkable ability to maintain internal body temperature (T_{core}) within a narrow range close to 37°C despite wide environmental temperature variations. The brain's neural "thermostat" is made up by central circuits in the hypothalamic preoptic area (POA), which orchestrate peripheral thermoregulatory responses to maintain T_{core}. How the POA detects temperature and integrates temperature information to achieve thermal balance is largely unknown.

I will present our recent findings that implicate TRP channels in hypothalamic thermoregulation. I will conclude with an outlook on future experiments geared to address key questions concerning internal temperature detection and thermoregulation.

UCP2 in astrocytes regulates the activation of NPY neurons to control feeding behavior

Cristina Garcia Caceres[1]

[1]INSTITUTE FOR DIABETES AND OBESITY, ASTROCYTE BIOLOGY UNIT, Business Campus Garching, Parkring 13, 85748 GARCHING, Germany

Research dissecting brain control over systemic metabolism traditionally focuses on neurons. However, our recent discoveries support that astrocytes respond to hormones and nutrients and cooperate with neurons to control energy homeostasis. Furthermore, we uncovered that high-fat high-sugar (HFHS) feeding rapidly induces astrogliosis in the arcuate nucleus of the hypothalamus (ARC), with changes in astrocytes interactions with blood vessels and neurons. We then interrogated if HFHS diet leads to mitochondrial alterations in ARC astrocytes that might affect their functionality and their crosstalk with neurons contributing to obesity pathogenesis. Although, in diet-induced obese (DIO) mice ARC astrocytes, the number of mitochondria unchanged, their size was significantly increased. Next, we identified uncoupling protein 2 (UCP2) as one of the most deregulated mitochondrial genes in primary hypothalamic astrocytes exposed to HFHS-derived blood borne metabolic factors. To explore if HFHS-induced mitochondria alterations in ARC astrocytes of DIO mice was due to alterations in astrocytic UCP2 function, we ablated UCP2 from hGFAP (glial fibrillary acidic protein)-positive astrocytes (GFAP-UCP2 KO mice) in adult mice fed with a chow diet. Interestingly, GFAP-UCP2 KO mice exhibited similar mitochondria morphological alterations in astrocytes than those observed in DIO mice. Likewise, those mice displayed hyperphagia, which was associated with an increased number of Neuropeptide Y (NPY) activated neurons (cFos positive NPY neurons) in the ARC versus their WT littermates. In addition, GFAP-UCP2 KO mice exhibited a reduced feeding response to ghrelin, which was consistent with a reduced increase in ghrelin-induced activation of ARC NPY neurons. We finally observed that the lack of astrocytic UCP2 accceraleted diet-induced obesity in mice. Overall these findings suggest that UCP2-dependent astrocytic metabolic processes play an important role for engaging appropriate hypothalamic responses to whole-body nutritional and endocrine status, presumably by regulating the activation of NPY neurons to control feeding behavior.

Remodeling of the hypothalamic vasculature upon hypercaloric feeding depends on astroglial HIF1α and VEGF

Tim Gruber, Chenchen Pan[4], Beata Legutko[1], Ali Ertürk[4], Cristina García-Cacéres[1], Tamas L. Horvath[5], Matthias H. Tschöp[1,2,3]

[1]Helmholtz Diabetes Center & German Diabetes Research, Helmholtz Center Munich
[2]Institute for Advanced Study, TUM, Munich, Germany
[3]Division of Metabolic Diseases, TUM, Munich, Germany
[4]Institute for Stroke and Dementia Research, Klinikum der Universität München
[5]Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT, USA

Obesity and diabetes exert deleterious impact on neurovascular beds, a phenomenon most prominently observable in the retina of the eye. We recently reported that high-fat high-sugar (HFHS) diet exposure induces neovascularization within the hypothalamus of mice and obese humans, a process partly reminiscent of diabetic retinopathy. In order to assess the impact of an obesogenic diets on the architecture of the mouse brain vasculature more globally we now combined fluorescent angiography, optical tissue clearing and 3D whole-brain imaging to reveal the hypothalamus being a particularly vulnerable brain region in a hypercaloric environment. Initially suspected to be a rather chronic process we found vascular remodelling to already coincide with early changes in body composition upon just two weeks of HFHS feeding. In order to identify which mechanisms are involved in the initiation of this pathologic vascularization during nutritional excess we interrogated bioenergetic changes within the hypothalamus. Here we provide first evidence that short-term HFHS diet exposure rapidly increases hypothalamic cellular respiration, which is associated with a reduced local oxygen availability, a phenomenon not found in other brain regions. This apparent mismatch of oxygen demand and supply was further found to correlate with significantly increased hypoxia-inducible factor 1α (HIF1α) protein levels in the hypothalamus as well as its downstream mediator, vascular-endothelial growth factor (VEGF). Interestingly, the diet-induced increase in VEGF immunoreactivity predominantly co-localized with glial-fibrillary acidic protein (GFAP)-positive astrocytes. Given that astrocytes are an integral part of the neurovascular interface we generated astrocyte-specific mouse models to postnatally interfere with components of the HIF1α –VEGF axis in those glial cells. Interestingly, we found that ablating HIF1α from astrocytes by using a tamoxifen-dependent loss-of-function mouse model entirely prevented the up-regulation of VEGF as well as the hypothalamic angiogenic response upon a HFHS diet. Likewise, the knock-down of VEGF in hypothalamic astrocytes by virus-mediated expression of small-hairpin RNA driven from a synthetic GFAP-promoter recapitulated these findings. Finally, viral overexpression of VEGF in hypothalamic astrocytes otherwise devoid of HIF1α induced marked hypervascularization corroborating the sufficiency of VEGF as primary angiongenic factor in-vivo. Overall these findings indicate that a hypercaloric environment leads to rapid bioenergetic changes within the hypothalamus while inducing the HIF1a-VEGF cascade in local astroglia, which we identified to be a prerequisite for obesity-associated angiopathy of the hypothalamus.

Applying unsupervised machine learning to study the lateral hypothalamic circuitry underlying motivated behaviour in freely moving mice

Hanna Elin van den Munkhof

[1]Max Planck Institute for Metabolism Research, Neuronal circuits and behaviour, Gleueler Str 50, 50931, Cologne, Germany

To understand the brain functions, it is crucial to relate neural activity to behaviour. While highly advanced tools exist to characterize neuronal activity in behaving animals, current methods for the analysis of behaviour rely on manual scoring or identification using supervised machine learning (1). Manual scoring is highly subjective, while supervised machine learning is automated but still assesses a limited set of behaviours predefined by a human observer. Since behaviours are regulated by changes in neuronal dynamics occurring at a fast, sub-second, time scale, we are in need of more refined and objective methods to analyse behaviour at high temporal resolution.

Here we apply MoSeq, an unsupervised machine learning algorithm (2,3), to automatically analyse mouse behaviour based on depth images. MoSeq allows for the unbiased identification of behaviour, uncovering novel behaviours with sub-second precision. Wiltschko et al. (2) have shown that mouse behaviour consists of a sequence of reused modules with defined transition probabilities, which can change depending on environment, genetic or neural manipulation.

We use MoSeq to study the lateral hypothalamic circuitry underlying motivated behaviour, with a focus on primary rewards, including feeding. The lateral hypothalamus (LH) comprises multiple cell types, each of which has a unique function in the regulation of innate behaviours. We have recently shown a role of GABA cells in the LH in feeding-related behaviours and arousal (4,5). To assess the functions of other neurochemically defined cell groups in the LH, we now manipulate activity of these cells using chemogenetics (DREADDs), while mice engage in a range of innate behaviours. We aim to gain insight into implication of these LH subpopulations in primary rewards. Ultimately, a better understanding of the neurocircuitry directing motivated behaviour will aid in treatment improvements for various psychiatric disorders, including eating disorders, addiction and depression.

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Symposium

S7: Short-term adaptation in early auditory processing: from synaptic depression to focal perception

S7-1 Regulation of auditory nerve synaptic function by activity
Matthew A. Xu-Friedman

S7-2 Building fast and resilient inhibitory synapses with Ca2+ nanodomains and microdomains
Henrique von Gersdorff, Dennis Weingarten, Nicolas Müller, Eckhard Friauf

S7-3 Novel form of synaptic plasticity: rebound effect at MNTB-LSO inputs
Elisa G Krächan, Martin Fuhr, Tatjana T Schmitt, Jennifer Winkelhoff, Isabell Paulußen, Eckhard Friauf

S7-4 Adaptation to stimulus statistics enhances the separability between interaural level differences on a population basis
Jörg Encke, Helge Gleiss, Andrea Lingner, Todd R. Jennings, Sonja Brosel, Lars Kunz, Benedikt Grothe, Michael Pecka

S7-5 Time course of stimulus-history dependent adaptation of auditory spatial perception
Andrea Lingner, Michael Pecka, Christian Leibold, Benedikt Grothe

S7-6 cortical mechanisms underlying stimulus-specific adaptation and deviance detection
Israel Nelken

Regulation of auditory nerve synaptic function by activity

Matthew A. Xu-Friedman[1]

[1]University at Buffalo, SUNY, Dept. Biological Sciences, 109 Cooke Hall, 14260 Buffalo, NY, USA

The auditory system is exposed to a wide range of activity conditions. This poses a problem for central auditory synapses, because they must maintain fidelity and avoid depletion by activity. We have found that synapses formed by the auditory nerve adapt to their level of activity, changing their levels of depression and the sizes of the readily releasable pool bidirectionally, depending on acoustic experience. Presynaptic changes in depression result from changes in calcium signalling. In addition, there are postsynaptic changes in excitability. We used dynamic clamp to show that both pre- and postsynaptic changes influence fidelity. These experiments provide important information about how synapses optimize themselves for different activity conditions. Our results may have implications for how abnormal acoustic activity can lead to problems such as tinnitus and language processing disorders.

Building fast and resilient inhibitory synapses with Ca2+ nanodomains and microdomains

Henrique von Gersdorff[1], Dennis Weingarten[1,2], Nicolas Müller[2], Eckhard Friauf[2]

[1]Vollum Institute, Oregon Health & Science University, Portland, Oregon, USA

[2]Animal Physiology Group, University of Kaiseslautern, Kaiserslautern, Germany

Excitatory synapses in the auditory brainstem can process sound information with exquisite temporal precision. However, less is known about how inhibitory synapses shape the processing of sound signals and how they develop after hearing onset. We have recently investigated synapses of the lateral superior olive (LSO) in the mouse auditory brainstem. Using whole-cell patch clamp recordings in acute brainstem slices we characterized inputs from the medial nucleus of the trapezoid body (MNTB) and the cochlear nucleus (CN) to principal neurons of the LSO. The MNTB-LSO synapse is glycinergic and CN-LSO synapse is glutamatergic. We used electrical afferent fiber stimulation to elicit EPSCs and IPSCs. Recordings were done at 36°C from pre-hearing mice at postnatal day P10-12 and young adults at P28-34. Using high-frequency stimulation at 50, 100 and 200 Hz the synaptic parameters could be determined. Like the calyx of Held synapse in the MNTB after hearing onset, CN-LSO synapses showed an increase in their number of readily releasable vesicles (RRP). However, MNTB-LSO glycinergic synapses showed a rapid form of short-term depression followed by robust facilitation. Surprisingly, the RRP of MNTB-LSO glycinergic synapses dropped from 600 vesicles at P10-12 to below 300 vesicles at P28-34. To counteract this reduced number of vesicles and rapid synaptic depression, these synapses developed a robust frequency-dependent vesicle replenishment not present in pre-hearing synapses. The slow Ca2+ buffer EGTA and the K+-channel blocker TEA had little effect on the extent of replenishment in young synapses. These immature synapses seem to exhibit active zones with docked vesicles tightly coupled to Ca2+ channels (Ca2+ nanodomain triggered exocytosis). However, mature synapses showed a two-fold higher vesicle replenishment with increased Ca2+ and a drop to ~50% of steady-state IPSC amplitude compared to P10-12 under EGTA. Mature synapses were thus surprisingly sensitive to EGTA. This was in stark contrast to previous findings in the developing calyx of Held synapse. In summary, mature MNTB-LSO glycinergic synapses develop a remarkably fast vesicle replenishment, specialized for faithful and sustained steady-state inhibition during high-frequency activity, using both Ca2+ nanodomain and microdomain mediated exocytosis.

Novel form of synaptic plasticity: rebound effect at MNTB-LSO inputs

Elisa G Krächan, Martin Fuhr[1], Tatjana T Schmitt[2], Jennifer Winkelhoff[1], Isabell Paulußen[1,3], Eckhard Friauf[1]

[1]TU Kaiserslautern, Department of Biology, Animal Physiology
[2]TU Kaiserslautern, Department of Biology, Physiology of Neuronal Networks
[3]Medical School University of Bonn, Department of Neurosurgery, Experimental Neurophysiology

Sound localization in the mammalian auditory brainstem is achieved by processing interaural time and level differences (ILD). Synapses involved in these tasks are capable of transmitting signals in a precise and reliable manner, even during sustained high-frequency activity. Such synapses, e.g. inner hair cell ribbon synapses and endbulbs and Calyces of Held, are equipped with specific morphological features. The lateral superior olive (LSO) is a hub in the ILD pathway and weighs excitatory against inhibitory signals from the ipsilateral and contralateral ear. LSO inputs lack such morphological specifications yet can also process binaural signals in the millisecond range over sustained periods. Here, we assessed the characteristics of inhibitory, glycinergic and excitatory, glutamatergic LSO inputs in response to continuous (60 s) high-frequency trains (50-200 Hz). For comparison, we offered bursts followed by gaps of silence, a more physiological pattern. We performed whole-cell voltage-clamp recordings in juvenile (P11) and young adult (>P20) mice, while electrically stimulating glutamatergic inputs from the cochlear nucleus (CN-LSO) or glycinergic inputs from the medial nucleus of the trapezoid body (MNTB-LSO). ePSCs of both input types showed frequency-dependent short-term depression (STD). Gaps reduced STD via within-gap replenishment of synaptic vesicles. Analysis of $ePSC_1$ in each burst, a readout of within-gap replenishment, revealed unexpected amplitude increases after initial STD. These increases, which we named rebound effect, were present at about 80% of P11 MNTB-LSO synapses, yet only at frequencies ≥100 Hz. The probability of its emergence as well as its extent depended on the gap length, shorter gaps increasing bot parameters. The gradual increase of $ePSC_1$ amplitudes began remarkably late (20 s), reaching a maximum 25 % higher than the maximal STD level. Furthermore, it lasted until the end of the 60-s train. It is of presynaptic origin because the quantal size was constant during the 60-s train, thus excluding postsynaptic receptor desensitization or saturation. Neither lowering $[Ca^{2+}]_o$ nor buffering residual Ca^{2+} via EGTA-AM affected the rebound. Whereas $eIPSC_1$s increased, subsequent ePSCs in each burst became reciprocally smaller. This resulted in unchanged cumulative ePSC amplitudes per burst and thus points to an increase in release probability (P_v). The assumption was confirmed when the instantaneous readily releasable pool (RRP) was calculated via forward extrapolation of $eIPSC_{1-3}$. RRP declined within the first 20 s and stayed constant thereafter. In contrast, P_v increased strongly from 20 % at the beginning to an steady state level of 80 % at 30 s. Increasing the initial Pv via TEA reduced the rebound extent by decreasing the dynamic range of P_v which seemed to seal at 80 %. The rebound effect increased the temporal precision by stabilizing $eIPSC_1$ peak latencies and decreasing latency jitter. The ratio of inputs displaying a rebound effect declined with age to 50 % at P20, whereas the extent and timing remained unchanged. CN-LSO inputs displayed no rebound effect, although heterogeneous plasticity was found. Collectively, we describe a novel form of plasticity at inhibitory MNTB-LSO synapses. It converts tonic responses to onset responses and may thereby enable efficient computation of repetitively offered sound bursts with high temporal precision.

Adaptation to stimulus statistics enhances the separability between interaural level differences on a population basis

Jörg Encke, Helge Gleiss[2], Andrea Lingner[2], Todd R. Jennings[2], Sonja Brosel[2], Lars Kunz[2], Benedikt Grothe[2], Michael Pecka[2]

[1]Bio-Inspired Information Processing, Technical University of Munich, Garching, Germany.

[2]Division of Neurobiology, Department of Biology II, Ludwig-Maximilians University Munich, Martinsried, Germany

Discriminating individual sound sources in complex environments is essential in daily life. It is often assumed that the underlying neuronal representation is optimized to encode the absolute location of a sound source. Recent findings, however, reported stimulus dependent adaptation of this representation which might result in a deterioration in the encoding of absolute sound location. Here, we investigate the impact of adaptation on the encoding of interaural level differences in naturalistic listening conditions. We show that neurons in the brainstem of gerbils exhibit pronounced dynamic range adaptation (DRA). Within individual neurons, this adaptation conveyed little information due to high response variability. However, if analyzed on a population level, the DRA focally improved source separability and maximized coding efficiency. Using intrinsic energy imaging and modeling, we demonstrate that this increase in efficiency was facilitated by slow negative feedback. These findings suggest that the neuronal representation of interaural level differences might be optimized for efficient sound source separability instead of absolute location.

Time course of stimulus-history dependent adaptation of auditory spatial perception

Andrea Lingner[1], Michael Pecka[1], Christian Leibold[2], Benedikt Grothe[1]

[1]Ludwig-Maximilians-Universitaet Munich, Division of Neurobiology, Department Biology II, Großhadernerstrasse 2-4, D-82152 Martinsried, Germany

[2] Bernstein Center for Computational Neuroscience Munich, Großhaderner Straße 2-4, D-82152, Martinsried, Germany

Traditionally, the auditory system is thought to serve reliable sound localization. Recent electrophysiological evidence however indicates that specific feed-back systems in the early binaural pathway cause the subjectively perceived location of sounds to be strongly influenced by immediately preceding stimuli. These findings contradict the canonical concept of sound localization and raise questions about the functional significance of these feed-back loops.
Here we present data from human psychoacoustical experiments investigating the nature and time course of context-dependent spatial sensitivity. In accordance with previous human psychophysical studies, we found pronounced shifts in the spatial perception of sounds away from a prior presented adapter sound, corresponding to a substantial miss-judgment of absolute sound source positions in the range of tens of degrees of auditory space. Intriguingly, these localization errors are confined to locations close to the adapter position. As a result of this spatial constraint, listeners experienced a selective dilation and compression of auditory perceptional space relative to the adapter location. Consequentially, human listeners reported a focal increase in spatial resolution near the adapter location on the expense of locations further away (Lingner et al., Sci Rep. 2018 May 29; 8(1):8335). Recently, we discovered that these pronounced shifts in the spatial perception of sounds are present over a time period lasting from hundreds of milliseconds up to a couple of seconds.

Together, our findings indicate the need for a new concept for the coding of auditory space which is based on the premise that spatial hearing in mammals serves relative separation rather than absolute sound localization.

cortical mechanisms underlying stimulus-specific adaptation and deviance detection

Israel Nelken[1]

[1]Hebrew University of Jerusalem, Edmond and Lily Safra Center for Brain Sciences, Edmond J. Safra Campus, 91904 Jerusalem, Israel

Stimulus-specific adaptation (SSA) is the reduction in the responses to a repeated stimulus that doesn't generalize to other stimuli. Using recordings with dense microelectrode arrays (Neuropixels electrodes) we studies SSA in the inferior colliculus, medial geniculate body (the auditory thalamus), and auditory cortex. SSA to pure tones is robust in all three structures, although in IC and MGB it tends to be concentrated in the non-leminscal subdivisions. SSA to broadband stimuli, however, is robust in auditory cortex, and is weak in subcortical stations of the auditory system. SSA in subcortical stations is largely compatible with cross-frequency integration of simply-adapting inputs (Adaptation in Narrowly Tuned Frequency channels, ANFC), while cortical SSA is not. Using intracellular recordings and optogenetic manipulations of inhibitory interneurons in auditory cortex, I will show how the cortical circuitry may participate in shaping SSA to both pure tones and broadband stimuli.

Symposium

S8: From astrocytes to behaviors: searching the cellular and molecular roots of emotion dysfunctions

S8-1 Partner loss impairs brain oxytocin signalling: physiological and emotional consequences in monogamous prairie voles
Oliver J. Bosch

S8-2 CB1 receptor signaling in the brain: the where matters
Giovanni Marsicano

S8-3 Astrocytic EphrinA impacts the distribution of synaptic AMPA receptors in health and depression
Barbara Di Benedetto

S8-4 Astrocyte regulation of neuronal excitability
Christine R. Rose

S8-5 Antidepressant drugs require astrocytes to prime an early synaptic pruning and remodelling in the prefrontal cortex
Celia Roman, Annette M. Vogl, Sebastian A. Giusti, Elisabeth Butz, Inga D. Neumann, Damian Refojo, Rainer Rupprecht, Barbara Di Benedetto

S8-6 Oxytocin rapidly affects astrocytic morphology
Carl-Philipp Meinung, Barbara Di Benedetto, Inga D Neumann

Partner loss impairs brain oxytocin signalling: physiological and emotional consequences in monogamous prairie voles

Oliver J. Bosch[1]

[1]University of Regensburg, Department of Behavioural and Molecular Neurobiology, Regensburg Center of Neuroscience, Universitätsstr. 31, 93053 Regensburg, Germany

Brain oxytocin (OT) promotes social behaviour and social bonds, which are vital for physical and mental health. The sudden loss of a partner, however, increases the susceptibility to emotional dysfunctions.
In monogamous prairie voles (Microtus ochrogaster), which form selective pair bonds, separation from the bonded opposite sex partner has detrimental effects on their physiology and emotionality. The physiological effects range from increased heart rate variability to elevated circulating stress hormone levels. With respect to emotionality, partner loss increases anxiety-related behaviour as well as passive stress-coping, indicative of depressive-like behaviour. Though the underlying mechanisms might be diverse, recent and ongoing studies help us to understand the complex interactions in the brain. Losing the partner activates the corticotropin releasing factor (CRF) system, which suppresses the oxytocin (OT) signalling on multiple levels from the paraventricular nucleus downstream to the nucleus accumbens shell (NAcS). Consequently, chronic intra-NAcS infusions of synthetic OT or a CRF receptor type 2 antagonist reverses the separation-induced effects on depressive-like behaviour. In confirmation, the emotionality of paired prairie voles becomes impaired by reducing OT receptor signalling or by activating CRF receptor type 2 in the NAcS. Due to possible interactions of both neuropeptide systems with microglia, together with their role in psychopathologies such as depression and anxiety, an involvement of microglia in separation-induced emotional dysfunctions is likely.
The negative emotional consequences of partner-loss, mediated via the CRF system supressing the OT system, is likely adaptive during short separations to encourage reunion with the partner. Therapeutic strategies targeting these systems should be explored in-depth for the treatment of social loss-mediated emotional dysfunctions.

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CB1 receptor signaling in the brain: the where matters

Giovanni Marsicano[1]

[1]Inserm, NeuroCentre Magendie, 146 rue Leo Saignat, 33077 Bordeaux, France

Cannabinoid drugs (e.g. the active principle of the plant cannabis, D9-tetrahydrocannabinol, THC) exert several effects on the brain via the activation of the G protein-coupled type-1 cannabinoid receptors (CB1). On the other hand, CB1 receptors are part of a physiological system (the endocannabinoid system, or ECS), through which the particular endogenous signaling molecules (the endocannabinoids) control a plethora of brain functions. The effects of exogenous cannabinoids and the physiological roles of the ECS are only partially overlapping. This is likely due to the fact that the ECS has patterns of activation that are extremely regulated in time and space, features that are obviously overcome by massive stimulation of CB1 receptors by exogenous drugs.

During the last years, my laboratory contributed to the understanding of the mechanisms underlying both pharmacological effects of cannabinoids and the physiological functions of the ECS. In particular, we dissected the impact of CB1 receptors expressed in different brain cell types and regions in these effects and functions.

More recently, we identified a novel mode of action of CB1 receptors, namely the direct control of mitochondrial bioenergetic activity as a mean to regulate brain cellular and cognitive processes.

Astrocytic EphrinA impacts the distribution of synaptic AMPA receptors in health and depression

Barbara Di Benedetto[1]

[1]University of Regensburg, Department of Psychiatry and Psychotherapy, Franz-Josef-Strauss Allee 11, 93053 Regensburg, Germany

Brains of major depressive disorder (MDD) patients show aberrant density and morphology of astrocytes and an altered glutamatergic activity in the prefrontal cortex (PFC), two core aspects of the disease which are present in an animal model of MDD, the high anxiety-related behavior (HAB) rats. We investigated astrocytic dysfunctions in MDD using electrophysiology, microarray, chromatin-immunoprecipitation (ChIP), qPCR, immunofluorescent-immunohistochemistry and the forced swim test to measure biomolecular, biochemical and behavioural changes in control and HAB rats. Increasing the astrocytic ephrinA signalling in acute brain slices impaired long-term potentiation at glutamatergic synapses, thus suggesting a role for the astrocyte/neuron ephrinA/EphA system in MDD. Furthermore, intracerebral injections of ephrinA induced a depressive-like behaviour in rats. Using ChIP/qPCR, in HAB primary culture astrocytes we revealed an endogenously increased ephrinA expression accompanied by an accumulation of the epigenetic mark H3K4me3 at its promoter, which was additionally confirmed in vivo in PFC astrocytes of HAB rat brains. Because the ephrinA/EphA system modulates synaptic formation/function, we examined synapses in neurons co-cultured with either ephrinA-enriched HAB astrocytes or with normal astrocytes. We observed no difference in total numbers of "silent" synapses, which corresponded to an unchanged relative enrichment of the AMPA receptor subunit GluR2 on total PSD95-positive spines. But we saw an increased relative number of "active" GluR1/PSD95-positive spines, which may indicate an altered homeostatic plasticity.
We propose that an increased astrocytic ephrinA expression may contribute to an altered synaptic structure which may induce MDD onset. A further exploration of its functional consequences might help to develop diagnostic tools or alternative therapeutic strategies for clinical interventions.

Astrocyte regulation of neuronal excitability

Christine R. Rose

[1] Institute of Neurobiology, Heinrich Heine University Düsseldorf

At tripartite synapses of the vertebrate brain, fine processes of astrocytes reach towards the synaptic cleft, enabling them to sense neuronal activity and transmitter release. For example, astrocytes are endowed with different primary and secondary active transporters for ions which regulate the ion composition of the extracellular space. Among those, the astroglial sodium/potassium ATPase (NKA), which is stimulated by K+ released from active neurons, is critical for the regulation of extracellular potassium concentrations ([K+]e). A weakening and/or failure of astroglial K+ uptake through the NKA results in its accumulation in the extracellular space in active brain regions, driving neuronal depolarization and over-excitability. In addition, astrocytes are responsible for the re-uptake of glutamate at synapses through excitatory amino acid transporters (EAATs). A change in transporter expression levels and/or a reduction in the driving force for glutamate uptake results in altered glutamate clearance in the extracellular space and thereby alters neuronal excitability. Astrocytes also express receptors ionotropic and metabotropic for glutamate and other transmitters. Activation of these transmitter receptors can result in astrocyte calcium signaling, causing the release of gliotransmitters which then feed back onto neuronal activity. Last, but not least, there is a tight metabolic relationship between astrocytes and neurons. Notably, neurons do not contain any significant energy stores. Astrocyte, in contrast represent the dominating glycogen is stores of the brain. It not surprising then that diverse metabolic interactions between these two cell types exist. A prominent example of neuro-metabolic coupling is the so-called astrocyte-neuron lactate shuttle. It proposes that astrocytes increase glucose uptake and glycolysis in response to neuronal activity and then supply neighboring neurons with lactate to support their metabolic needs. This talk will present an overview of these different aspects of astrocyte function at synapses. Moreover, their relevance for neuronal function and excitability in the healthy brain as well as under different pathological conditions will be discussed.

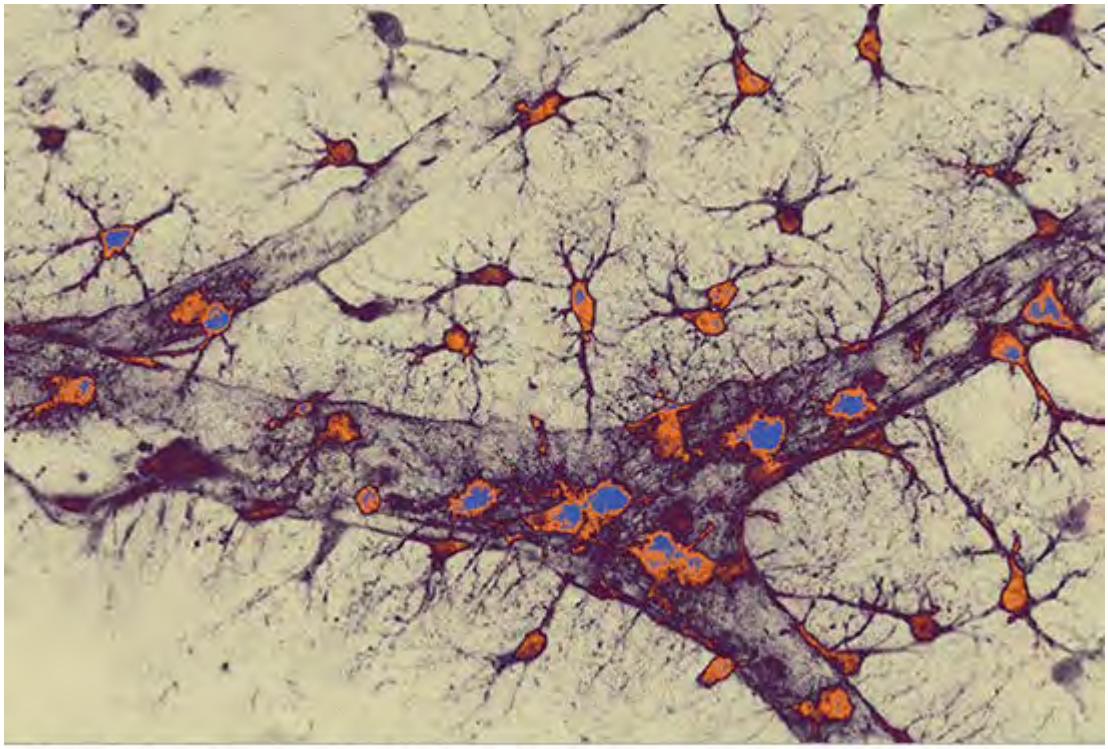


Fig. 1: Astrocytes surrounding a blood vessel in the mouse brain (N. J. Gerkau & J. Meyer; Institute of Neurobiology, HHU Düsseldorf)

Antidepressant drugs require astrocytes to prime an early synaptic pruning and remodelling in the prefrontal cortex

Celia Roman, Annette M. Vogl[2], Sebastian A. Giusti[2], Elisabeth Butz[2], Inga D. Neumann[3,4], Damian Refojo[2], Rainer Rupprecht[1,2,4], Barbara Di Benedetto[1,4]

[1]University of Regensburg, Department of Psychiatry and Psychotherapy, Franz-Josef-Strauß-Allee 11 , 93053 Regensburg, Germany[2]Max Planck Institute of Psychiatry, Germany[3]Department of Neurobiology, University Regensburg, Germany[4]Regensburg Center of Neuroscience, Germany

Astrocytes are non-professional phagocytes that engulf synapses to remodel neuronal circuits. Neuropsychiatric disorders such as major depressive disorder (MDD) are characterized by disrupted synaptic communication and neuronal connectivity, which are reversed by antidepressants (ADs). This suggests a role for astrocyte-mediated phagocytosis in the pathogenesis of these disorders and in response to ADs. Aim of this work was to identify astrocyte-dependent molecular triggers of MDD and examine their impact on neuronal synapses upon AD treatments. Recently, MEGF10 emerged as a mediator of astrocyte-dependent synapse elimination in the developing mammalian brain. MEGF10 activation depends on the induction of the ERK/MAPK pathway, a regulator of cellular plasticity. Our results showed that treatment of astrocytes and neurons with different antidepressants (ADs) led to opposite ERK1/2 activation patterns and a “re-juvenalization” effect in these two cell types. Furthermore, we observed a reduction in neuronal synaptic densities after AD treatment, but only in the presence of intact astrocytes. This astrocyte-dependent synaptic pruning also occurred in the adult rat prefrontal cortex after short-term treatment with the AD fluoxetine and correlated with increased MEGF10 expression in cortical astrocytes. We therefore propose that ADs favour the remodelling of neuronal circuits in the adult brain by reactivating a ‘’juvenile-like plasticity program’’ characterized by an astrocyte-mediated synaptic reshaping and an elevated expression of MEGF10. We suggest MEGF10 as a potential novel candidate to develop alternative treatment options for diseases characterized by synaptic aberrancies, such as MDD.

Oxytocin rapidly affects astrocytic morphology

Carl-Philipp Meinung, Barbara Di Benedetto[2], Inga D Neumann[1]

[1]University of Regensburg, Behavioural and Molecular Neurobiology, Regensburg, Germany
[2]University Hospital Regensburg, Neuro-Glial Pharmacology, Regensburg, Germany

The brain oxytocin (OXT) system has generated a lot of research interest due to its general anxiolytic and prosocial properties. In contrast to neurons, very little is known about the intracellular consequences of astrocytic oxytocin receptor (OXTR) activation. As part of the tripartite synapse, astrocytes modulate neuronal communication, a spatial relationship that is critically set by astrocytic morphology. Therefore, we investigated the effects of OXT-stimulation on astrocytic morphology and proteins involved in the regulation of the astrocytic cytoskeleton in vitro. To this end, synthetic OXT was applied to cultured rat primary cortical astrocytes (isolated from newborn pups postnatal day 1-3). OXT (from 1nM-1µM) caused a dose-dependent increase in both, the length and number of cellular processes as early as 10 min post-administration. Mechanistically, this elongation and process formation was sensitive to pre-treatment with a PKC-inhibitor and- and a MEK-inhibitor, respectively. Also, pre-treatment with an OXTR-antagonist (L368,899; 10µM) prevented the OXT-induced effects on astrocyte morphology. In contrast, the closely related neuropeptide vasopressin (AVP) did not affect the examined parameters. These findings indicate the involvement of OXTR-specific Gq-protein coupled signaling. Furthermore, OXT exposure led to changes in the expression level of proteins involved in cytoskeletal dynamics. Since we did not observe similar effects in neuronal cells, our data point toward a mode of action characteristic to OXT, but not AVP, that specifically affects astrocytic morphology. However, detailed underlying mechanisms involved remain to be revealed.

Supported by the Deutsche Forschungsgemeinschaft DFG GRK2174.

Symposium

S9: Resolving the cognitive function of prefrontal circuits: from neurons to behavior

S9-1 Quantitative whole brain mapping of the monosynaptic input to four different cell types in the mouse medial prefrontal cortex
Marie Carlén

S9-2 Thalamo-Prefrontal Interactions in Working Memory
Christoph Kellendonk

S9-3 Connectivity reveals prefrontal cortical circuit homologies between rodents and primates
Sarah Rachel Heilbronner

S9-4 Prefrontal Cortex Circuits as a Hub for Flexible Learning and Attentional Filtering of Goal-Irrelevant Information
Thilo Womelsdorf

S9-5 Microglia inhibition rescues developmental hypofrontality in a mouse model of cognitive impairment
Mattia Chini, Christoph Lindemann, Jastyn A. Pöpplau, Laura Carol-Perdiguer, Marilena Hnida, Xiaxia Xu, Joachim Ahlbeck, Sebastian H. Bitzenhofer, Christoph Mulert, Ileana L. Hanganu-Opatz

S9-6 Low frequency oscillatory bursts in the macaque prefrontal cortex predict spontaneous transitions in the content of consciousness
Abhilash Dwarakanath

Quantitative whole brain mapping of the monosynaptic input to four different cell types in the mouse medial prefrontal cortex

Marie Carlén[1]

[1]Karolinska Institutet, Neuroscience, Biomedicum, Quarter 4B, 17177 Stockholm, Sweden

My presentation focus on the prefrontal cortex (PFC), and particularly the medial PFC (mPFC) of the mouse (*mus musculus*). Theories on the architecture and local computations of frontal cortical networks are at large based on extrapolations of knowledge derived from investigations of sensory cortical areas, although major differences are well known, including in the cytoarchitecture, and in the functional repertoire. Computations in the mPFC of the mouse are thought to be central to cognitive operations, including decision making and memory. In cortical networks, functionally distinct cell types build local circuit structures enabling computational operations. It is widely held that the cell types' local and long-range connectivity are the most important determinants of the functional repertoire (of any brain areas), but the knowledge regarding the structure and function of the PFC is lagging.

I will present a novel rabies tracing system, and a whole-brain mapping of the afferent connections of four functionally distinct cell types in the mPFC of the mouse (inhibitory VIP-; SST-, and PV-expressing interneurons, respectively, and CaMKII-expressing excitatory neurons, 166, 299 input neurons mapped). I will discuss mPFC's connectivity with key brain structures, and how the cell types' differentiated circuit functions relate to their respective input system. A first mapping of local connectivity using rabies tracing will be presented, revealing possible novel circuit motifs. If time allows, technical challenges and pitfalls in rabies tracing will be covered, including the presence of tracing resistant connectivity.

Thalamo-Prefrontal Interactions in Working Memory

Christoph Kellendonk[1]

[1]Columbia University, Department of Psychiatry, 1051 RIVERSIDE DRIVE, KOLB ANNEX BUIDLING 40 HAVEN, 10032 New York, USA

Traditionally the thalamus has been described as a relay station for sensory information. Accumulating evidence, however, suggests that higher order thalamic nuclei regulate complex behaviors via their interactions with the cortex. Using the mouse as an animal model we recently have shown that inputs from the mediodorsal thalamus to the prefrontal cortex are critical for sustaining cortical delay activity and working memory. Still, the thalamocortical circuit architecture that regulates working memory needs to be determined. For example, it is known that the mediodorsal thalamus projects to superficial and deep layers of the medial prefrontal cortex, but the involvement of superficial cortical layers in working memory is still unclear and will be addressed here. Understanding how thalamo-prefrontal circuits regulate behavior has high relevance for psychiatric disorders including schizophrenia, where thalamo-prefrontal hypoconnectivity has been associated with deficits in working memory.

Connectivity reveals prefrontal cortical circuit homologies between rodents and primates

Sarah Rachel Heilbronner[1]

[1]University of Minnesot, Department of Neuroscience, 2-164 Jackson Hall 321 Church St SE, 55455 Minneapolis, USA

The prefrontal cortex (PFC) is a complex structure with multiple functionally distinct subdomains. Understanding the neural mechanisms of normal and abnormal PFC function requires the use of rodent models; however, it is not always clear how different subdomains map onto each other across species. I am using connectivity with conserved structures (such as the striatum) to establish PFC homologies across rodents, nonhuman primates, and ultimately humans. I analyzed the relationship between conserved areas within the striatum (such as the shell of the nucleus accumbens) and anatomical projections from the anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC) to assess network similarities across rats and monkeys. I showed that anatomical connectivity reveals that some subregions within the PFC are strongly homologous: for example, the infralimbic cortex (rodent) and area 25 (primate) share nearly identical connectivity profiles. In addition, medial-lateral connectivity differences support cross-species similarities in OFC topography. However, dorsal ACC had different connectivity profiles across species. Finally, I will present diffusion tractography connectivity results demonstrating how these homology analyses might be extended to humans, including patient populations. Along with segmenting the striatum and identifying striatal hubs of overlapping inputs, these results help to translate findings between rodent models and human pathology.

Prefrontal Cortex Circuits as a Hub for Flexible Learning and Attentional Filtering of Goal-Irrelevant Information

Thilo Womelsdorf[1]

[1]Vanderbilt University, Psychology, 111 21st Ave South, 37240 Nashville, USA

Neural circuits in the prefrontal cortex (PFC) endow behavior a high degree of flexibility to adjust to changing environmental demands. This flexibility is likely originating from neural computations that learn from past experiences about which environmental features are relevant and irreleavant for ongoing behavioral goals. PFC representations of which features are relevant can than guide (and exert control over) attention and choices to increase behavioral success.
This talk will describe how neuronal activity in nonhuman primate PFC maps onto different stages of reinforcement learning and attentional filtering of distraction. We will describe how these learning- and filtering- related activities in the PFC synchronize to neuronal circuits in other brain circuits in the basal ganglia that more closely reflect actual choices during goal directed behavior. These results suggest specific neural circuit mechanisms underlying flexible learning and efficient attentional allocation.

Microglia inhibition rescues developmental hypofrontality in a mouse model of cognitive impairment

Mattia Chini, Christoph Lindemann[1], Jastyn A. Pöpplau[1], Laura Carol-Perdiguer[1], Marilena Hnida[1], Xiaxia Xu[1], Joachim Ahlbeck[1], Sebastian H. Bitzenhofer[1], Christoph Mulert[2], Ileana L. Hanganu-Opatz[1]

[1]ZMNH - University Medical Center Hamburg-Eppendorf, Developmental Neurophysiology , Falkenried 94 , 20251 Hamburg, Germany

[2]Psychiatry Neuroimaging Branch (PNB), Department of Psychiatry and Psychotherapy, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany

Cognitive deficits, core features of mental illness, largely result from dysfunction of prefrontal-hippocampal networks. This dysfunction emerges already during early development, before a detectable behavioral readout, yet the cellular elements controlling the abnormal maturation are still unknown. Combining *in vivo* electrophysiology and optogenetics with neuroanatomy and behavioral investigation during development in mice mimicking the dual genetic – environmental etiology of psychiatric disorders, we identified pyramidal neurons in layer II/III of the prefrontal cortex as key elements causing disorganized oscillatory entrainment of local circuits in beta-gamma frequencies. We showed that their abnormal firing rate and timing result from sparser dendritic arborization and lower spine density. Moreover, we developed a machine-learning classifier that, using features derived from this population of neurons, predicts with high accuracy whether mice belong to the control or the model group. Transient pharmacological modulation of aberrantly hyper-mature microglia rescues morphological, synaptic and functional neuronal deficits and restores cognitive abilities. Accordingly, the classifier predicts these mice as belonging to the control group. Elucidation of the cellular substrate of developmental miswiring related to later cognitive deficits opens new perspectives for identification of neurobiological targets, amenable to therapies.

Low frequency oscillatory bursts in the macaque prefrontal cortex predict spontaneous transitions in the content of consciousness

Abhilash Dwarakanath

[1]Max Planck Institute for Biological Cybernetics, Physiology of Cognitive Processes, Max-Planck-Ring 8, 72076 Tübingen, Germany

[2]Department of Organismal Biology and Anatomy, University of Chicago, USA

[3]5Cognitive Neuroimaging Unit, CEA, DSV/I2BM, INSERM, Universite Paris-Sud, Universite Paris-Saclay, Neurospin Center, 91191 Gif/Yvette, France

In multistable perception, the content of consciousness alternates spontaneously between mutually exclusive or mixed interpretations of competing representations. Identifying neural signals predictive of such intrinsically driven perceptual transitions is fundamental in resolving the mechanism and localizing the brain areas giving rise to visual consciousness. Here we employed a no-report paradigm of binocular motion rivalry based on the optokinetic nystagmus reflex read-out of spontaneous perceptual transitions coupled with multielectrode recordings of local field potentials and single neuron discharges in the macaque prefrontal cortex. We show that an increase of oscillatory bursts in the delta-theta (1-9 Hz), and a decrease in the beta (20-40 Hz) bands, along with significant perceptual modulation of single neurons during periods of dominance and perceptual switches, are predictive of spontaneous transitions in the content of visual consciousness. These results suggest that the balance of stochastic prefrontal fluctuations is critical in refreshing conscious perception, casting doubt on a posterior cortical mechanism for visual awareness.

Symposium

S11: The 4Rs in animal-based neuroscience research: Refinement, Reduction, Replacement, Responsibili

S11-1 Brain organoids as ideal replacements of animal models in neuroscience? – Chances and limitations of a brain in a dish.
Michael Heide

S11-2 Navigating ethics and evidence in preclinical neuroscience research
Ulrich Dirnagl

S11-3 The Reproducibility Opportunity
Malcolm R Macleod

S11-4 Responsibility includes communication and transparency about animal research
Roman Stilling, Stefan Treue

Brain organoids as ideal replacements of animal models in neuroscience? – Chances and limitations of a brain in a dish.

Michael Heide[1]

[1]Max Planck Institute of Molecular Cell Biology and Genetics, Huttner lab, Pfotenhauerstr. 108, 01307 Dresden, Germany

Brain organoids have quickly established themselves as powerful models of normal and pathophysiological brain development. These *in vitro* systems model many aspects of brain, especially cortical development, but until now they have not succeeded in fully emulating *in vivo* cortical development. Nevertheless, brain organoids have the potential to replace or in certain cases even surpass animal models. In this talk, I would like to discuss (i) how the two major classes of brain organoids are generated and what are the key differences between them; (ii) aspects of *in vivo* cortical development that are recapitulated and aspects that are not (fully) recapitulated *in vitro* by organoids; (iii) cases in which brain organoids can replace or even surpass animal models and cases in which animal models are still superior.

Navigating ethics and evidence in preclinical neuroscience research

Ulrich Dirnagl[1]

[1]Charite Universitätsmedizin Berlin, Department of Experimental Neurology, , Chariteplatz 1, 10117 Berlin, Germany

Millions of people worldwide currently suffer from serious neurological diseases and injuries for which there are few, and often no, effective treatments. The paucity of effective interventions is, no doubt, due in large part to the complexity of the disorders, as well as our currently limited understanding of their pathophysiology. The bleak picture for patients, however, is also attributable to avoidable impediments stemming from quality concerns in preclinical research that often escape detection by research regulation efforts. I will connect the dots between these concerns about the quality of preclinical research and their potential ethical impact on the patients who volunteer for early trials of interventions informed by it. I will do so in hopes that a greater appreciation among preclinical researchers of these serious ethical consequences can lead to a greater commitment within the research community to adopt widely available tools and measures that can help to improve the quality of research.

The Reproducibility Opportunity

Malcolm R Macleod[1]

[1]CAMARADES, University of Edinburgh[1], United Kingdom

It is important for research users to know how likely it is that reported research findings are true. Early definitions of “Reproducibility” related to the re-analysis of existing data following the same analytical procedures. “Replication” was held to require the collection of new data, following the same methods, and applying the same analytical procedures. However, the interchangeable use of these terms (and others) is such that “Reproducible research” has come to have broader meaning than perhaps initially intended. This issue is addressed by Goodman et al (http://stm.sciencemag.org/content/8/341/341ps12).
Articulating this broader definition, and borrowing from Goodman, one might consider a hierarchy of characteristics which might give confidence in the “truth” of a research finding. First, “reproducibility” as originally described, based on reanalysis of an existing dataset (“reproducibility of analysis”). Secondly, the collection of new data in experiments as identical as possible to the first (“reproducibility of experimental findings”). Thirdly, the deliberate variation of experimental conditions or analytical approaches to establish whether the same conclusions can be drawn (“robustness”). Goodman considers 2 more levels, inferential reproducibility (making the same evidentiary claims for the same analytical findings), and generalisability (the extent to which predictions made by experiments are true outside of a research or laboratory setting).
The main focus of recent concern relates to “reproducibility of experimental findings”. This has been studied in retrospective observational (eg at Amgen and at Bayer) and prospective (the various Reproducibility or Replication projects) studies; the findings of these have been that it has not been possible to confirm many findings previously considered to be “true”.
Failed replication (“reproducibility of experimental findings”) in biomedical research may occur if the originator study was falsely positive (by chance, or because the experimental design placed the study at risk of bias); if our understanding of the literature is polluted by publication bias; or in the presence of some unknown (latent) independent variable which influences the phenomenon under study. In this case, what was intended as a test of “replication of experimental findings” was a test of “robustness”. This latter explanation promises the discovery of a previously unknown facet of the process being studied.
Sensible approaches might seek to (1) increase the probability that published research is true (through the development of organised research improvement strategies); (2) establish a framework to select efficiently which research findings we should attempt to replicate (by establishing whether there are study characteristics which predict whether or not a key research finding can be replicated); and (3) develop strategies to evaluate the robustness of key research findings (based on pre-registered, probably multicentre studies, with deliberate heterogeneity). This would provide an opportunity substantially to increase the value of existing and of future research.

Responsibility includes communication and transparency about animal research

Roman Stilling[1], Stefan Treue[1,2]

[1]Informationsinitiative "Tierversuche verstehen", Münster, Germany
[2]German Primate Center - Leibniz Institute for Primate Research, Göttingen, Germany

The past and current success of much of biomedical research critically relies on the combination of many methods, including the use of animals – to study fundamental biological processes or as models for human disease. At the same time, animal welfare in research facilities and studies has made significant progress in the last decades. However, animal-based research has a notoriously bad reputation throughout society and is facing the imminent threat to be further restricted or even stopped altogether based on the political perception of this controversy. We argue that, to a large extent, this situation is due to a lack of transparency and openness to communicate about the necessity, the regulatory framework, and significance of animal research despite the obligation to justify the use of taxpayers' funds.
In this talk we will highlight best-practice measures to increase pro-active communication about animal research at the international, national and local level and will share first-hand experiences with this approach.
Finally, we will showcase the German initiative "Tierversuche verstehen". This platform is supported by the "Alliance of Science Organisations in Germany" and was launched in 2016 to comprehensively and transparently inform the public about all aspects of experimental methods using animals in research.

Symposium

S12: Breaking News

Descending control of two coupled locomotor systems

Felix Clotten, Carmen Smarandache-Wellmann[1]

[1]University of Cologne, Zoological Institute, Animal Physiology , Zülpicher Str. 47b, 50674 Köln, Germany

Locomotion is variable and is utilized in a broad range of behavioral contexts. Thus, the evoked motor output needs to be adapted to specific requirements, i.e. initiated or terminated, or modulated in timing, strength, or direction. In several model systems, both vertebrates and invertebrates, descending input were shown to control the neural micro-circuits underlying locomotion. Even more, in animals those are able of performing different forms of locomotion, for example swimming and walking, it could be shown that the same descending input can control more than one form of locomotion or even the transition between two different locomotor outputs.

The signal crayfish, *Pacifastacus leniusculus*, has two anatomically separated locomotor systems. Paired limbs (swimmerets) on the abdomen can be used for forward swimming and additional pairs of limbs (walking legs) on the thorax are used during walking. Interestingly, during walking, the swimmerets are also rhythmically active to lift up the animal's abdomen and to help balancing the animal on an uneven substrate. The effect of walking activity on the swimmeret system has been previously studied and descending input, i.e. 'command neurons', controlling the motor output has been described for both locomotor systems, respectively. However, so far no information is available about the neural targets of these inputs within the neural micro-circuits.

In this study separated axon bundles in the connectives of the nerve cord were stimulated electrically and the fictive motor outputs of both the swimmeret and the walking system were recorded simultaneously. Histological identification of the stimulation sides revealed that the locations of the stimulated axon bundles were consistent with previously described locations of excitatory and inhibitory 'command neurons'. In addition, observed stimulations effects on either one or both of the systems indicated stimulation of these 'command neurons'.

In a vast majority of preparations the swimmeret system spontaneously expressed rhythmic activity. In contrast to that, the state of activity in the walking system was more diverse and ranged from silent preparations, preparations expressing tonic activity, to preparations that expressed single bouts of rhythmic activity. Electrical stimulations could induce, terminate, or enhance rhythmic activity in both systems, depending on the stimulated axon bundle and the preparation's state of activity described before. The stimulation effects were either restricted to one system, or both systems were affected simultaneously.

Both motor neurons (MN) and presynaptic interneurons of the pattern-generating micro-circuits (CPG-IN) are possible targets of descending input. Therefore, intracellular recordings of MNs and CPG-INs of the swimmeret system were performed to investigate changes in their membrane potential during transition between two states of activity. Both MNs and CPG-INs were affected by stimulation of descending input and showed similar changes in membrane potential compared to spontaneous transition. In general, the changes suggest that CPG-INs rather than the MNs directly are targeted by descending input. However, sub-threshold stimulations as well as individual MN recordings indicate that sub-populations of MNs might be affected differently by descending input.

Testosterone derivatives increase sensitivity of P2X receptors to ATP and antagonize the effect of ivermectin on deactivation

Sonja Sivcev, Barbora Slavikova[3], Marian Rupert[1], Milorad Ivetic[1], Eva Kudova[3], Hana Zemkova[1]

[1]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic;[2]Faculty of Science, Charles University, Prague, Czech Republic;[3]Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic

Extracellular ATP (adenosine-5´-triphosphate) and other nucleotides activate two types of purinergic P2 receptors: ionotropic P2X receptors (P2XRs) and G-protein coupled P2Y receptors. Family of P2XRs comprises seven subunits (P2X1-7) which can assemble as homo- or hetero-trimers. They are ubiquitously expressed and unevenly distributed in the central and peripheral nervous systems, immune system and many other tissues. Although steroids have been already identified to modulate ATP-gated cationic channel activity, the detailed mechanisms that underlie this modulation are poorly understood. Here, we describe a library of testosterone derivatives that effectively modulate the rat P2XRs. We designed and synthesized new derivatives of testosterone to increase the steroid-induced modulatory effect, and performed the structure-activity relationship analysis. The effect of drugs was examined electrophysiologically in HEK293 cells expressing recombinant P2X2R, P2X4R and P2X7R, and pituitary cells or hypothalamic neurons endogenously expressing these receptors. Our measurements showed that 1–30 μM 17β ester derivatives of testosterone modulate positively within 1-min the 1 μM ATP-evoked currents in P2X2R and P2X4R, but not agonist-evoked current in P2X7R. The comparison of chemical structures and whole-cell recordings revealed that the interactions of testosterone derivatives with P2XRs depend on lipophilicity and the length of alkyl chain at position C-17 on the D-ring of testosterone. Most of the tested testosterone derivatives are more potent modulators than the endogenous testosterone. Pretreatment with testosterone butyrate or valerate increases receptor sensitivity to ATP 3.4 fold, reduces the rate of P2X4R desensitization, accelerates resensitization and enhances ethidium uptake by HEK293 cells expressing the P2X4R. The effect of ivermectin, P2X4R-specific allosteric modulator, on deactivation is antagonized by testosterone derivatives in concentration-dependent manner suggesting a binding to position related to ivermectin binding site within transmembrane domain. Our results revealed structural requirements of putative allosteric steroid site(s) for proper P2XR-mediated interactions that might serve as a guide for synthesis of new molecules.

Acknowledgements: This work was supported by grants from the Charles University Grant Agency (928517), the Grant Agency of the Czech Republic (16-12695S, 18-05413S and 304/12/G069), the Ministry of Education, Youth and Sports of the Czech Republic within the LQ1604 National Sustainability Program II (Project BIOCEV-FAR) and by the project BIOCEV (CZ.1.05/1.1.00/02.0109).

A luminance-sensitive cell type in Drosophila facilitates visual contrast computation

Madhura Ketkar, Katja Sporar[1], Lennart Schlieder[3], Marion Silies[1]

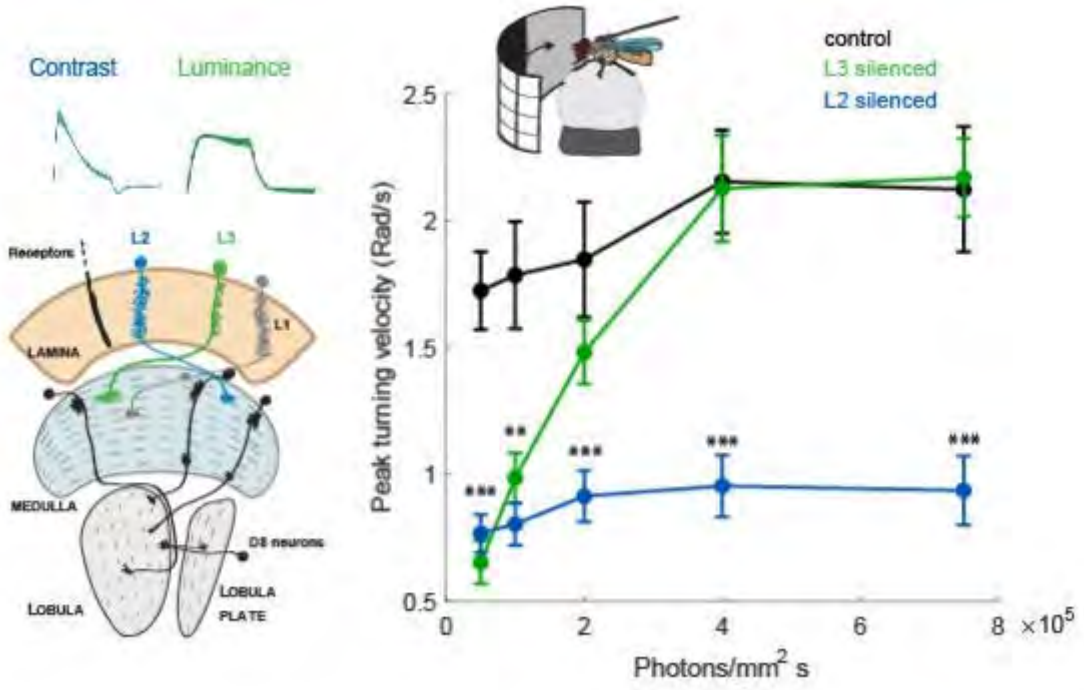
[1]Johannes Gutenberg-Universität Mainz, Institute for Developmental and Neurobiology, Hanns-Dieter-Hüsch-Weg 15, 55128 Mainz, Germany

[2]International Max Planck Research School for Neurosciences, Grisebachstr.5, 37077 Göttingen, Germany

[3]Georg-August-Universität Göttingen, Germany

Contrast, a spatial or temporal difference in luminance, is one of the most informative features of visual cues. For example, motion computation relies on spatiotemporal comparison of contrasts. Photoreceptor cells themselves already extract contrast from visual scenes. However, a fraction of photoreceptor output is carried forward as raw luminance by downstream neurons possessing sustained response profiles, parallel to or in the same neurons that amplify and relay contrast information. Advantages of retaining the luminance information have been speculated, but not verified behaviorally.

Here, we report that a luminance sensitive pathway exists in the visual system of Drosophila and plays a role in behaviorally relevant contrast computation. Downstream of photoreceptors, the L1 and L2 neurons convey contrast information to the ON and OFF pathways respectively. Using *in vivo* two-photon calcium imaging in combination with visual stimulation, we found another OFF pathway neuron L3 to be luminance-sensitive and most active in the dark. Consistent with its physiological specialization, L3 enhances behavioral responses to motion in dim environments. This enhancement is observed when an L2 signal under-represents contrast salience, potentially as a result of constraints in photoreceptor contrast computation. On the other hand, presence of L3 inhibits behavioral responses to motion of small-contrast stimuli. Taken together, these evidences suggest that luminance information, preserved parallel to the contrast information, helps refining the contrast computation in downstream circuitry so as to match behavioral significance of the stimulus. Our work shows how different visual features are combined to facilitate robust behavioral responses to relevant sensory inputs.


Contrast
Luminance
Receptors
L2
L3
L1
LAMINA
MEDULLA
DS neurons
LOBULA
LOBULA PLATE
control
L3 silenced
L2 silenced
Peak turning velocity (Rad/s)
Photons/mm2 s
×10^5
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Olfactory object recognition based on fine-scale stimulus timing in *Drosophila*

Aarti Sehdev, Yunusa Garba Mohammed[1], Tilman Triphan[2], Paul Szyszka[1]

[1]Universität Konstanz, Neurobiology, Universitätstrasse 10, 78464 Konstanz, Germany
[2] University of Leipzig, Institute of Biology, Department of Genetics, Leipzig, 04103, Germany

Odorants of behaviorally relevant objects (e.g., food sources) intermingle with those from other sources. Therefore, to sniff out whether an odor source is good or bad – without actually visiting it – animals first need to segregate the odorants from different sources. To do so, animals could use temporal cues, since odorants from one source exhibit correlated fluctuations, while odorants from different sources are less correlated. However, it remains unclear whether animals can rely solely on temporal cues for odor source segregation. Here we show that fruit flies can use temporal differences in odorant arrival down to 5 milliseconds to segregate mixtures of attractive and aversive odorants, and odor source segregation works for odorants with innate, as well as learned valences. Thus, the insect olfactory system can use stimulus timing for olfactory object segregation, similar as mammalian auditory or visual systems use stimulus timing for concurrent sound segregation and figure-ground segregation.

A combination of GABA- and glutamate-gated chloride channels mediates ON selectivity in the Drosophila visual system

Sebastian Mauricio Molina-Obando, Juan Felipe Vargas Fique[1], Burak Gür[1], Miriam Henning[1], Junaid Akhtar[2], Marion Silies[1]

[1]European Neuroscience Institute, Göttingen, Germany
[2]Institute of Development and Neurobiology, Mainz, Germany

In different sensory system, information is processed in distinct ON and OFF pathways. One example is the Drosophila visual system, in which the neuronal circuits that extract motion cues have recently been mapped, and separate into parallel pathways that respond to moving contrast increments (ON) or decrements (OFF). How specific aspects of motion computation are implemented at the molecular or biophysical level is not known. Here, we aimed to understand how cell types of motion detection circuits become contrast selective.
ON and OFF selectivity emerges at the second visual synapse, where the first order interneuron L1 is the major input to the ON pathway, whereas L2 and L3 give input to the OFF pathway. As L1, L2 and L3 depolarize to OFF and hyperpolarize to ON, a sign inversion is needed for cell types downstream of L1 to become ON-selective. Since L1 is glutamatergic, inhibitory glutamate receptors were the main candidates. Using in vivo2-photon calcium imaging, we recorded visual responses of L1's postsynaptic cells and downstream direction-selective neuron. A pharmacological approach using high concentrations of picrotoxin (PTX) suggested that ionotropic (GluCls) and not metabotropic glutamate receptors were involved. However, low PTX concentrations that were thought to not affect GluCls but to selectively block GABA-A receptors already abolished ON responses. This suggested that PTX was already inhibiting GluCls at lower concentrations as compared to other systems, or raised the possibility that a combination of glutamate and GABA-gated chloride channels mediates ON responses in the fly visual system. To distinguish the specific contribution of GABA-A-Rs and GluCls, we used Crispr/Cas9 technology to generate a PTX-insensitive allele of *GluClα* and also used a PTX-insensitive of *Rdl*, the major Drosophila GABA-A-R gene. Interestingly, both alleles could partially rescue visual ON responses, arguing that a combination of different chloride channels indeed mediates ON selectivity in the fly. Moreover, a pancellular *GluClα* loss of function abolished ON but not OFF responses. Strikingly, specific manipulations of GluCls and GABA-A-Rs seem to affect different temporal components of the response, hinting towards a functionally distinct role of the two chloride conductances. We are currently using genetic approaches to disentangle cell-autonomous and non-autonomous functions of *Rdl* and *GluClα*. To this end, our work elucidated the biophysical mechanisms of a core visual computation. We furthermore revealed a novel mechanism for the implementation of a sign inversion in sensory pathway splitting, relying on a combinatorial use of two different chloride channels.

Multiplexing motor functions and impulsive traits is molecularly dissociated by subthalamic metabotropic glutamate receptor 4.

Andreea Constantinescu, Lukasz Piszczek[1,3], Dominic Kargl[1], Anton Pekcec[2], Janet Nicholson[2], Wulf Haubensak[1]

[1]Research Institute of Molecular Pathology, Haubensak Group, Campus Vienna Biocenter 1, 1030 Wien, Austria
[2]Boehringer Ingelheim Pharma GmbH & Co. KG, Div. Research Germany, Biberach an der Riss, Germany
[3]Equal Contributions

Behaviors are highly contingent on environmental settings, for instance conditionally withholding stereotypic responses in time for later execution. Impulsivity, the underlying trait, is a key feature of cognitive control, linked to incentive salience, attention and the ability to suppress and/or cancel prepotent responses. Impairment in this function contributes to the pathobiology of disorders such as attention deficit/hyperactivity disorder (ADHD) and addiction. Despite its ethological and clinical significance, neuronal mechanisms specifically controlling impulsive responding remained largely unknown. Here, we combined a Go/No-Go task (GNG), a behavioral assay for assessing impulsivity in both humans and rodents, with functional imaging to delineate neuronal circuitry linked to impulsivity. This screen identified the subthalamic nucleus (STN), a relatively small node of the basal ganglia circuitry primarily linked to general motor control. Interestingly, we found that STN additionally encodes specific GNG parameters. Low-intensity STN optogenetic inhibition of the in the GNG task increased impulsivity, while not affecting motor output. shRNAi mediated knocking down of STN mGluR4 in high impulsive animals reduced impulsivity in the GNG task without affecting general motor behavior. Conversely, administering a positive allosteric modulator of the metabotropic glutamate receptor 4 (mGluR4) modulates STN firing and mimicked the impulsivity phenotype. Taken together, our study suggests that STN circuitry controls both motor and impulsive traits, and that this function is dissociated at the molecular level by the mGluR4, a novel biomedical target at the intersection of motor and cognitive functions.

Volitional Control of Spatiotemporal patterns of Neuronal Synchrony via Brain-Machine Interface"

Eilon Vaadia[1]

[1]The Hebrew University, ELSC[1], Hebrew University, Eilon Vaadia, 6428711 Tel Aviv, Israel

Neuronal networks change during sensorimotor learning, to optimize the associations between action and perception. We examine if and how the brain harnesses neuronal patterns pertaining to the current action-perception state. We compared spatiotemporal patterns of neuronal activity under different types of Brain-Machine-Interfaces (*BMI-states*) and under *movements-state*. We found that (1) neuronal patterns during the *BMI-states* were markedly different from the *movement-state*. (2) The subjects could volitionally control their brain activity, and generate new spatiotemporal patterns in the local cortical circuit (oscillations, synchronization, firing rate od single units and local-network connectivity).
We conclude that during BMI learning, the brain can generate patterns pertaining to the current action-perception state to achieve a reward. The Subjects' ability to directly modulate specific patterns of in a neuronal circuit provides a powerful approach for understanding neuronal processing in relation to behavior and for the use of BMIs in a clinical setting.

Dissecting Key Mechanisms of Gut-to-Brain Signalling

Ahmed Shaaban, Benjamin H. Cooper[1,2], Michelle Chan[1], Nils Brose[1], Cordelia Imig[1]

[1]Max Planck Institute for Experimental Medicine, Neurobiology, Hermann-Rein-Straße, 3, 37075 Göttingen, Germany
[2]Equal contribution

Regulated fusion of vesicles at synapses in the brain is orchestrated by a complex and highly conserved molecular machinery that determines the speed and fidelity of synaptic transmission. Interestingly, most components of this release machinery are also present in other secretory cell types that operate on a much slower time-scale by secreting hormones via volume-transmission that often act on target organs at a far distance. Enteroendocrine cells (EECs) are scattered throughout the gut epithelium and comprise a group of secretory 'sensory' cells that function as chemo- and mechanoreceptors and respond to distinct nutrients, metabolites, and physical forces by releasing a variety of peptide hormones and bioamines, which are critical for the control of food intake, appetite, gut motility, and metabolism. Recent studies have shown that EECs may express a large number of protein components of the neuronal presynaptic release machinery, form 'axon-like' processes and 'synaptic-like' contacts with sensory afferents, and utilize the classical neurotransmitter glutamate to send signals to the brain via the vagus nerve. It is therefore tempting to speculate that EECs release substances using similar mechanisms as synapses that mediate fast synaptic transmission between neurons in the brain. However, such potential 'synaptic-like' contacts between EECs and neurons have so far not been directly observed at the ultrastructural level and their physiological relevance is unclear given that EECs have a very high turnover rate and are thought to release hormones from so-called large dense core vesicles (LDCVs, 80-500nm diameter) that usually fuse with slower kinetics than small synaptic vesicles. Correspondingly, the overall goal of this study is to investigate whether EECs signal in a synaptic-like fashion with sensory afferents to mediate gut-brain-communication and to determine the functional properties of their hormone and transmitter release process, the molecular composition of their vesicle fusion machinery, and the nature and extent of their connectivity with other cell types. Using a multidisciplinary approach combining genetic mouse mutants, 3D-intestinal stem cell-derived organoid cultures, correlative light- and electron microscopy, immunohistochemistry, as well as electrophysiology and -chemistry, we are focussing on the analysis of one distinct EEC type, namely serotonergic enterochromaffin cells (ECs), that release the vast majority (>90%) of systemic serotonin. Our preliminary findings support the hypothesis that hormone release from EECs is mediated by components of the neuronal presynaptic neurotransmitter release machinery and that our experimental approach outlined above will provide very standardized experimental conditions for the analysis of mutants to determine key signalling processes along the gut-brain-axis.

Finite Element Simulations of active Electroception

Thede Witschel, David Emanuel Vetter[1], Hanspeter A. Mallot[1]

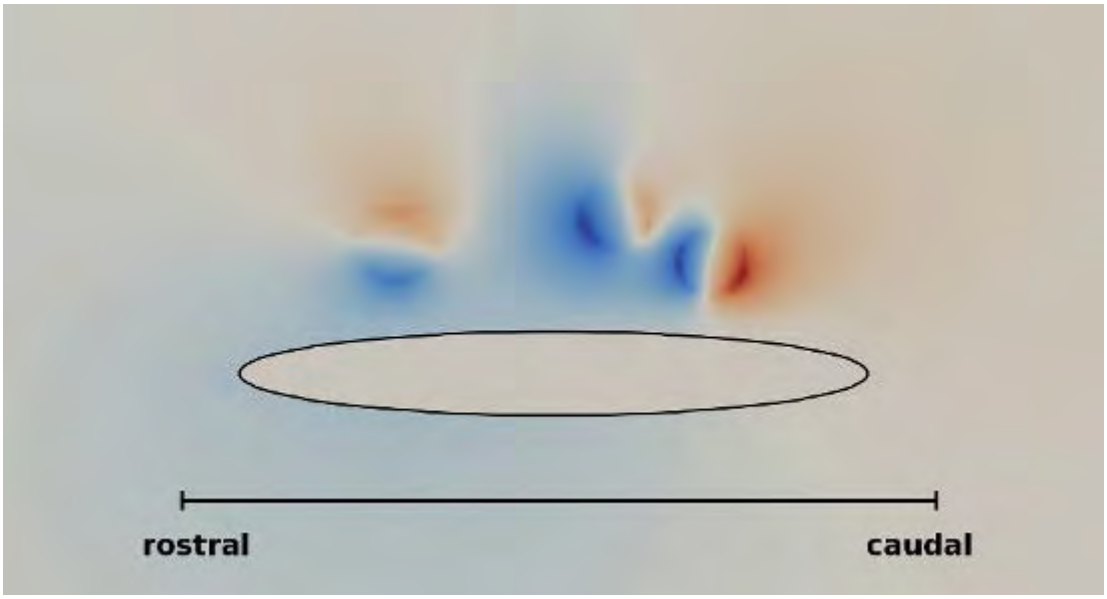
[1]University of Tübingen, Cognitive Neuroscience, Auf der Morgenstelle 28, 72076 Tübingen, Germany

The concept of optic flow has been around for several decades (Gibson, *The perception of the Visual World*, 1950) and has found support in electrophysiological evidence of flow field usage in insects and vertebrates (Kubo et al., *Neuron*, 2014).
In an accompanying poster we present a computational analysis of optic flow processing in zebrafish (Mallot et al).

In principle, the changes in a series of images can be described with a flow field, with distinct patterns resulting from characteristic motion events. A focus of expansion would indicate a forward translation, a uniform lateral change would occur as a result of yaw rotation.
In light of their wide spread usage and biological relevance, we set out to find other sensory systems, which allow the use of flow fields.

In order to extend optic flow to other sensory systems, a candidate system must be able to produce flow fields.
A viable system appears to be the active electroception of wave-type weakly electric fish, as it is found in animals of the *Gymnotiformes* order.
Active electroception uses the difference between the observed and the expected unperturbed transdermal current. This difference can be interpreted as an electric image.
We built a software package that allows the simulation of an arbitrary conductive environment by approximating a solution to the Poisson equation using the finite element method.
The figure shows the simulated difference between the unperturbed potential and the perturbation introduced by three spherical conductors in the vicinity of a fish.
To confirm the results of the simulation we compared it to a similar approach using an analytical solution of the Poisson equation and measurements of a real, submerged electric dipole with field perturbations.

Simulated electric images will be used to generate a database of realistic spatial temporal pattern impinging on the fish surface as the fish explores a cluttered environment.
From this database stimulus statistics of electric environments will be analysed and used in the neural network model of optimal encoding.


rostral
caudal

Sound encoding at individual inner hair cell synapses

Özge Demet Özcete, Tobias Moser[1,2]

[1]Max Planck Institute for Experimental Medicine, Göttingen, Germany
[2]Institute for Auditory Neuroscience, University Medical Center Göttingen, Göttingen, Germany
[3]International Max Planck Research School for Neurosciences, Göttingen, Germany

The auditory system enables us to perceive sound over broad frequency and pressure range. Sound is encoded at the ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) in the cochlea. Sound-induced mechanical stimuli depolarize IHCs, which is followed by synaptic Ca^{2+} influx through $Ca_V1.3$ channels. This leads to IHC glutamate release onto postsynaptic SGNs.[1] While the receptor potential of IHCs represent the full sound pressure range, each SGN encodes only one fraction of it. SGNs differ in their spontaneous activity and sound thresholds even in comparable frequency tuning. Several mechanisms were proposed to explain the SGN diversity. Presynaptically, it was shown that IHCs encode sound from a single receptor potential through heterogeneous presynaptic Ca^{2+} signaling. The active zones along the IHC abneural/neural axis differ in both amplitude and voltage dependence of activation.[2] While postsynaptically, a recent study shows different molecular compositions of SGNs innervating IHCs preferentially along the IHC abneural/neural axis. [3] How heterogeneous presynaptic signaling is encoded into postsynaptic, diverse SGN spiking is still unknown. Here, we investigate sound encoding at individual inner hair cell synapses by paired optical recordings of presynaptic, subcellular Ca^{2+} influx and corresponding glutamate release, using the genetically encoded glutamate sensor, iGluSnFR.[4]

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C-terminal splicing of presynaptic calcium channels contributes to the variability of neurotransmitter release

Jennifer Heck, Pierre Parutto[4], Anna Ciuraszkiewicz[1,5], Arthur Bikbaev[1,8], Romy Freund[1], Jessica Mitlöhner[2], Maria Andres-Alonso[3], Anna Fejtova[3,5,7], David Holcman[4,6], Martin Heine[1,5,8]

[1]Leibniz Institute for Neurobiology, Research Group Molecular Physiology, Magdeburg, Germany
[2]Leibniz Institute for Neurobiology, Department Neurochemistry and Molecular Biology, Magdeburg, Germany
[3]Leibniz Institute for Neurobiology, Research Group Presynaptic Plasticity, Magdeburg, Germany
[4]Ecole Normale Superieure, Paris, France
[5]Center for Behavioral Brain Sciences–CBBS, Otto-von-Guericke-University, Magdeburg,Germany
[6]Churchill College, CB30DS, United Kingdom
[7]Department of Psychiatry and Psychotherapy, University Hospital, Friedrich-Alexander-University, Erlangen, Germany
[8]Institute of Developmental Biology and Neurobiology, Johannes Gutenberg University, Mainz, Germany

Neuronal communication at chemical synapses is initiated by vesicular release of neurotransmitters, a process triggered by the transient influx of calcium ions. To successfully induce vesicle exocytosis, spatial proximity between voltage-gated calcium channels (VGCCs) and vesicular calcium sensors is essential. However, using single-particle tracking photoactivated localization microscopy (sptPALM) VGCCs where found to be surprisingly mobile within presynaptic compartments. Despite their key function in synaptic transmission, little is known about the molecular determinants of this mobility and its impact on synaptic function. Super resolution imaging revealed distinct dynamics for individual $Ca_V2.1$ C-terminal splice variants and an activity-dependent modulation of presynaptic $Ca_V2.1$s. Further, we localized $Ca_V2.1$s within active synapses and found that they transiently dwell (100-200 ms) in nanodomains of 100 nm in size. Based on functional imaging and paired whole-cell recordings of hippocampal neurons, we identified differences in evoked glutamate release of synapses populated by different $Ca_V2.1$ splice variants. Unexpectedly, the shorter C-terminal splice variant, lacking the currently described scaffold-mediated tethering of the calcium channel to the vesicle, showed multi-vesicular release and induced larger postsynaptic currents compared to $Ca_V2.1$ expressing the full C-terminus. Furthermore, repetitive stimulation with varying frequencies revealed $Ca_V2.1$ splice variant-dependent differences in the expression of short-term plasticity.
Reversible optogenetic immobilization of $Ca_V2.1$s resulted in a general enrichment of presynaptic calcium channels. However, only the long $Ca_V2.1$ variant was potent to increase evoked glutamate release and short-term depression in the clustered state. Our results suggest that a variable equipment of the presynapse with $Ca_V2.1$ splice variants dictates the functional architecture of presynaptic calcium domains. Concluding, we propose that rapid on/off binding of $Ca_V2.1$ variants to active zone scaffolds is required for rapid regulation of synaptic release and can be modulated by alternative splicing.

Executive eye movement impairment in presymptomatic amyotrophic lateral sclerosis mutation carriers

Daniel G. Schmidt, Martin Gorges[1], Martin Kunz[1], Antje Knehr[1], Dorothée Lulé[1], Elmar H. Pinkhardt[1], Jochen Weishaupt[1], Albert C. Ludolph[1], Jan Kassubek[1]

[1]Ulm University, Dept. of Neurology, Oberer Eselsberg 45, 89081 Ulm, Germany

Background: Behavioral changes including cognitive impairment are a common feature of amyotrophic lateral sclerosis (ALS). Multimodal evidence from presymptomatic ALS cases suggested that the pathological process initiate in a yet unknown period of time before disease-defining symptoms onset.
Objective: To study behavioral performance in presymptomatic and affected ALS mutation carriers.
Methods: Videooculographic and sociodemographic data from 39 presymptomatic and 39 symptomatic ALS carriers – both groups carrying one of ALS-associated mutations – were compared to age-, gender-, and education-matched healthy controls. Presymptomatic subjects, at the time of enrollment, did no display any symptoms or signs of manifest disease and were recruited from first-degree family members with a known ALS-associated gene mutation.
Results: Executive oculomotor dysfunctions were observed at the group level in presymptomatic subjects including increased anti-saccade errors ($p<0.001$). As previously investigated, oculomotor performance was substantially worse in the symptomatic ALS cohort compared to matched healthy controls including executive and basal oculomotor functions (Gorges et al., 2015). Executive dysfunctions at the group level resulted from 21% of presymptomatic subjects and 56% of affected individual whereas all other subjects (i.e. 69% presymptomatic, 44% affected individuals) performed within normal range as compared to controls. A differentiation for genotype was not performed yet due to limited sample sizes of single mutations.
Conclusion:
The oculomotor data support a sequential development of eye movement disturbances with executive dysfunctions as a possible but inconstant early manifestation.

Gorges et al. Eye Movement Deficits Are Consistent with a Staging Model of pTDP-43 Pathology in Amyotrophic Lateral Sclerosis. PLoS One. 2015;10:e0142546.

Symposium

S13: Breaking News

S13-1 Exploring cortical brain networks with flexible LCP microelectrode arrays in parallel to two-photon imaging of anaesthetized and awake mice
Michael Schweigmann, Laura Caudal, Gebhard Stopper, Anja Scheller, Klaus Peter Koch, Frank Kirchhoff

S13-2 Design of an ultra-fast switching mouse melanopsin variant with a narrow action spectrum
Raziye Karapinar, Dennis Eickelbeck, Stefan Tennigkeit, Till Rudack, Klaus Gerwert, Stefan Herlitze

S13-3 A flexible and transparent electrode array for closed-loop optogenetic stimulation
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Exploring cortical brain networks with flexible LCP microelectrode arrays in parallel to two-photon imaging of anaesthetized and awake mice

Michael Schweigmann, Laura Caudal[1], Gebhard Stopper[1], Anja Scheller[1], Klaus Peter Koch[2], Frank Kirchhoff[1]

[1]University of Saarland, CIPMM, Department of Molecular Physiology, Kirrberger Str. 100, 66421 Homburg, Germany

[2]Trier University of Applied Sciences, Department of Electrical Engineering, Schneidershof, 54293 Trier, Germany

Neurons and astrocytes are highly interconnected and form a complex cellular network for signal processing in the brain. The electrical activity of neurons and astroglial Ca^{2+} transients are tightly coupled. Parallel recordings of electrical activity and Ca^{2+} signaling can help to identify the molecular mechanisms of neuron-glia communications. Here, we describe flexible liquid crystal polymer (LCP) microelectrode arrays for electrical recordings and stimulations during two-photon laser-scanning microscopy (2P-LSM).

The arrays were designed for standard craniotomies used for cortical 2P-LSM *in vivo* imaging. Being thin, flexible and of low weight they can be easily positioned onto the dura. Three different designs were constructed: arrays (1) with eight circular electrodes (arranged in a matrix of three by three elements, sparing the center), (2) with sixteen circular electrodes (four by four matrix) or (3) with eight rectangular electrodes (placed in four groups of 2 single sites). In addition to recordings, the electrodes can also be used to stimulate neurons in deeper layers of the mouse cortex. The contact sites of gold are coated with nanoporous platinum to decrease the tissue contact impedance. The round-shaped electrodes have a diameter of 150 µm whereas the rectangular shaped electrode are 400 µm x 200 µm in size. The biocompatibility of the electrodes was tested with immunohistochemistry.

Electrical and Ca^{2+} recordings were performed in mice with neuronal or astroglial expression of the Ca^{2+} indicator GCaMP3 (Nex-Cre x R26-CAG-lsl-GCamP3$^{fl/wt}$ or GLAST-CreERT2 x R26-CAG-lsl-GCamP3$^{fl/fl}$). With the sixteen channel electrode arrays, we could obtain a spatially resolved pattern of the electrical activity inside the cranial window. The eight channel arrays were used for simultaneous acquisition of Ca^{2+} (using 2P-LSM) and electrical signals. In addition, we could elicit Ca^{2+} signals by electrical stimulation. Using different stimulation intensities and depth of anesthesia (isoflurane) we could observe the change of brain activity during transition from anesthetized to awake.

In conclusion, we successfully generated novel LCP electrodes for simultaneous recording of electrical activity and in vivo imaging to study complex brain functions.

Acknowledgement

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Design of an ultra-fast switching mouse melanopsin variant with a narrow action spectrum

Raziye Karapinar, Dennis Eickelbeck[1], Stefan Tennigkeit[2], Till Rudack[2], Klaus Gerwert[2], Stefan Herlitze[1]

[1]Ruhr-University Bochum, Department of General Zoology and Neurobiology , Universitätsstraße 150, 44801 Bochum, Germany

[2]Ruhr-University Bochum, Department of Biophysics , Universitätsstraße 150, 44801 Bochum, Germany

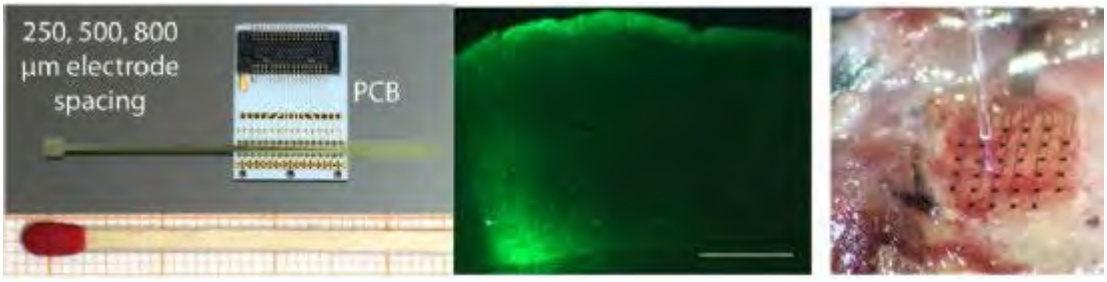
Optogenetics combines the use of light-sensitive proteins and genetical targeting strategies to allow for precise light-controlled manipulation of cell function and signaling in living tissue. We characterized the mouse melanopsin isoform mOpn4L (*mus musculus*) as a novel optogenetic tool *in vivo* and *in vitro* and identified its unique biophysical characteristics and G-protein coupling. Melanopsin functions as a selective molecular light switch for G-protein coupled receptor pathways. We could previously demonstrate that melanopsin is able to sustainably activate and deactivate $G_{i/o}$ as well as $G_{q/11}$ pathways by using short low intensity light pulses for precise activation and deactivation. In the next step we introduced point mutations in mOpn4L to obtain an excitation wavelength shift for future combination with other opsins in tandem activation experiments. Our aim was to create a blue-shifted melanopsin variant maintaining the advantages of mouse melanopsin. Here we show *in vitro* and *in vivo* in the cerebellar cortex that the Y211F mouse melanopsin variant, compared to wild type mouse melanopsin, displays a 20 nm blue-shifted absorption maximum combined with faster on and two-fold faster off kinetics while retaining the high light sensitivity of WT melanopsin. Thus, Y211F offers higher temporal precision together with a narrower excitation bandwidth, being an ideal tool to control intracellular G-protein signals with minimal phototoxicity.

A flexible and transparent electrode array for closed-loop optogenetic stimulation

Marcel Brosch, Michael Thomas Lippert[2], Martin Deckert[3], Theresa Weidner[4], Kentaroh Takagaki[5], Frank W Ohl[6], Bertram Schmidt[7]

[1]LIN, SPL NRO, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]LIN, SPL NRO, Brenneckestr. 6, 39118 Magdeburg, Germany
Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany
[3]Institute of Micro and Sensor Systems, University Magdeburg, Germany
[4]LIN, SPL NRO, Brenneckestr. 6, 39118 Magdeburg, Germany
[5]LIN, SPL NRO, Brenneckestr. 6, 39118 Magdeburg, Germany
[6]LIN, SPL NRO, Brenneckestr. 6, 39118 Magdeburg, Germany
Institute of Biology, Otto-von-Guericke University, Magdeburg, Germany
Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany
[7]Institute of Micro and Sensor Systems, University Magdeburg, Germany

Synchronized activity of functionally linked neurons in the neocortex gives rise to mesoscopic activity patterns that can be recorded as local field potentials (LFP) at the cortical surface. Here we present a novel multi-electrode array to measure such mesoscopic signals at high spatial resolution. The flexible array contains 32 recording sites in a transparent polyimide substrate. A novel roughening process reduces electrode impedance and leads to extended long-term stability of the array, allowing months-long use. Its low thickness renders the array substrate transparent to blue light, making it suitable for closed-loop optogenetic stimulation and optical imaging. In our work, we evaluate long-term signal quality in the auditory cortex of awake Mongolian gerbils and demonstrate the combination of electrical recordings with optogenetic stimulation in anesthetized animals. Furthermore, we compare LFP recordings in the visual cortex of anesthetized rats with simultaneous voltage sensitive dye imaging.



Nanosensor-based Imaging of Presynaptic Dopamine Release

Sofia Elizarova, Meshkat Dinarvand [3], Nils Brose[1], Sebastian Kruss[3], James A. Daniel[1]

[1]Max Planck Institute for Experimental Medicine, Department of Molecular Neurobiology, Hermann-Rein-Straße 3, 37075 Göttingen, Germany

[2]Göttingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences (GGNB), University of Göttingen, Germany

[3]Institute for Physical Chemistry, University of Göttingen, Germany

Dopamine (DA) is a key neurotransmitter (NT) in the central nervous system that regulates reward processing, cognition and motor control. DA is particularly associated with prevalent human diseases such as Parkinson's disease, schizophrenia and drug addiction. Despite its importance in brain function and disease, our knowledge with respect to the molecular regulation of DA secretion is limited and largely restricted to proteins that mediate DA production, vesicular storage and uptake. The extent to which the molecular machinery that mediates the secretion of other, well-studied NTs is involved in the secretion of DA is yet to be understood. It is possible that molecular regulation of DA secretion differs from typical, fast-acting NTs, since transmission via DA acts in a slow spill-over fashion (volume transmission). DA can be secreted from multiple types of release sites, including synaptic and non-synaptic axonal structures, but also somatodendritic regions. To investigate the function and molecular composition of these different synaptic populations, it is essential to monitor the kinetics of DA release from individual release sites. Current electrophysiological and optical methods, however, are limited regarding their spatial resolution and number of probes per cell.

To address this methodological problem, we developed DA nanosensors for optical DA detection based on single-walled carbon nanotubes. These DA nanosensors are fluorescent in the near infrared, detect DA in the sub-nanomolar range, and selectively report the presence of DA over other catecholamines. Due to their small size, recordings from thousands of DA nanosensors per cell can be acquired in parallel, allowing the identification and comparison of individual DA release sites across large regions of the cell. We used this method to reliably detect hot spots of DA release in the membrane of chemically-stimulated PC12 cells. Furthermore, we employed this method in order to identify key molecules that facilitate and regulate DA secretion from distinct neuronal structures at the level of individual synapses. To do this we have developed a suitable neuron-culture system that supports the growth of DA neurons directly on the DA nanosensor array. In addition, we have validated various synaptic proteins present in DA neurons.

Through this new method we aim to gain new insights into the basic biology of DA secretion and the underlying molecular machinery in wildtype neurons, as well as in disease-related models. Ultimately, this will lead to a better understanding of how alterations in these processes lead to DA-linked diseases.

Manipulation of intracellular cAMP and membrane potential using light activated adenylyl cyclases and CNG channels

Oana Constantin, Shiqiang Gao[2], Shang Yang[2], Georg Nagel[2], Thomas G. Oertner[1], Christine E. Gee[1]

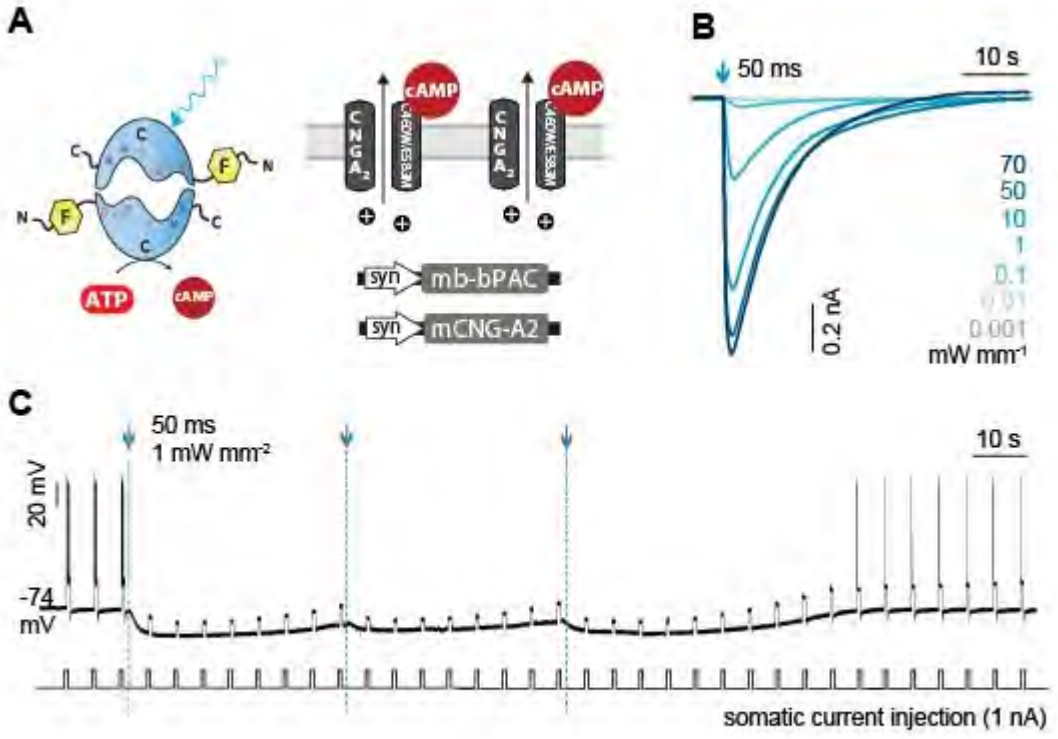
[1]Institute for Synaptic Physiology, University Medical Center Hamburg-Eppendorf, 20251 Hamburg

[2]Biocenter, University of Würzburg, Julius-von-Sachs-Platz 2, 97082 Würzburg

The ubiquitously-expressed cyclic nucleotide cAMP, was discovered almost six decades ago to be an intracellular signaling molecule. It is a molecule of vital importance initiating complex intracellular signaling cascades in response to extracellular stimuli. It is present in essentially every type of cell and is involved in processes including gene transcription and cell division. Cyclic AMP has also been implicated in synaptic transmission and synaptic plasticity. Although much is known about the role of cAMP in complex cellular processes, it remains difficult to selectively manipulate and quantify cAMP in intact cells and tissues. Pharmacological tools lack cell-specificity and are prone to off-target effects. To facilitate investigation of the vast spectrum of processes modulated by cAMP, we are improving optogenetic tools such as photo-activated cyclases and cAMP sensors that allow for cell-specific manipulation and measurement of cAMP with high spatial and temporal resolution.

We report the development of highly effective, very low dark activity, blue light-activated, soluble and membrane-bound photoactivatable adenylyl cyclases (PACs) with strong specificity for producing cAMP (Figure 1A). By expressing these tools together with cAMP preferring CNG channels in hippocampal pyramidal neurons, we detected rapid, dramatic elevations of intracellular cAMP using short pulses of blue or green light (Figure 1B). Using these PACs we can manipulate the intracellular concentration of cAMP specifically in the genetically targeted cells in order to study the effects of cAMP on cellular processes (i.e. synaptic plasticity).

We also describe the engineering of a novel light-gated K^+ specific channel by fusing bPAC to cyclic nucleotide-gated channels with high permeability for K^+. The optimized fusion construct strongly hyperpolarizes rat hippocampal neurons when blue light is applied (50 ms, 470 nm, 1 mW/mm^2) and completely blocked action potentials induced by 1 nA somatic current injections for around 1 minute after each flash (Figure 1C). Based on the narrow activation spectra in blue range and high light sensitivity of bPAC, it should be possible to combine the new tandem constructs with red light-activated Chrimson for precisely spiking or inhibiting action potentials in the targeted neuron(s) with light of two colors.


A
C
F
N
ATP
cAMP
CNGA2
CABD(W/E)533M
syn
mb-bPAC
mCNG-A2
B
50 ms
10 s
70
50
10
1
0.1
0.01
0.001
mW mm-1
0.2 nA
C
50 ms
1 mW mm-2
10 s
20 mV
-74
mV
somatic current injection (1 nA)

Real-time neurofeedback in freely behaving rats: training a network to study a network

Golan Karvat, Michael Tangermann[2,4,6], Ilka Diester[1,3,4,5]

[1]Optophysiology - Optogenetics and Neurophysiology
[2]Brain State Decoding Lab
[3]Bernstein Center for Computational Neuroscience
[4]Brain Links Brain Tools
[5]Faculty of Biology
[6]Department of Computer Science, Albert Ludwigs University, Freiburg, Germany

In order to understand sophisticated systems such as the brain, there are two main approaches: first, observe its activity during natural stimuli, in a task or even during free behavior. Second, the system's behavior can be observed upon applying external stimuli, e.g. electrical, optogenetic or pharmacological. Recent years have witnessed great improvements in stimulation techniques, but it is never clear, if the resulting output is natural, i.e. if the behavior could be observed also from a non-stimulated system.
A more natural approach for manipulation is to use neurofeedback training: here, a desired neural activity pattern is positively reinforced, while behavior is observed passively. With neurofeedback we can be sure, that the brain has produced the observed neural activity of interest by itself. However, although neurofeedback has been used for decades for clinical research in humans, its use in basic science on rodents is sparse.
One of the main targets for neurofeedback is oscillatory local field potential (LFP) activity, yet the real-time nature of the technique on the one hand, and the time/ frequency resolution trade-off on the other hand, pose a great challenge. For example, activity in the beta band (15-30 Hz), which influences motor preparation and sensory perception, is known to appear in bursts shorter than 150 ms and to focus around bands as narrow as 1 Hz. Therefore, traditional techniques such as sliding window Fourier transform are hardly feasible.
In this study, we developed an online data analysis algorithm for estimating narrowband power and phase, which was optimized for minimal delay and maximal resemblance to traditional, offline time-spectral analyses: the system includes 32 real-time finite-impulse-response filters in steps of 1 Hz, with a fixed delay of 130 ms. In real-time, the acquisition system detects peaks and troughs in the filtered data, which serve for power- and phase estimates, at a time resolution of half the period of each frequency. The phase-linearity of the system allows for a direct comparison between different frequencies in 1 Hz resolution, and for the detection of transient bursts.
As a proof of concept, we trained a rat in a first experimental phase to increase the activity in one of the frequencies in the beta band (20-25 Hz in steps of 1 Hz). The rat was implanted with a 32-channel silicone probe in the primary motor cortex (M1). Reward was given for events of significantly high power (>98th percentile, adapted over a history of 15 s) which lasted for at least 70 ms. Artifacts were detected and rejected online, and the rat was taught to avoid them by negative reinforcement (white noise and time-out periods).
To our surprise, not only did the rat significantly increase the number of high beta events after one week of training, but also the average power of the target frequencies significantly increased over monitored 30 min periods. In a second phase, we tested if the induced change could be reverted by teaching the rat to avoid beta-events. We observed a power reduction back to pre-conditioning levels.
To the best of our knowledge, this is the first demonstration of a real-time neurofeedback with high temporal- and frequency resolution in a freely behaving rodent. Since the technique can easily be modified to other frequencies and/ or brain areas, we propose to add neurofeedback to the

neuroscientist’s toolbox, to decipher the effect of physiological patterns on behavior.

Hearing Colors: Evaluation of Frequency Representation in Optogenetic Midbrain Implants

Meike Marie Rogalla, Kai Jannis Hildebrandt[1]

[1]Cluster of Excellence Hearing4all, Neurobiology of Hearing Group, Department for Neuroscience, School of Medicine and Health Sciences, University of Oldenburg

Partial restoration of auditory function in patients with disabling hearing impairments is achieved by hearing aids or implants (cochlea or auditory brainstem implants). The state of the art approaches for building such implants suffer from the drawback of limited temporal and spectral resolution. Electrical field spread depends on the impedance of the surrounding medium, impeding spatially focused electrical stimulation in neural tissue (Hernandez et al. 2014, Kral et al. 1998). To overcome these technical limitations, optogenetic stimulation could be used in such prosthesis to achieve a more focal and precise stimulation with enhanced resolution. Despite scatter and refraction, this method may allow for precise and specific stimulation of nearby sites within the same nervous tissue of the auditory pathway. Previous experiments have provided proof-of-principle of detection of excitation at a single outlet in the inferior colliculus (IC) in a rodent model. However, the feasibility of discrimination of stimulation at different sites remains to be shown and will be the next steps towards a differential stimulation of the tonotopic axis.
In this study we aimed to perform behavioral tests of the ability of mice to discriminate stimulation at two different points within the IC. To this end, we devised an optogenetic midbrain-implant, which stimulates two points within different layers of the IC. We aimed to provide a proof-of-principle for stimulation of neural populations that represent different acoustic frequencies. Pre-surgery, animals were trained in a frequency oddball task in which they had to indicate a change in frequency within a continuous sequence of tone pips. We then implanted two well-separate excitatory outlets at two sites in the IC (unilateral) with a distance of 700µm in depth after injection of rAAV5-CAG-ChR2-GFP. Post-surgery, auditory stimuli were converted into optical stimulation: the mouse had to report changes from frequent stimulation at one outlet to the respective other. Discrimination performance was highly significant for both acoustic and optogentic stimulation. Results from the behavioral tests and from modeling of light spread and neural activation indicate that distances of the outlets < 100µm should be well discriminable, thus establishing the first steps towards continuous frequency stimulation in auditory brain implants.

Hernandez, V. H., Gehrt, A., Reuter, K. (2014): Optogenetic stimulation of the auditory pathway. The Journal of Clinical Investigation, 124, 1114-1129.

Kral, A., Hartmann, R., Mortazavi, D. and Klinke, R. (1998): Spatial resolution of cochlear implants: the electrical field and excitation of auditory afferents. Hearing Research, 121, 11-28.

Chemogenetic silencing: synaptic mechanisms and long-term effects at Schaffer collateral synapses

Mauro Pulin, Céline D. Dürst[2], Thomas G. Oertner[2], J. Simon Wiegert[1]

[1]RG Synaptic Wiring and Information Processing, Center for Molecular Neurobiology Hamburg (ZMNH), Hamburg, Germany
[2]Institute for Synaptic Physiology, Center for Molecular Neurobiology Hamburg (ZMNH), Hamburg, Germany

Chemogenetic manipulation of neuronal activity, utilizing a mutual exclusive relationship between an engineered G-protein coupled receptor (GPCR) and a non-natural (synthetic) ligand (Clozapine-N-Oxide), has become an invaluable method in the field of neurosciences. The recently developed “Designer Receptors Exclusively Activated by Designer Drugs” (DREADDs) are based on different human muscarinic receptors, and depending on which GPCR signaling cascade they are linked to (Gi/o, Gs or Gq) their activation will lead to inhibition or excitation of neuronal activity. The M4 variant hM4Di has become a widely used chemogenetic tool for inhibition of defined neuronal circuits. However, how hM4Di exactly mediates silencing of neuronal transmission remains a matter of debate.

Here, we show in organotypic hippocampal slice cultures that hM4Di activation did not significantly affect the resting membrane potential and thus firing properties of CA3 pyramidal neurons. In these cells, we did not observe GIRK channel-mediated hyperpolarization, as initially proposed. In contrast, by using genetically encoded, fluorescent glutamate and calcium indicators, we demonstrate that activation of hM4Di specifically reduces glutamate release from presynaptic terminals due to suppression of presynaptic calcium influx.

Since hM4Di activation blocks synaptic release without affecting action potential firing in CA3 cells, we combined this DREADD with channelrhodopsin-2 (ChR2) to tightly control activity of a selected subset of Schaffer collateral synapses. Two-photon calcium imaging at single dendritic spines confirmed that acute application of CNO blocked excitatory postsynaptic calcium transients (EPSCaTs). Electrophysiological recordings revealed that silencing of transmission was selective for evoked release, while spontaneous synaptic transmission was unaffected.

Based on our previous observation that higher synapse elimination after LTD was associated with low synaptic release probability, we tested whether chronically reducing transmission at identified synapses without plasticity induction had a similar impact on their lifetime. Notably, hM4Di-mediated silencing of synaptic transmission was only transiently effective despite the continuous presence of CNO, indicating that chronic DREADD-mediated strategies require close monitoring of network and synaptic activity in order to avoid misinterpretation due to unexpected side effects. Under these conditions, when synapses were silenced for approximately 72h, their lifetime was not changed, suggesting that reduced evoked transmission alone could not account for increased synapse elimination.

Optogenetic spike-timing-dependent plasticity (oSTDP)

Margarita Anisimova, Bas van Bommel[1], Thomas G. Oertner[1], Christine E. Gee[1]

[1]Center for Molecular Neurobiology Hamburg, University Medical Center, Hamburg, Germany

Modification of synaptic strength after coincident activity in the pre- and postsynaptic neurons is a candidate mechanism for information storage in the brain. This process is commonly referred to as a spike-timing dependent plasticity (STDP) and may underlie memory formation in the hippocampus. Investigating the long-term consequences of STDP has been difficult due to the invasive nature of intracellular recordings, routinely used to control pre- and postsynaptic spiking. We present an all-optical method to induce STDP that allows assessing changes in synaptic strength days later. We induced timing-dependent plasticity at Schaffer collateral synapses in rat organotypic hippocampal slices transfected with channelrhodopsins that are activated by different wavelengths of light. An adeno-associated virus was used to express ChrimsonR in a small subset of CA3 neurons and single-cell electroporation was used to express CheRiff in several CA1 pyramidal neurons per slice. We found that red light flashes (300 at 5 Hz) paired with bursts of 3 violet light flashes (50 Hz) induced long term depression or long term potentiation depending on the timing interval. We then constructed illumination towers, each containing independently controlled, collimated, red (630 nm) and violet (405 nm) high-power LEDs so that ChrimsonR and CheRiff-expressing neurons could be stimulated with either causal (pre before post) or anti-causal (post before pre) pairing in the incubator. At the read-out point (3 hours to 3 days) we sequentially recorded EPSCs from CA1 neurons, including at least 2-3 non-transfected neurons, while re-activating ChrimsonR-expressing CA3 neurons with a condenser-coupled orange laser. To determine relative input strengths, the initial EPSC slope from each CheRiff neuron was divided by the average EPSC slope from the non-transfected neurons in that slice. At 3 hours after pairing, input strength was not significantly changed by either causal or anti-causal pairing, although a trend towards potentiation after causal pairing was apparent. Surprisingly, three days after pairing, input strength to the CheRiff neurons was significantly larger in slices that underwent not only causal, but also anti-causal pairing. As described for STDP, tLTP was abolished when NMDA type glutamate receptors were blocked during pairing. Reducing the pairing frequency from 5 Hz to 0.1 Hz also prevented plasticity induction. Interestingly, when spiking was blocked with tetrodotoxin in the period from 3 hours to two days after causal pairing, input strength no longer increased. These experiments suggest that the synaptic memory of a short episode of coincident activity may be actively maintained and amplified in the cultures, perhaps by replaying the activity pattern. In conclusion, our all-optical method allows us to induce plasticity at defined hippocampal synapses and to assess changes in synaptic strength days after induction. In the future we will use this method to compare molecular and structural properties of the paired synapses with neighboring synapses on the same cells.

Optogenetic stimulation of VTA dopaminergic neurons in a rodent model of depression

Yixin Tong, Wen Zhao[1], Volker Arnd Coenen[1], Mate Daniel Döbrössy[1]

[1]Unviersity Freiburg Medical Center, Laboratory of Stereotaxy and Interventional Neurosciences, Department of Stereotactic and Functional Neurosurgery, Breisach Str. 64, 79106 Freiburg im Breisgau, Germany

Introduction: Major depressive disorder (MDD) is one of the most common mental disorders, and more than 300 million of people suffer from depression worldwide. Approximately 30 % of patients are resistant to conventional treatments such as anti-depressant medication or psychotherapy. Recent clinical trials found that Deep Brain Stimulation (DBS) of the Medial Forebrain Bundle (MFB) can have rapid onset and long-term antidepressant effects with Treatment Resistant Depressive patients. However, the mechanisms of action are elusive. In this study, we focus on i.) the potential anti-depressive effects of selective optogenetic stimulation of the Ventral Tegmental Area's (VTA) dopaminergic (DA) neurons passing through the MFB and ii.) compare the therapeutic effects of different stimulation patterns.

Methods: 30 female TH:Cre rats (10-12 wks old) were matched into two groups according to their baseline behavioral performance. Animals in each group received either AAV-EF1α-DIO-ChR2-EYFP (the virus expressing Channelrhodopsin) or AAV-EF1α-DIO-EYFP (Control virus) into the VTA bilaterally, followed by the implantation of the optic fibers in the MFB. After recovery, the animals were exposed to the Chronic Mild Unpredictable Stress (CMUS) paradigm once or twice daily for 6 weeks to induce the depressive-like phenotype. During the CMUS induction period, half the animals received optic stimulation during 6 weeks (n=12; "Preventive Stim" group; 8 pulses 30 Hz 5ms pulse width for 30 mins, 2 times a week), or they received optic stimulation only after the 6 weeks CMUS induction period (n=18; "Post Stim" group; same number and stimulation parameter as the other group). Behavior tests classically sensitive to depressive-like phenotype such as the Open Space Swimming Test (OSST), the Elevated Plus Maze (EPM), the Social Interaction Test (SIT), the Sucrose Preference Test (SPT), the Novel Object Recognition test (NOR), Ultrasonic Vocalization (USV) and Corticosterone levels were monitored.

Results: Animals from both stimulation conditions showed initial higher mobility in OSST compared to the Controls', which suggests a stimulation mediated rescue from the depressive-like phenotype. However, whilst the effect in the Preventive Stimulation group was present only during Day1, the Post Stimulation animals had higher activity up until Day 3. On the other hand, the Preventive group showed lower anxiety compared to the Post stimulation group in the EPM. Also, the Preventive stimulation group had lower Corticosteone levels compared with the Control animals who received the same stimulation parameters.

Conclusions: CMUS induced a depressive-like phenotype as assessed by behavioral testing, however, both the Preventive and the Post optogenetic stimulation of the VTA dopaminergic projections could "rescue" some to the behaviors. The Preventive stimulation condition had better anti-anxiety effect, while the Post stimulation pattern had more impact on motivation.

Symposium

S14: Adaptivity and inhomogeneity in neuronal networks - two sides of the same coin?

S14-1 Self-organized network inhomogeneity governs spontaneous activity dynamics
Samora Okujeni, Ulrich Egert

S14-2 Cell assembly formation and non-random connectivity in networks subject to homeostatic structural plasticity
Júlia V Gallinaro, Stefan Rotter

S14-3 Self-organization of neuronal dynamics by plasticity and adaptation
Anna Levina, Viola Priesemann, Oleg Vinogradov, Felix Effenberger

S14-4 Dopamine blocks homeostatic excitatory synaptic plasticity in immature dentate granule cells of entorhino-hippocampal tissue cultures
Christos Galanis, Andreas Strehl, Tijana Radic, Stephan Wolfgang Schwarzacher, Thomas Deller, Andreas Vlachos

Self-organized network inhomogeneity governs spontaneous activity dynamics

Samora Okujeni[1,2], Ulrich Egert[1,2]

[1] Biomicrotechnology, Department of Microsystems Engineering, University Freiburg, Germany
[2] Bernstein Center Freiburg, University Freiburg, Germany

The spatial patterns of neurons and neurites determines the balance of long-range and recurrent local connectivity, and the degree of modularity in neuronal networks. Network formation is subject to homeostatic control thought to stabilize some activity-related target parameter.
Here, we studied how outgrowth, cell migration and activity interact in shaping neurite trees and network structure. The homeostatic regulation of neurite formation depends on protein kinase C, which modulates cytoskeletal turnover and stability in response to neuronal activity. Developmental manipulation of protein kinase C activity in cultured networks produced more homogeneous or more clustered cell body distributions with neurite outgrowth anti-correlated with the degree of modularity.
Despite large-scale differences in network architecture, morphogenesis ceased after three weeks in vitro, suggesting that homeostatic processes had established target activity levels. In contrast to theoretical studies on activity dependent growth but consistent with predictions for modular networks, spontaneous activity and rates of synchronized burst increased with the degree of clustering, whereas peak firing rates in bursts increased in highly interconnected homogeneous networks. This suggests that average firing rate is not the target function of homeostatic growth and migration.
A pivotal link between activity and growth dynamics is the intracellular Ca^{2+} concentration. The amplitude of Ca^{2+} transients increased exponentially with network recruitment during bursts, the corresponding peak firing rates and the resulting depolarization levels. In consequence, long-term estimates of Ca^{2+} levels converged for different architectures in mature networks. We show that simple rules for Ca^{2+}-dependent growth and migration are sufficient to explain different degrees of modularity in developing network architectures.

Cell assembly formation and non-random connectivity in networks subject to homeostatic structural plasticity

Júlia V Gallinaro[1], Stefan Rotter[1]

[1]University of Freiburg, Bernstein Center Freiburg, Hansastrasse 9a, 79104 Freiburg, Germany

The interaction between Hebbian and homeostatic plasticity in neuronal networks has recently received a lot of attention. Hebbian synaptic plasticity like STDP, known for its associative properties, leads to instabilities in recurrent networks, and different homeostatic mechanisms have been proposed to stabilize the learning process. While slow homeostatic plasticity has been observed in experiments, a mechanism fast enough to compensate instabilities on short time scales remains to be found [1]. The goal of this work is to contribute another aspect to the understanding of this interaction: could associative properties also emerge from a rule based on homeostatic principles [2]? We consider the maturation of networks in the primary visual cortex (V1) of mice as an example. In contrast to the situation right after eye-opening, neurons in adult V1 are more likely to connect to other neurons that have similar preferred orientations (PO) [3]. We simulate this maturation process in a recurrent network of leaky integrate-and-fire neurons, in which excitatory to excitatory connections are subject to a structural plasticity rule based on the homeostasis of firing rates [4,5]. We found that upon stimulation that emulates early visual experience [6], the connection probability is indeed modulated according to the PO of neurons. Moreover, we could show that this effect is long-lasting and the emerging structure decays only slowly when the specific external stimulation is turned off. Our results demonstrate very clearly that associative properties can also emerge from a plasticity rule that is only based on firing rate homeostasis in single neurons, and that is not explicitly dependent on correlations between the activity of neurons.

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Self-organization of neuronal dynamics by plasticity and adaptation

Anna Levina[1], Viola Priesemann[2], Oleg Vinogradov[1], Felix Effenberger[3]

[1]Universtity of Tübingen, Computer Science, Max-Planck-Ring 8, 72076 Tübingen, Germany
[2] Max Planck Institute for Dynamics and Self-Organization, Department of Nonlinear Dynamics, Am Faßberg 17, 37075 Göttingen, Germany
[3] Frankfurt Institute for Advanced Studies (FIAS), Ruth-Moufang-Str. 1, 60438 Frankfurt/Main, Germany

It is widely believed that the structure of neuronal circuits plays a major role in brain functioning. Although the full synaptic connectivity for larger populations is not yet assessable even by current experimental techniques, available data show that neither synaptic strengths nor the number of synapses per neuron are homogeneously distributed. Several studies have found long-tailed distributions of synaptic weights with many weak and a few exceptionally strong synaptic connections [1]. Little is known about how inhomogeneities could arise in the developing brain and we hypothesize that there is a self-organizing principle behind their appearance. We show how structural inhomogeneities can emerge by a combination of simple synaptic plasticity mechanisms from an initially homogeneous network [2].

The neuronal dynamics in dissociated cultures is dominated by intermingled periods of very low and very high activity, forming population bursts. Classical models of neuronal firing and plasticity are aiming at capturing the asynchronous state devoid of such burst [3]. We discuss how the parameters of these models shell be altered to obtain dynamics similar to hippocampal cultures. Along the way we discover, that adaptation is required to capture the essential features of the neuronal dynamics.

What can be a goal-state of such adaptation and plasticities? We discuss the information-theoretical evaluation of the development in dissociated cultures. We observe that information transfer and active information storage increase with development beyond the bounds explained by the increase in rate.

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Dopamine blocks homeostatic excitatory synaptic plasticity in immature dentate granule cells of entorhino-hippocampal tissue cultures

Christos Galanis, Andreas Strehl[2], Tijana Radic[2], Stephan Wolfgang Schwarzacher[2], Thomas Deller[2], Andreas Vlachos[1]

[1]Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany

[2]Neuroscience Center, Institute of Clinical Neuroanatomy, Goethe-University Frankfurt, 60590 Frankfurt, Germany

Homeostatic plasticity plays a fundamental role in maintaining neural networks in a stable state. Among the best studied forms is homeostatic synaptic plasticity, which adjusts synaptic strength in a compensatory manner to changes in network activity. In recent years, numerous studies have addressed the cellular and molecular mechanisms of homeostatic synaptic plasticity in various experimental conditions. Yet, its relevance in neural development and maturation remains not well-understood. In this context, we hypothesized that homeostatic plasticity, i.e., negative feedback mechanisms which aim to stabilize neural networks by preventing major changes in network structure and function, may be suppressed in immature neurons. To test this hypothesis, we employed single cell recordings of immature and mature dentate granule cells of entorhino-hippocampal tissue cultures. Consistent with our hypothesis, mature dentate granule cells show a robust synaptic scaling response in 4-week-old entorhino-hippocampal cultures, while in the same set of cultures immature granule cells do not adjust their excitatory synaptic strength in a compensatory manner. However, in 1-week-old entorhino-hippocampal cultures both mature and immature granule cells scale their excitatory synapses. In search of the mechanisms we show that dopamine blocks synaptic scaling via D1/5-receptor activation, specifically in immature granule cells of 1-week-old tissue cultures. Together, these results disclose the ability of immature granule cells to express homeostatic synaptic plasticity during early postnatal development. They reveal a novel role of dopaminergic signaling pathways, which may gate activity-dependent changes of newly born neurons by blocking homeostasis.(CG and AS contributed equally to this work.)

Symposium

S15: The brain oxytocin system - its complex impact on autism, social behavior, and stress

S15-1 Social Reinforcement Learning and its Neural Modulation by Oxytocin in Autism Spectrum Disorder
Martin Schulte-Rüther, Jana Kruppa, Anna Gossen, Eileen Oberwelland, Nicola Großheinrich, Hannah Cholemkery, Christine Freitag, Gregor Kohls, Gereon R. Fink, Beate Herpertz-Dahlmann, Kerstin Konrad

S15-2 Oxytocin and social contact reduce anxiety
Adam Steven Smith

S15-3 Oxytocin: its signaling of action and receptor signalling in the brain
Marta Busnelli

S15-4 The brain oxytocin system and its complex impact on stress and anxiety
Benjamin Jurek

S15-5 Oxytocin alters the morphology of hypothalamic neurons via the transcription factor myocyte enhancer factor 2A (MEF-2A)
Magdalena Meyer, Ilona Berger, Julia Winter, Inga Neumann, Benjamin Jurek

S15-6 Brain-Derived Neurotrophic Factor modulates synaptic properties of ovBNST neurons via TrkB receptors
Dominik Fiedler, Manju Sasi, Robert Blum, Christopher Klinke, Marta Andreatta, Hans-Christian Pape, Maren Denise Lange

Social Reinforcement Learning and its Neural Modulation by Oxytocin in Autism Spectrum Disorder

Martin Schulte-Rüther[1,2], Jana Kruppa[1,2], Anna Gossen[1,2], Eileen Oberwelland[1,2], Nicola Großheinrich[1], Hannah Cholemkery[3], Christine Freitag[3], Gregor Kohls[4], Gereon R. Fink[2,5], Beate Herpertz-Dahlmann[4], Kerstin Konrad[2,4]

[1]University Hospital RWTH Aachen, Translational Brain Research in Psychiatry and Neurology, JARA-Brain, Aachen, Germany

[2]Institute of Neuroscience and Medicine (INM-3), Jülich Research Center, Germany
[3]Department of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy, Goethe- University Frankfurt, Germany
[4] Department of Child and Adolescent Psychiatry, University Hospital RWTH Aachen, Germany
[5]Department of Neurology, University Hospital Cologne, Germany

Background: Oxytocin (OXT) has recently been shown to enhance motivation and attention to social stimuli. These effects may have the potential to enhance social reinforcement learning (SRL), the core mechanism of behavioral interventions. It is unclear whether OXT can compensate for possible deficits in SRL in ASD and whether such compensatory mechanism is related to an increase in saliency towards social stimuli per se or to a modulation of the brains' reward circuitry (especially nucleus accumbens; NAcc), which is specific for social feedback. These questions are important for future interventions aiming to combine OXT and behavioral treatments in ASD.
Objectives: We investigated the potential of OXT to compensate for deficits in socially reinforced learning in ASD and its underlying neural mechanism in a social learning task, which allowed for the differentiation of social feedback stimuli and social stimuli as the target of learning.
Methods: Using functional Magnetic Resonance Imaging we assessed brain activation during performance of a probabilistic reinforcement learning task in 24 typically developing controls (TDC) and 15 patients with ASD (18-26 years) in a double-blind placebo-controlled cross-over design. Participants indicated whether social or non-social stimuli belong to category A or B and social or non-social feedback with non-100% contingencies was provided. Data were analyzed using computational modeling according to the Q-Learning model. From the behavioral data, trial-by-trial reward-prediction error (RPE) values were calculated. We assessed the correlation of brain activation with RPE values during feedback and brain activation related to the anticipation of reward during choice. Based on previous studies of RPE learning and anticipation of reward, we focused on brain activation in the nucleus accumbens using an ROI approach ($p<.05$, voxel level corrected for ROI).
Results: In the ASD group, OXT enhanced the correlation of the RPE signal with activation in the NAcc during social feedback despite the learning target being non-social, whereas in the TDC group this effect was found in the PLC condition. The learning target being social showed a similar patterns in ASD, whereas in TDC, a reduced correlation was found for social learning targets during OXT. Behaviorally, subjects from both groups demonstrated significant learning during the task across conditions. Individuals with ASD showed OXT-induced enhanced learning when the learning target or feedback was social as compared to the non-social condition.
Conclusion: Our results demonstrate that in ASD, OXT selectively enhances reinforcement learning in social contexts, along with an enhanced correlation of the RPE with ventral striatal brain activation during social feedback. In the TDC group, OXT had a rather attenuating effect on RPE signals. Future studies should employ combined OXT-behavioral interventions for the treatment of ASD with a focus on providing opportunities for learning in social contexts and employ immediate reinforcing feedback.

Oxytocin and social contact reduce anxiety

Adam Steven Smith[1]

[1]University of Kansas, Department of Pharmacology & Toxicology and Program in Neuroscience, Lawrence, KS 66045, USA

Brain oxytocin (Oxt) regulates aspects of sociality, emotionality, and stress coping, releasing in various hypothalamic and extrahypothalamic brain areas in response to intimacy, conflict, and stress. It has been speculated that one of the advantages to social living, the anxiolytic effects of social contact with a bonded partner, could be propagated by an Oxt-mediated neural pathway. In a series of experiments, we investigated in female prairie voles (*Microtus ochrogaster*) the (1) anxiolytic effects of social buffering, (2) capacity for a social partner to promote Oxt neuronal circuitry, and (3) functional role of Oxt in mediate stress-induced activity of the hypothalamic-pituitary-adrenal (HPA) axis. In Experiment 1, distressed female voles who were allowed to interact with their bonded male partner demonstrated a reduction in stress-related behaviors and HPA output, namely a reduction in circulating corticosterone levels. In Experiment 2, social interaction with a partner after stress promoted release of Oxt in the paraventricular nucleus of the hypothalamus (PVN), the catalyzing site of the HPA axis. Pharmacological administration of Oxt into the PVN reduced the display of anxiety-like behaviors on the elevated plus maze (EPM) and plasma corticosterone levels. In addition, the social buffering effect during contact with a male partner after stress was eliminated if an Oxt receptor antagonist was administered into the PVN. In Experiment 3, ante-stress injections of Oxt in the PVN limited the elevated platform stress-induced (EPS) rise in circulating corticosterone levels, increase of c-Fos expression in corticotrophin-releasing hormone (CRH) immunoreactive (-ir) neurons in the PVN, and anxiety-like behaviors on the EPM while promoting c-Fos expression in GABA-ir neurons in the PVN. The anxiolytic effects of intra-PVN injections of Oxt were blocked with concurrent injects of a GABAA receptor antagonists. Together, our data demonstrate that social interaction during periods of distress can promote the release of Oxt in the PVN, and this signaling can inhibit the HPA axis stress response through excitation of GABA neurons which may function as an inhibitory tone on CRH neurons. This provides direct evidence that Oxt mediates aspects of the neurocircuitry regulating social buffering of stress.

Oxytocin: its signaling of action and receptor signalling in the brain

Marta Busnelli[1]

[1]National Research Council (CNR), Institute of Neuroscience, Via Vanvitelli 32, 20129 Milano, Italy

The hypothalamic neuropeptide oxytocin is increasingly recognized as an important regulator of human social behaviors, including social decision making, evaluating and responding to social stimuli, mediating social interactions, and forming social memories. Given the effect of oxytocin on these basic interpersonal interactions, there has been a growing body of research on the possible involvement of oxytocin in the pathophysiology and treatment of neuropsychiatric disorders that impact social functioning, such as autism, schizophrenia, and depression. However, the molecular cascade of events involved in mediating such effects are largely unknown. Both the distribution and the number of oxytocin receptors in the brain affect the type and degree of behavioral responses. The oxytocin receptors, expressed on neuronal and glial cells, transduce the oxytocin signal intracellularly via the activation of G proteins and downstream effectors. In vitro studies, performed in several cell lines, reported that oxytocin receptor can exist as monomer, homodimers or heterodimers and that can activate a number of signaling pathways that, depending on the cell context, may mediate synergistic or opposite effects. Understanding the various signaling mechanisms mediating oxytocin receptor-induced cell responses in the brain is difficult but crucial to determine the different responses in different cell types and brain regions, and the success of oxytocin and oxytocin-derived analogues in the treatment of neurodevelopmental and psychiatric diseases depends on how well we can control such responses.

Trying to determine the role of the oxytocin receptor in the nervous system, we first developed oxytocin receptor biased agonists, that inducing selective receptor conformations are active on a single specific signaling pathway (Busnelli et al. 2012; Passoni et al. 2016; Reversi et al. 2006). One of these, the atosiban, a selective OTR/Gi3 agonist, inhibited the effect of oxytocin on the firing properties of “sensory wide dynamic range” (WDR) neurons in the deep laminae of the spinal cord (Eliava et al. 2016). The use of the biased agonist atosiban contributed not only to clarify the involvement of an OTR/Gi pathway in a key function such as the regulation of analgesia at spinal cord level but also give rise to a new class of therapeutic agents.

Moreover, to determine the presence and the functional role of oxytocin receptor dimers in the brain, we generated a new class of oxytocin bivalent ligands, where two oxytocin-derived agonists were joined by a carboxylic spacer. Using these bivalents ligands we demonstrated that they induced the oxytocin receptor/Gq activation at a concentration that is 1,000 times less than that required by their monovalent counterparts and promoted sociability in mice and zebrafish at doses that are respectively 100 times lower than those of endogenous oxytocin and 40 times lower than those of endogenous isotocin, thus indicating that oxytocin receptor dimers are also present in the CNS, where they are involved in social processing (Busnelli et al. 2016).

In conclusion, our data demonstrate that the newly developed oxytocin-analogs not only can help to define how the specific cellular responses and behavioral effects are generated but also have a great potential in paving the way for the development of advanced therapeutic targeting the oxytocin receptor in important diseases such as autism, schizophrenia and other neurological disorders.

The brain oxytocin system and its complex impact on stress and anxiety

Benjamin Jurek[1]

[1]University of Regensburg, Department of Behavioral and Molecular Neurobiology, Universitaetsstr. 31, 93051 Regensburg, Germany

In recent years, the neuropeptide oxytocin (OXT) attracted considerable attention in science and public due to its ability to modulate aspects of socio-emotional behavior and stress. Studies on intranasal OXT in human patients or healthy probands are accumulating, reporting a barrage of effects depending on the context, sex, dosage, and duration of the treatment. This is in part due to the plethora of signaling cascades that are coupled to the OXT receptor (OXTR), such as the MEK1/2, p38, ERK5, CamK, PI3K, Calcineurin, eEF2, or PKA/PKC. The activity of these cascades depend on the sex, species and duration of treatment (Jurek and Neumann, 2018), while signal/target specificity is brought about by the exclusive or combined activation of a yet unknown subset of signaling cascades. OT's concerted effects on specific cascades lead to altered activity of a defined set of nuclear transcription/translation factors, (e.g. CREB, CRTC3, MEF-2, or eEF2, see graphic below) to alter gene expression, but also cytoplasmic targets, like channel proteins, to alter neuronal excitability (van den Burg et al., 2015) or morphology (Meyer et al., 2018). Our research aims to determine how the combination of nuclear and cytoplasmic effects interact to lead to the observed behavioural effects of OXT.
We could unravel the role of the MAPK pathways in OT's anxiolytic effect (Jurek et al., 2012), as well as the involvement of the CREB/CRTC (Jurek et al., 2015) and MEF-2A/C (Meyer et al., 2018) pathways in OT's transcriptional regulation of stress-related factors, such as corticotropin releasing factor (CRF), CRF receptor 2, or NPY5R. The transcriptional regulation of those factors manifests then in altered anxiety-like behavior. With this work we hope to contribute to a better understanding of the underlying mechanisms of altered socio-emotional behavior, which is essential for the development of effective and safe treatment of anxiety- or stress-related disorders.

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Oxytocin alters the morphology of hypothalamic neurons via the transcription factor myocyte enhancer factor 2A (MEF-2A)

Magdalena Meyer, Ilona Berger[2], Julia Winter[1], Inga Neumann[1], Benjamin Jurek[1]

[1]University of Regensburg, Institute of Zoology, Department of Behavioral and Molecular Neurobiology, Germany

[2]Technische Universität Dresden, University Hospital, Department of Internal Medicine III, Dresden, Germany

Oxytocin (OT) has gained attention not only as anxiolytic drug and as potential treatment option for autistic children; it also acts as a growth and differentiation factor in neuronal cells. While behavioral effects of OT have been studied in detail, knowledge about the cellular effects of OT is relatively sparse. In this study, we present evidence for three hypotheses: 1) OT leads to neurite retraction in hypothalamic neurons via the OT receptor (OTR) 2) The transcription factor MEF-2A is a central regulator of OT-induced neurite retraction, and 3) The MAPK pathway is critical for OT-induced MEF-2A activation. Incubation of rat hypothalamic H32 cells with 10 nM to 1 µM OT, vasopressin, and the specific OTR agonist TGOT, over the course of 12 h resulted in a time-dependent, significant retraction of neurites. In addition, the size of the nuclear compartment increased, whereas the overall cell size remained unchanged. OT treatment for 10 h increased the cellular viability significantly, and this effect could be blocked by a specific OTR antagonist, providing evidence for a specific and pro-active effect of OT on neurite retraction, and not as an unspecific side effect of apoptosis. The molecular mechanism that controls OT-induced neurite retraction includes a reduced phosphorylation of the transcription factor MEF-2A at Serine 408 (S408). This dephosphorylation is under the control of the OTR-coupled MAPK pathway, as blocking MEK1/2 by U0126 inhibited MEF-2A activation and subsequent neurite retraction. The siRNA-mediated knockdown of MEF-2A prevented the OT-induced neurite retraction, providing direct evidence for a role of MEF-2A in morphological alterations induced by OT treatment. In summary, the present study reveals a previously unknown OTR-coupled MAPK-MEF-2A pathway, which is responsible for OT-induced neurite retraction of hypothalamic neurons.

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Brain-Derived Neurotrophic Factor modulates synaptic properties of ovBNST neurons via TrkB receptors

Dominik Fiedler, Manju Sasi[2], Robert Blum[2], Christopher Klinke[3], Marta Andreatta[3], Hans-Christian Pape[1], Maren Denise Lange[1]

[1]Institute of Physiology I, Westfälische Wilhelms-University Münster, Germany
[2]Institute of Clinical Neurobiology, University Hospital Würzburg, Germany
[3]Institute of Psychology I, Julius-Maximilians-University Würzburg, Germany

The neurotrophin brain-derived neurotrophic factor (BDNF) is well known to contribute to neuronal development, growth and differentiation. In addition, it plays an essential role in memory formation and is associated with fear learning and extinction. Further, BDNF is highly present in the bed nucleus of the stria terminalis (BNST), a key region contributing to stress-modulated fear. However, the mechanisms by which BDNF modulates synaptic transmission as well as synaptic plasticity in local BNST networks are not well understood.

Immunohistochemically staining revealed the existence and regional distribution of BDNF in the BNST, with high levels in the oval nucleus (ovBNST). To examine if BDNF may modulate ovBNST neurons, electrophysiological in vitro whole cell patch clamp recordings were performed. Synaptic plasticity of ovBNST neurons was examined as long-term depression (LTD). Robust LTD in ovBNST neurons was initiated by low frequency stimulation (10 Hz for 10 min). LTD was absent by functional blocking the BDNF receptor TrkB either by its antagonist ANA-12 or by the kinase inhibitor K252a. Further, LTD was absent when BDNF was captured by a BDNF scavenger (TrkB-Fc chimera). These results demonstrate that this form of synaptic plasticity is BDNF dependent. Intracellular BAPTA also abolished this form of LTD indicating its presynaptic nature. The investigation of synaptic transmission in ovBNST neurons revealed no effect of BDNF application on frequency or amplitude of spontaneous evoked postsynaptic currents. However, BDNF application caused a significant hyperpolarizing shift of the membrane potential from resting values, indicating a lower excitability of ovBNST neurons, mediated via TrkB receptors.

In summary, BDNF was demonstrated to be highly present within the ovBNST. We further revealed substantial effects of BDNF on synaptic plasticity, but not basal synaptic activity in ovBNST neurons, mediated via TrkB. These findings point out the critical impact of BDNF TrkB mediated signaling on synaptic properties of ovBNST neurons, apt to affect long lasting fear memory.

Symposium

S16: Mitochondrial dysfunction in neurodegeneration

Proteolytic control of mitochondrial dynamics and neurodegeneration

Thomas Langer[1]

[1]Max-Planck-Institute for Biology of Ageing, AG Langer, Joseph-Stelzmann-Straße 9B, 50931 Cologne, Germany

Mitochondria are functionally and structurally dynamic organelles that play key roles in metabolism and participate in cellular signaling. A decline in mitochondrial activities is associated with ageing and neurodegeneration. The i-AAA protease YME1L in the mitochondrial inner membrane acts as gatekeeper of mitochondrial quality control, regulates mitochondrial phospholipid trafficking and balances fusion and fission of mitochondria by processing of the dynamin-like GTPase OPA1. Homozygous recessive mutations in human YME1L trigger mitochondrial fragmentation and cause a neuromuscular disorder with intellectual disability, motor developmental delay, optic atrophy as well as ataxia and movement deficiencies. Mice lacking Yme1l in the nervous system manifest ocular dysfunction with microphthalmia and cataracts and develop deficiencies in locomotor activity due to specific degeneration of spinal cord axons, which relay proprioceptive signals from the hind limbs to the cerebellum. The analysis of this mouse model provides new insights into the relationship of mitochondrial dynamics and disturbed mitochondrial proteostasis for neuronal survival.

CLUH is a post-transcriptional regulator of mitochondrial function

Elena Rugarli[1]

[1]University of Cologne, Institute of Genetics and CECAD, Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany

Mitochondria are dynamic and plastic organelles, which flexibly adapt morphology and metabolic function to meet extrinsic challenges and demands. The mitochondrial proteome is encoded by both the nuclear and the mitochondrial genomes. Well-characterized transcriptional cascades control the expression of nuclear encoded mitochondrial proteins and thus regulate mitochondrial biogenesis. However, these broad and slow transcriptional responses can hardly explain the fine-tuned adaptation of mitochondrial function, which is required during certain physiological conditions. Recently, we discovered that the CLUH (clustered mitochondria homologue) protein is an evolutionary conserved RNA-binding protein specific for a subset of transcripts encoding mitochondrial proteins. CLUH protects target mRNAs from decay and promotes translation. In addition, CLUH plays a crucial role in allowing an efficient mitochondrial catabolic response at the foetal-neonatal transition and during starvation. We will discuss how CLUH mechanistically regulates mitochondrial gene expression, and the physiological implications for the nervous system.

Mitochondrial turnover and homeostasis in ageing and neurodegeneration

Nektarios Tavernarakis[1]

[1]Foundation for Research and Technology-Hellas, Institute of Molecular Biology and Biotechnology, N. Plastira 100, Vassilika Vouton, PO Box 1385, 70013 Heraklion, Crete, Greece

Ageing is driven by the inexorable and stochastic accumulation of damage in biomolecules vital for proper cellular function. Although this process is fundamentally haphazard and uncontrollable, senescent decline and ageing is broadly influenced by genetic and extrinsic factors. Numerous gene mutations and treatments have been shown to extend the lifespan of diverse organisms ranging from the unicellular Saccharomyces cerevisiae to primates. It is becoming increasingly apparent that most such interventions ultimately interface with cellular stress response mechanisms, suggesting that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Key determinants of this capacity are the molecular mechanisms that link ageing to main stress response pathways, and mediate age-related changes in the effectiveness of the response to stress. How each pathway contributes to modulate the ageing process is not fully elucidated. A better understanding of the dynamics and reciprocal interplay between stress responses and ageing is critical for the development of novel therapeutic strategies that exploit endogenous stress combat pathways against age-associated pathologies. Mitochondria, the main energy hub of the cell, are highly dynamic organelles, playing essential roles in fundamental cellular processes. Mitochondrial function impinges on several signalling pathways modulating cellular metabolism, cell survival and healthspan. Maintenance of mitochondrial function and energy homeostasis requires both generation of newly synthesized and elimination of dysfunctional mitochondria. Impaired mitochondrial function and excessive mitochondrial content are major characteristics of ageing and several human pathophysiological conditions, highlighting the pivotal role of the coordination between mitochondrial biogenesis and mitophagy. However, the cellular and molecular underpinnings of mitochondrial mass homeostasis remain obscure. Our findings unravel a homeostatic feedback loop that allows cells to adjust their mitochondrial population in response to environmental and intracellular cues. Age-dependent decline of mitophagy both inhibits removal of dysfunctional or superfluous mitochondria and impairs mitochondrial biogenesis resulting in progressive mitochondrial accretion and consequently, deterioration of cell function.

The origin of sleep defects in Parkinson disease

Jorge De Sousa Valadas[1]

[1]VIB-KU Leuven , Center for Brain & Disease Research, 3000, Campus Gasthuisberg, Herestraat 49 Leuven, Belgium

Parkinson's disease is the second most common neurodegenerative disorder, characterized by typical motor symptoms including tremor and difficulties to initiate movements. However, more than a decade before the onset of motor symptoms, most patients already suffer from disturbed sleep patterns that persist throughout the course of the disease. Although sleep disturbances are extremely debilitating and not reverted by the most commonly used symptomatic treatment for Parkinson's disease: L-DOPA, the study of non-motor symptoms in Parkinson's have attracted relatively little attention. We have used different fruit fly models of familial Parkinson's disease and identify circadian rhythm and sleep defects that are in nature similar to those seen in patients. We have mapped the defects to specific neurons in the brain and identify key molecular and organellar defects at the level of ER-mitochondrial contacts that explain neuronal dysfunction. Similarly, induced neurons derived from skin cells from Parkinson patients show identical defects to those we observe in fly sleep neurons, indicating our findings are evolutionarily conserved and pathologically relevant. Based on our findings we propose specific therapeutic avenues we will pursue in future work.

ROLE OF RABCONNECTIN-3A IN VESICLE ACIDIFICATION, TRAFFICKING AND NEURODEGENERATION

Sindhuja Gowrisankaran, Andrea Raimondi[2], Nicolas de Roux[3], Ira Milosevic[1]

European Neuroscience Institute (ENI), Synaptic Vesicle Dynamics Group; University Medical Center Göttingen (UMG), Göttingen, Germany [1]

Advanced Light and Electron Microscopy BioImaging Center (ALEMBIC), Hospital San Raffaele, Milano, Italy[2]

Inserm U1141, Hospital Robert Debré, University Paris Diderot, Paris, France [3]

The regulation and timing of vesicle acidification is essential for numerous cellular processes, from macro-molecule degradation in lysosomes to refilling of synaptic vesicles (SVs) at the neuronal synapse. Acidification of vesicles is achieved by vacuolar ATPases (vATPases), a family of proton pumps that controls the pH gradient across organelle membranes. Despite their critical importance at the synapse and in many intracellular trafficking routes, the regulation of vATPase activity is poorly understood. In a search for the vATPase regulators, we cloned human Dmxl2 gene encoding Rabconnectin-3a (Rbcn-3a). An alteration in the gene dosage of Dmxl2 in human patients resulted in a complex pathology called as poly endocrine-polyneuropathy syndrome (PEPNS). Rbcn-3a encodes a large 340kDa protein, whose function at the mammalian synapse remains largely unknown.

We found it to be present on every organelle that acidifies, including SVs. Loss of Rbcn-3a in mice resulted in early embryonic lethality. When Rbcn-3a is eliminated from neuronal cells in culture, neurons developed normally, yet their activity was perturbed. Neurons without Rbcn-3a failed to fully acidify SVs, and showed altered SV recycling. Curiously, the synapses of neurons without Rbcn-3a also accumulated lysosomes-like structures and lysosomal markers, suggesting an unanticipated connection between the machinery for endocytosis, acidification and cellular homeostasis.

Lysosomal and mitochondrial crosstalk: a case for neurodegeneration in LSDs?

King Faisal Yambire

[1]Universitätsmedizin Göttingen, Institute of Cellular Biochemistry, Humboldtallee 23, 37073 Göttingen, Germany
[2]Synaptic Vesicle Dynamics, European Neuroscience Institute, Grisebachstr. 5, 37077 Göttingen, Germany

Metabolic defects result in various diseases, with neurodegeneration as one of the most prevalent consequences. Mitochondria and lysosomes are essential for cellular metabolism. In addition to being cellular 'powerplants', mitochondria play crucial roles in cellular signaling by contributing to cellular stress responses like autophagy, apoptosis or cell proliferation. Lysosomes have evolved beyond their role as cellular 'incinerators' to coordinate major processes such as autophagy and nutrient sensing. Contrary to previous views, recent evidence suggest the existence of functional interdependent networks between lysosomes and mitochondria. In this study, we elucidate mechanisms of crosstalk beyond autophagy (mitophagy) between lysosomes and mitochondria, and show that lysosomal defects affect mitochondrial function.

To identify the mechanism(s) regulating lysosomal and mitochondrial crosstalk, we employed two biochemically similar models of lysosomal lipid storage disorders (LSDs) with distinct etiologies: Acid sphingomyelinase- and Niemann-pick type C1- deficient mouse tissues, which are respective models for Niemann-Pick types A and C diseases. We also evaluated the effects of chronic lysosomal malfunction on mitochondrial fitness and function in cells from patients of these disorders.

We found in Niemann-Pick disease patient cells that impaired S1PR1 signaling engages transcriptional programs, via KLF2 and ETV1, to repress mitochondrial biogenesis and function. Moreover, *in silico* experiments from microarray datasets of brain and liver samples of a mouse model of Niemann-Pick disease confirmed the induction of KLF2 and ETV1, and the concomitant repression of mitochondrial biogenesis. Interestingly, mechanisms of KLF2 and ETV1 downregulation, including siRNA-mediated silencing or enhanced S1PR1 signaling, are enough to promote mitochondrial biogenesis. These findings highlight the involvement of a transcriptional network in the regulation of lysosomal and mitochondrial crosstalk and the therapeutic potential of modulating S1PR1 signaling in Niemann-Pick disease.

Symposium

S17: Dissection of a central brain circuit: structure, plasticity and functions of the drosophila mushroom body

S17-1 Mechanisms underlying age-induced memory impairment in relation to mushroom body function
Stephan Sigrist

S17-2 Serotonergic Modulation of Memory Circuits
Lisa Scheunemann, Clément Hua, Thomas Preat

S17-3 Function of the anterior paired lateral (APL) neuron in associative olfactory learning in larval *Drosophila*
Nino Mancini, Michael Schleyer, Bertram Gerber

S17-4 Reward signaling in a recurrent circuit of dopaminergic neurons and Kenyon cells in the *Drosophila* larva
Radostina Lyutova, Maximilian Pfeuffer, Dennis Segebarth, Jens Habenstein, Astrid Rohwedder, Felix Frantzmann, Mareike Selcho, Christian Wegener, Andreas S. Thum, Dennis Pauls

S17-5 Modelling the mechanisms of learning in the mushroom body
Barbara Webb

S17-6 Mechanisms to diversify learning rules in parallel memory circuits
Yoshinori Aso, Gerald Rubin

Mechanisms underlying age-induced memory impairment in relation to mushroom body function

Stephan Sigrist[1]

[1]Freie Universität, Genetics, Takustrasse 6, 14195 Berlin, Germany

Macroautophagy is a cellular maintenance program conserved from yeast to humans, meant to protect the brain from premature aging and neurodegeneration. How neuronal autophagy, usually loosing efficacy with age, intersects with neuronal processes mediating brain maintenance remains to be explored. Here, we show that impairing autophagy in the Drosophila learning center (mushroom body, MB) but not in other brain regions triggered changes normally restricted to aged brains: impaired associative olfactory memory as well as a brain-wide ultrastructural increase of presynaptic active zones ("metaplasticity"), a state non-compatible with memory formation. Mechanistically, decreasing autophagy within the MBs reduced expression of an NPY-family neuropeptide, and interfering with autocrine NPY signaling of the MBs provoked similar brain-wide metaplastic changes. Our results in an exemplary fashion show that autophagy-regulated signaling emanating from a higher brain integration center can execute high-level control over other brain regions to steer life-strategy decisions such as whether or not to form memories.

Serotonergic Modulation of Memory Circuits

Lisa Scheunemann[1,2], Clément Hua[1], Thomas Preat[1]

[1]ESPCI Paris, Genes and Dynamics of Memory Systems, CNRS, 10 rue Vauquelin, 75005 Paris, France
[2]Sigrist Group, Institut of Biology - Genetics, Department of Biology, Chemistry and Pharmacology, Freie Universität Berlin, Takustraße 6, 14195 Berlin, Germany

An important feature of animal behavior is to remember positive or negative experiences and adapt their behavior by an avoidance or attraction response towards the associated stimuli. In many species, dopamine transmits the valence of an experience, yet little is known how positive and negative stimuli are encoded in the brain and integrate into memory circuits. In Drosophila, the input of dopaminergic signals to the memory center, the mushroom body (MB), has been dissected to a single cell level (Aso et al., 2014). Thereby, the rewarding signals as well as the aversive input to the MB have been identified, which are organized by a specific network of dopaminergic projections onto the MB and MB output neurons that tile the MB into structural and functional compartments (Owald and Waddell, 2015). This plastic organization is thought to skew MB output synapses towards an approaching or avoidance behavior.
How the opposing valences are encoded upstream of the dopaminergic system remains still largely elusive. A previous body of work indicates an interaction between serotonin and dopamine in the control of conditioned appetitive and aversive behavior. In a recent study, we have shown that in Drosophila, aversive long-term memory is consolidated only if a pair of serotonergic neurons, the SPN, is activated and stimulates downstream oscillations in the dopaminergic neurons MP1, which allows LTM processes in the MB (Scheunemann et al., 2018). Inhibition of the SPN directly after aversive LTM training strongly impairs LTM. However, we now find that the same inhibition after appetitive training leads to increased LTM scores. Since dopaminergic signaling from MP1 was shown to be indispensable for both aversive and appetitive LTM expression, we hypothesize that the internal state of the animal is changing plasticity properties of the SPN-MP1-MB axis. These results could provide further insight into how information is evaluated in a state-dependent manner and directly acts on cognitive function.

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Owald, D., and Waddell, S. (2015). Olfactory learning skews mushroom body output pathways to steer behavioral choice in Drosophila. Curr. Opin. Neurobiol. 35, 178–184.
Scheunemann, L., Plaçais, P.Y., Dromard, Y., Schwärzel, M., and Preat, T. (2018). Dunce Phosphodiesterase Acts as a Checkpoint for Drosophila Long-Term Memory in a Pair of Serotonergic Neurons. Neuron.

Function of the anterior paired lateral (APL) neuron in associative olfactory learning in larval *Drosophila*

Nino Mancini, Michael Schleyer[1], Bertram Gerber[1]

[1]Leibniz Institute for Neurobiology (LIN), Genetics of Learning and Memory, Brenneckestraße, 6, 39118 Magdeburg, Germany

Inhibitory systems are important controllers of sensory systems and behaviour, allowing the processing of relevant information against environmental noise, and the selection of adaptive motor actions from a pool of competing behavioural options. Several studies have shown the critical role of GABAergic synaptic inhibition in odour processing, olfactory learning and behavior in invertebrates, including *Drosophila melanogaster*.

In this project, we focus on a single, GABAergic anterior paired lateral (APL) neuron, identified in both adult and larval *Drosophila*. Although the role of APL in memory acquisition and retrieval has been investigated in adults, the lack of a defined circuitry limits the interpretation of behavioural and physiological data. Larval *Drosophila*, however, offers such possibilities because of its simple olfactory system that is well characterized at synaptic resolution and without cellular redundancy. Using a combination of behavioural analysis, optogenetics and connectomics, we aim to understand how APL inhibitory processes are organized in the larval brain and how they modulate associative olfactory memory formation and retrieval.

We discovered, surprisingly, that activating APL optogenetically is sufficient to establish a reward memory. We follow up on this asking whether this rewarding effect requires intact GABA synthesis in APL and working in collaboration with colleagues from Würzburg University contributing to physiological expertise.

Reward signaling in a recurrent circuit of dopaminergic neurons and Kenyon cells in the *Drosophila* larva

Radostina Lyutova, Maximilian Pfeuffer[2], Dennis Segebarth[2,4], Jens Habenstein[2,5], Astrid Rohwedder[3], Felix Frantzmann[2], Mareike Selcho[2], Christian Wegener[2], Andreas S. Thum[3], Dennis Pauls[2]

[1]Julius-Maximilians Universität Würzburg, Dept. of Neurobiology and Genetics, Am Hubland, Biocenter, 97074 Würzburg, Germany

[2]Neurobiology and Genetics, Theodor-Boveri Institute, Biocenter, University of 10 Würzburg, D-97074 Würzburg, Germany

[3] Department of Genetics, University of Leipzig, D-04103 Leipzig, Germany

[4]Institute of Clinical Neurobiology, University Hospital of Würzburg, D-97078 Würzburg, Germany (present address)

[5]Department of Behavioral Physiology and Sociobiology, Theodor-Boveri Institute, Biocenter, University of Würzburg, D-97074 Würzburg, Germany (present address)

Dopaminergic neurons in the brain of the *Drosophila* larva play a key role in mediating reward information to the mushroom bodies during appetitive olfactory learning and memory. Using optogenetic activation of Kenyon cells we provide evidence that a functional recurrent signaling loop exist between Kenyon cells and dopaminergic neurons of the pPAM cluster. An optogenetic activation of Kenyon cells paired with an odor is sufficient to induce appetitive memory, while a simultaneous impairment of the dopaminergic pPAM neurons abolishes memory expression. Thus, dopaminergic pPAM neurons mediate reward information to the Kenyon cells, but in turn receive feedback from Kenyon cells. We further show that the activation of recurrent signaling routes within mushroom body circuitry increases the persistence of an odor-sugar memory. Our results reveal that sustained activity in the underlying circuitry is a conserved mechanism in insects and vertebrates to consolidate memories.

Modelling the mechanisms of learning in the mushroom body

Barbara Webb[1]

[1]University of Edinburgh, School of Informatics, 10 Crichton St, EH8 9AB Edinburgh, United Kingdom

An established function of the mushroom body (MB) in Drosophila and other insects is to support associative learning, e.g., to modulate approach or avoidance of odours depending on their previous pairing with attractive or aversive foodstuffs. The general architecture of this circuit has long been interpreted as implementing a fairly straightforward learning rule, in which co-occurrence of a sparse activation pattern across MB intrinsic neurons with the presence of a reinforcement signal carried by dopaminergic inputs will alter the strength of the connection from the intrinsic neurons to output neurons that control behaviour. Recent neuroanatomical and neurogenetic studies have largely supported this picture but reveal a number of additional complexities that suggest a more sophisticated function; and modelling studies have shown that this basic learning mechanism cannot account for all the experimental data. We draw on this new information to hypothesise alternative learning mechanisms and explore their capacity in computational models, agents and robots.

Mechanisms to diversify learning rules in parallel memory circuits

Yoshinori Aso[1], Gerald Rubin[1]

[1]Janelia Research Campus, , 19700 Helix Drive, 20147 Ashburn, USA

Animals discriminate stimuli and learn their predictive value based on temporal correlations with reward or punishment. Such associative learning entails lasting changes in connections between neurons. These changes are referred to as memory traces or engrams. It is now clear that even a simple form of associative learning induces multiple, distributed engrams. Do these multiple engrams serve different functions? How are they integrated for guiding memory-based action selection?
My lab has been studying the circuit of the mushroom body (MB) in Drosophila brains as a model system of parallel memory circuits. Sparse activity in the 2,000 Kenyon cells of the mushroom body represents the identity of sensory stimuli. Along the parallel axonal fibers of Kenyon cells, we have shown that dopaminergic neurons and MB output neurons form 16 matched compartmental units. These anatomically defined units are also units of associative learning. Our latest optogenetic activation experiments demonstrated that individual dopaminergic neurons independently write and update memories in each unit with cell-type-specific rules. We find extensive differences in the rate of memory formation, decay dynamics, storage capacity and flexibility to learn new associations across different units. Thus individual memory units within the mushroom body store different information about the same learning event. I will talk about our latest transcriptome data from individual dopaminergic neuron cell types and how cotransmitters of dopaminergic neurons diversify memory dynamics in the mushroom body.

Symposium

S18: From normal brain development to pathology: what role does the environment play?

S18-1 Developmental emergence of adult neural stem cells: Unravelling the influence of the niche
Scott A. Yuzwa, Danielle Jeong, Michael J. Borrett, Brendan T. Innes, Anastassia Voronova, Troy Ketela, David R. Kaplan, Gary D. Bader, Freda D. Miller

S18-2 Alteration of serotoninergic system alters neuroplastic mechanisms from postnatal development until adulthood.
Paola Brivio, Giulia Sbrini, Judith Homberg, Natalia Alenina, Francesca Calabrese

S18-3 Early active intercellular signaling networks in the developing human brain
Simone Mayer

S18-4 Stress hormones during pregnancy and fetal brain development: what we can learn from perinatal tissues and in vitro models
Cristiana Cruceanu, David S Fischer, Leander Dony, Simone Roeh, Anthodesmi Kontira, Silvia Martinelli, Maik Koedel, Rossella DiGiaimo, Silvia Cappello, Fabian J Theis, Elisabeth Binder

S18-5 Prevention of schizophrenia deficits via non-invasive adolescent frontal cortex stimulation in rats
Rebecca Winter, Ravit Hadar, Henriette Edemann-Callesen, Franziska Wieske, Bettina Habelt, Niranjan Khadka, Elizabeth Barroeta Hlusicka, Janine Reis, Klaus Funke, Nadine Bernhardt, Michael Nitsche, Christine Winter

S18-6 Maternal inflammation during pregnancy and fetal brain development
Claudia Buss, Alice Graham, Jerod Rasmussen, Sonja Entringer, John Gilmore, Martin Styner, Pathik Wadhwa, Damien Fair

Developmental emergence of adult neural stem cells: Unravelling the influence of the niche

Scott A. Yuzwa[1], Danielle Jeong[2,4], Michael J. Borrett[2,4], Brendan T. Innes[5,6], Anastassia Voronova[2], Troy Ketela[3], David R. Kaplan[2,4,6], Gary D. Bader[5,6], Freda D. Miller[2,4,6,7]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada, [2]Program in Neuroscience and Mental Health, Hospital for Sick Children, Toronto, Canada, [3]Princess Margaret Genomics Centre, University Health Network, Toronto, Canada, Institute of Medical Science[4], The Donnelly Centre[5] and Departments of Molecular Genetics[6] and Physiology[7], University of Toronto, Toronto, Canada M5G 1A8

Adult neural stem cells (NSCs), found in the ventricular/sub-ventricular zone region lining the lateral ventricles of the forebrain, are known to derive from radial precursor cells (RPs) which build the developing brain. Little, however, has been known with respect to how NSCs are established developmentally from RPs in the embryonic brain. Recent studies have shown that NSCs arise from RPs around mid-gestation and that this embryonic RP to NSC transition is characterized by RPs adopting a 'slow-dividing' or quiescent state thereby allowing these cells to persist throughout life in the adult brain as NSCs. The molecular mechanisms controlling this 'slow-dividing' state and therefore the transition from RPs to NSCs are largely unknown. Here I will describe how we have captured this RP to NSC transition at the single-cell level using high-throughput single-cell RNA-sequencing (scRNA-seq) carried out at multiple time points across embryonic cortical development. Using these data, we show that cortical RPs are relatively similar at the transcriptional level from E13.5 onwards, suggesting that it is likely that extrinsic cues from the niche, as opposed to intrinsic factors, are critical in controlling the establishment of the 'slow-dividing' state and thus the transition from RPs to NSCs. Further, I will describe how we have leveraged our scRNA-seq data to identify and characterize a cell-surface protein which acts as a proliferation 'on/off' switch by controlling the influence of the niche environment on 'slow dividing' RPs in culture and in vivo. Understanding how the microenvironment controls the proliferation of 'slow-dividing' RPs could enable the development of therapeutic strategies aimed at activating NSCs following injury or disease.

Alteration of serotoninergic system alters neuroplastic mechanisms from postnatal development until adulthood.

Paola Brivio, Giulia Sbrini[1], Judith Homberg[2], Natalia Alenina[3], Francesca Calabrese[1]

[1]Department of Pharmacological and Biomolecular Sciences, Universita' degli Studi di Milano, Milan, Italy

[2] Department of Cognitive Neuroscience, Centre for Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen, The Netherlands

[3] Max-Delbrueck-Center for Molecular Medicine (MDC), Berlin, Germany

Serotonin (5-HT) is a neurotransmitter that plays a central role in the brain development as well as in the regulation of the central nervous system (CNS) and alterations in the serotonergic system may contribute to the pathophysiology of mood disorders. Serotonin transporter (SERT) and tryptophan hydroxylase 2 (TPH2) knockout rats allowed us to investigate whether the vulnerable genotypes that induce hyperserotonergia, due to strongly reduced serotonin reuptake, and hyposerotonergia, due to lack of serotonin synthesis, respectively, may be associated with alterations of neuronal plasticity from the early stage of brain maturation until adulthood. Using SERT-/- and TPH2-/- rats at different ages, we highlighted a specific developmental pattern of the expression of the neuroplasticity's marker Brain Derived Neurotrophic Factor (BDNF) in rats' prefrontal cortex. The massive concentration of 5-HT in the synaptic cleft, due to the deletion of SERT, led to a reduction in Bdnf expression that originated early in life and worsened till adulthood, pointing out the negative effect of a chronic upregulated serotoninergic transmission. In contrast, in TPH2-/- rats we observed an increase in Bdnf expression starting post-weaning, that persisted until adulthood, indicating that the brain during its maturation activates compensatory mechanisms to deal with a lack of serotonin.
In summary, our results suggest that the perturbation of the serotoninergic system affects neuroplastic mechanisms throughout postnatal development, indicating that 5-HT has critical implications for brain plasticity across the lifespan.

Early active intercellular signaling networks in the developing human brain

Simone Mayer[1,2,3]

[1]Hertie Institut für klinische Hirnforschung, Molecular Brain Development, Otfried-Müller-Strasse 27, 72076 Tübingen, Germany

[2]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA, USA.

[3]Department of Neurology, University of California, San Francisco, CA, USA

Molecular fingerprints of diverse cell types have been characterized recently in the human neocortex using single-cell sequencing approaches. Using a single-cell RNA-Sequencing dataset and single-molecule in situ hybridization, we show that prior to the formation of the majority of cortical neuronal networks and synapses, progenitor cells and differentiating neurons express a plethora of neurotransmitter receptors. Activation of these receptors induces membrane currents and intracellular calcium elevations. By combining Calcium imaging and RNA-Sequencing in the same single cells, we identify that responses to neurotransmitters occur in a cell type-specific manner. This implies that neurotransmitters may specifically modulate the proliferation of progenitor cells and the differentiation of neurons and glial cells. The dynamic nature of neurotransmitter signaling along the neural lineage means that even small alterations caused by environmental or genetic disturbances may affect brain development and contribute to neurodevelopmental disorders or the predisposition to psychiatric disorders

Stress hormones during pregnancy and fetal brain development: what we can learn from perinatal tissues and in vitro models

Cristiana Cruceanu[1], David S Fischer[2], Leander Dony[1,2], Simone Roeh[1], Anthodesmi Kontira[1], Silvia Martinelli[1], Maik Koedel[1], Rossella DiGiaimo[3,4], Silvia Cappello[3], Fabian J Theis[2], Elisabeth Binder[1,5]

[1]Max Planck Institute of Psychiatry, Translational Research, Kraepelinstr. 2-10, 80804 Muenchen, Germany
[2]Institute of Computational Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg 85764, Germany;
[3]RG Developmental Neurobiology, Max Planck Institute of Psychiatry, Germany;
[4]Department of Biology, University of Napoli, Complesso Universitario Monte S. Angelo, 80126 Napoli, Italy;
[5]Department of Psychiatry and Behavioral Sciences, Emory University School of Medicine, USA.

The brain undergoes important growth and plasticity during prenatal development, and increased glucocorticoid (GC) exposure is one of the main factors mediating stress effects during this time, likely through epigenetic and transcriptional trajectories. Questions regarding human-specific early neurodevelopmental molecular pathways of prenatal stress cannot be addressed with current animal model tools. Top investigate these processes in a potentially superior system, we used iPSC-derived 3-dimensional brain organoids to model prenatally mediated risk and response to stress. We performed RNA sequencing (RNAseq) across nine organoid developmental stages (day 17 to 158) to determine this model's suitability as a fetal brain. To investigate GC effects, we tested dose- and time-curve stimulation in organoids with GC-receptor agonist dexamethasone, Finally, to determine specific cell type responses to GCs, we profiled 15,000 individual cells' transcriptomes using single cell RNAseq (scRNAseq) across organoid development (days 30, 60, 90). We identified key neurodevelopment markers expressed in the cerebral organoid model – including SOX2, PAX6, FOXG1, MAP2, with trajectories consistent with increasing neuronal differentiation over time. In a time-course dose-response experiment, 100nM dexamethasone was identified as the optimal GC-stimulation paradigm as it robustly elicited an effect on GR-regulated gene expression (e.g. FKBP5, SGK1). To explore cell type-specific GC response patterns, we used scRNAseq following dexamethasone treatment both acutely, chronically, and using a two-hit model of stress. We found dynamic expression of genes associated with GC-responsive pathways with differential response in specialised brain cells. Finally, we explored long-lasting epigenetic changes in DNA methylation and chromatin conformation to understand the molecular pathways whereby a vulnerability to stress may be imprinted. We found that cerebral organoids follow developmental trajectories of the human brain and show responsiveness to glucocorticoids consistent with in vivo data, including cell-type specific responses. The identified pathways may shed light on risk genes moderating the effects of prenatal stress in humans, and we are currently investigating these potentially stress-mediating mechanisms in perinatal cohorts of mothers and their babies.

Prevention of schizophrenia deficits via non-invasive adolescent frontal cortex stimulation in rats

Rebecca Winter, Ravit Hadar[1,2], Henriette Edemann-Callesen[1,2,3], Franziska Wieske[2], Bettina Habelt[1,2], Niranjan Khadka[4], Elizabeth Barroeta Hlusicka[1,2], Janine Reis[5], Klaus Funke[6], Nadine Bernhardt[1], Michael Nitsche[7,8], Christine Winter[1,2]

[1]Department of Psychiatry and Psychotherapy, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Germany
[2]Department of Psychiatry and Psychotherapy, Charité University Medicine Berlin, Campus Mitte, Germany
[3]International Graduate Program Medical Neurosciences, Charité Universitätsmedizin Berlin, Germany
[4]Department of Biomedical Engineering, The City College of New York, CUNY, New York, New York, 10031, USA
[5]Department of Neurology, University Medical Center, Albert Ludwigs University Freiburg, Germany
[6]Department of Neurophysiology, Medical School of the Ruhr-Universität Bochum, Bochum, Germany
[7]Department Psychology and Neurosciences, Leibniz Research Centre for Working Environment and Human Factors at TU Dortmund, Germany
[8]Department Neurology, University Hospital Bergmannsheil, Ruhr-Universität Bochum, Germany

Schizophrenia presents with a wide range of severe alterations in behaviour, emotion and cognition, such as delusions and disorganized speech. The onset of symptoms is typically in early adulthood. The maternal immune stimulation (MIS) animal model has been proven valid to study behavioural deficits as seen in schizophrenic patients. As schizophrenia is considered a neurodevelopmental disorder in which pathological processes build up over the developmental course and result in symptom manifestation after adolescence, a possibly critical point for treatment intervention to prevent the occurrence of symptoms is the vulnerable period of adolescence.

The aim of the present study was to examine cognitive functions to detect the onset of behavioural alterations usually seen in schizophrenia in the MIS animal model after transcranial direct current stimulation during adolescence in a longitudinal design. Mother rats were injected with 0.4mg/kg poly I:C (Sigma, Germany) dissolved in saline or saline only respectively into the tail vein on gestation day 15 to induce the phenotype in the offspring. After weaning, all rats were randomly divided in three stimulation groups (2x3 design): anodal tDCS (with the anode placed over the mPFC), cathodal tDCS (with the cathode placed over the mPFC) and sham stimulation (e.g. no current was applied) and stimulation conducted on postnatal days (PND) 35 to 47 for 20 minutes twice daily. The animals underwent behavioural testing at the age of 90 days over a course of twelve weeks including the Prepulse Inhibition test (PPI), the Sucrose Consumption Test (SCT), the Social Interaction test (SI), the Reversal Learning paradigm (RL) and the Amphetamine-induced Activity paradigm (AIA).

Anodal tDCS to the mPFC during adolescence successfully prevented the manifestation of sensorimotor gating deficits as seen in the PPI and abnormal rapid reversal learning as well as enhanced mesolimbic dopaminergic neurotransmission in the AIA paradigm, which are thought to reflect positive symptoms in schizophrenia. As for structural alternations, both anodal and cathodal tDCS prevented the enlargement of lateral ventricles. Moreover, sucrose consumption and social interaction were unaffected by the treatment. Anhedonia and deficits in social behaviour are among the negative symptoms of schizophrenia.

We conclude that anodal tDCS over the mPFC is a means to prevent positive symptoms of schizophrenia in the MIS animal model thus posing a alternative non-invasive treatment

strategy.Alleviation of negative symptoms with tDSC however may require either alternative target localization, application protocols or treatment techniques. In addition due to observed tDCS induced deficits in control animals, a biomarker would be needed to carefully select the patient group suitable to this intervention for the translation into clinical practice.

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Maternal inflammation during pregnancy and fetal brain development

Claudia Buss[1,2], Alice Graham[3], Jerod Rasmussen[2], Sonja Entringer[1,2], John Gilmore[4], Martin Styner[4], Pathik Wadhwa[2], Damien Fair[3]

[1]Charité Universitätsmedizin Berlin, Medical Psychology, Luisenstrasse 57, 10823 Berlin, Germany

[2]Development, Health and Disease Research Program, University of California, Irvine, 837 Health Sciences Drive, Irvine, California, 92697, United States

[3]Department of Behavioral Neuroscience, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd., Portland, OR, 97239, United States

[4]Department of Psychiatry, University of North Carolina, 333 South Columbia Street, Chapel Hill, North Carolina, 27514, United States

Maternal inflammation during pregnancy increases risk for offspring psychiatric disorders and other adverse long-term health outcomes. The influence of inflammation on the developing fetal brain is hypothesized as one potential mechanism. Empirical evidence will be provided in support of an association between maternal inflammation during pregnancy and the developing human fetal brain. Mother-offspring dyads were part of a prospective longitudinal study that started in early pregnancy. A biological indicator of maternal inflammation (interleukin-6 [IL-6]), which has been shown to influence fetal brain development in animal models, was quantified serially in early, mid and late pregnancy. Structural and functional MRI as well as and diffusion tensor imaging was acquired in neonates shortly after birth.

Higher average maternal IL-6 concentration during pregnancy was prospectively associated with neonatal amygdala volume as well as amygdala structural and functional connectivity. The observed IL-6-associated neural alterations at birth predicted cognitive and affective function during the first 2 years of life.

These findings provide new evidence in humans linking maternal inflammation during pregnancy with newborn brain and emerging behavioral phenotypes relevant for psychiatric disorders. A better understanding of intrauterine conditions that influence offspring disease susceptibility is warranted to inform targeted early intervention and prevention efforts.

Symposium

S19: From clinical symptoms to motoneuron pathobiology: most recent insights into amyotrophic lateral sclerosis (ALS)

S19-1 Clinical Translation of the Neuroanatomy of ALS
Albert Christian Johannes Ludolph

S19-2 From ALS genes to pathogenic principles and targets for individualized therapies
Jochen H. Weishaupt

S19-3 Molecular mechanisms of ALS - from nuclear transport defects to protein aggregation
Dorothee Dormann

S19-4 TDP-43 aggregation - implications for ALS
Karin Danzer

S19-5 Neuroinflammation in a mouse model of amyotrophic lateral sclerosis with FUS gene mutation and effects of standard and new therapies.
Diana Ivanovna Babaevskaia, Johannes de Munter, Alexander Trofimov, João Costa-Nunes, Dmitry Pavlov, Ekaterina Veniaminova, Margarita Oplatchikova, Anna Gorlova, Klaus-Peter Lesch, Erik Wolters, Daniel Anthony, Tatyana Strekalova

S19-6 Replicative reprogramming in the context of physiological CNS aging and age-related neurodegeneration
Diane Penndorf

Clinical Translation of the Neuroanatomy of ALS

Albert Christian Johannes Ludolph[1]

[1]University of Ulm, Neurology, Oberer Eselsberg 45, 89081 Ulm, Germany

Clinical Translation of the Neuroanatomy of ALS

In 2013, Braak and Brettschneider introduced a new concept of staging for the most frequent motor neuron disease, amyotrophic lateral sclerosis. Like in Alzheimer's and Parkinson's disease, Braak showed that the pathogenesis of ALS followed an anatomical stereotyped pattern of propagation. Specifically, a preclinical period was not found, the disease spread along the association fibers and initiated in the motor cortex. If cortical neuronal populations were affected which were connected by monosynaptic fibers reaching subcortical neuronal populations, the latter showed also TDP-43 neuropathology.

These results show that ALS is rather a multisystem degeneration than a pure motor neuron disease. Like in Parkinson's and Alzheimer's, this description forced clinicians to re-think their concept of ALS. In the meantime, it could be demonstrated, that the sequential affection of corticoefferent fibers can be reproduced by MRI techniques and that neuropsychological findings in ALS can be explained by the anatomical framework that Braak described. Disorders of ocular movements can be explained and most importantly, staging could be reproduced in vivo in a sequential pattern. This new anatomical and clinical understanding of ALS paves the way for disease modifying therapies.

From ALS genes to pathogenic principles and targets for individualized therapies

Jochen H. Weishaupt[1]

[1]Universität ulm, Neurologie, Albert-Einstein-allee 11, 89081 Ulm, Germany

Moderne Methoden der Hochdurchsatzsequenzierung hat in den vergangenen wenigen Jahren die Entdeckung neuer ALS-Gene enorm beschleunigt. Mittlerweile sind hochpenetrante ALS-verursachende Mutationen in ca. 30 verschiedenen Genen bekannt. Darüber hinaus finden sich zunehmend auch häufigere genetische Varianten mit geringerer Effektstärke die auch in Kohorten von sporadischen ALS-Patienten angereichert sind. Diese tragen wahrscheinlich zu einer polygenen Vererbung bei, und weisen auf eine signifikante Rolle von genetischen Veränderungen auch für die Mehrzahl der ALS-Patienten mit negativer Familienanamnese hin. Während die jüngst identifizierten Gene die Grundlage für die Entwicklung Gen- oder Signalweg-spezifischer molekularer Therapien z.B. mit Antisense-Oligonukleotiden sind, stellen diese auch die Ausgangslage für das weitergehende Verständnis der molekularen Pathophysiologie der Erkrankung dar. Ein genaueres Bild davon, welche zellulären Prozesse von den jeweiligen ALS-assoziierten Mutationen betroffen sind entsteht für die jüngst entdeckten ALS-Gene jedoch gerade erst. Dieser Vortrag wird einige der neuesten Entwicklungen bei der Translation von humangenetischen Entdeckungen in neue (Tier-)Modelle sowie daraus resultierende mechanistische und therapeutische Erkenntnisse darstellen.

Molecular mechanisms of ALS - from nuclear transport defects to protein aggregation

Dorothee Dormann[1]

[1]Ludwig-Maximilians-Universität München, BioMedical Center (BMC), Cell Biology, Grosshaderner Str. 9, 82152 Planegg-Martinsried, Germany

Over the last years, mechanistic studies in cellular and in vitro models have provided key insights into the molecular mechanisms underlying ALS. Pathological protein aggregates containing the RNA-binding proteins TDP-43 and FUS are a central hallmark of almost all ALS cases as well as some patients suffering from frontotemporal dementia (FTD). TDP-43 and FUS are usually located in the cell nucleus, whereas in neurons and glial cells of ALS/FTD patients, they are partially lost from the nucleus and accumulate in large cytoplasmic inclusions. Which molecular defects cause TDP-43 or FUS mislocalization and aggregation in ALS/FTD patients and how we can possibly prevent or revert them are central questions that we try to address in my lab.

For FUS, we have successfully used cellular and in vitro models combined with neuropathological analysis of human post-mortem brains to identify key pathomechanisms that cause FUS mislocalization and aggregation in ALS patients: In ALS-FUS patients, genetic mutations in the nuclear localization signal (NLS) of FUS cause impaired binding to the nuclear import receptor Transportin (TNPO1). This defect impairs nuclear import of FUS and promotes pathological phase separation and aggregation of FUS, as TNPO1 normally suppresses phase separation/aggregation of FUS and other RNA-binding proteins and ensures their proper nuclear import. More recently, we obtained evidence that defects in nuclear import receptors and/or abnormal post-translational modifications may also play a role in ALS associated with a hexanucleotide repeat expansion in C9orf72 as well as in ALS with TDP-43 aggregates and hence are key molecular defects that should be targeted in new therapeutic approaches.

TDP-43 aggregation - implications for ALS

Karin Danzer[1]

[1]Ulm University, Neurology, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Aggregation of TDP43 and related cytotoxicity play a central role in the development of sporadic ALS and a large subset of frontotemporal lobar degeneration. Recent evidence implicates oligomeric and pre-fibrillar forms of TDP43 to be released from neuronal cells with a subsequent nucleating process of TDP43 in recipient cells. This might be the molecular correlate of the systematic symptom spreading observed in ALS progression. Moreover, a highly predictable spatial progression of TDP43 pathology in ALS brains has been described in human ALS end stage post mortem tissue, which might be the result of cell-to-cell spreading of TDP43 oligomers. The mechanism of TDP43 oligomerization and aggregation is poorly understood. Under physiological conditions TDP43 is located predominantly in the nucleus. When mutated or under stress condition, TDP43 translocates to the cytoplasm, where it participates in the formation of stress granules (SGs) and eventually becomes hyperphosphorylated and part of insoluble, ubiquitin positive aggregates typical for a broad spectrum of ALS/FTD patients. In this symposium, we report on the latest results in modulating cell-to-cell transfer and aggregation of TDP43 using pharmacological intervention. We also discuss relevant genes and keyplayers in TDP43 propagation and try to shed light on the role of dysfunctional stress granules and TDP43 secretion. We will point to the potential crosstalk between neurons releasing TDP43 and glial cells via microvesicles and discuss strategies to study TDP43 propagation using in vivo models.

Neuroinflammation in a mouse model of amyotrophic lateral sclerosis with FUS gene mutation and effects of standard and new therapies.

Diana Ivanovna Babaevskaia[1], Johannes de Munter[2,3], Alexander Trofimov[1,2], João Costa-Nunes[1], Dmitry Pavlov[1,2], Ekaterina Veniaminova[1,2], Margarita Oplatchikova[1], Anna Gorlova[1,2], Klaus-Peter Lesch[1,2,4], Erik Wolters[3], Daniel Anthony[5], Tatyana Strekalova[1,2,4]

[1]I.M. Sechenov First Moscow State Medical University (Sechenov University), Laboratory of Psychiatric Neurobiology, Institute of Molecular Medicine

[2]Department of Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands

[3]Neuroplast, Maastricht Netherlands

[4]Division of Molecular Psychiatry, Laboratory of Translational Neuroscience, Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany

[5]Department of Pharmacology, Oxford University, Oxford, UK

A novel transgenic mouse line, which is based on the mutation of Fused in sarcoma protein (FUS), DNA/RNA-binding factor, was recently proposed and validated as a paradigm of amyotrophic lateral sclerosis (ALS). Here, we investigated behavioral and physiologic parameters, alone with inflammatory markers in the CNS and blood of FUS-transgenic (FUS-tg) female mice and their wildtype littermates. Subgroups of mice corresponding to a pre-symptomatic age of FUS-tg mutants, where treated with chronic dosing with riluzole (8 mg/kg/day, p.o.), a standard therapy of ALS, or selective COX2 blocker celecoxib (30 mg/kg/day p.o.) during six weeks, or a single i.c.v. administration of human stem cells (Neuro-Cells, 500 000 CD34 in 10 mkl). Separate groups were acutely challenged with low dose 0.1mg/kg of lipopolysaccharide (LPS). We found multiple emotional and cognitive aberrations in FUS-tg mice at their pre-symptomatic stage, plasma interleukin 1-beta and interleukin-6 were elevated. There was increased behavioral response to the LPS challenge, as well as greater increases of pro-inflammatory cytokines, e.g. interleukin 1-beta, as compared with wild type controls. Pro-inflammatory changes were more pronounced in the brain than in the spinal cord. In naïve mutant mice studied at their symptomatic phases of the ALS pathology, profound increases of brain and spinal cord levels of pro-inflammatory markers including Iba-1, were accompanied by increases of apoptotic markers such as GSK3 alpha and beta. FUS-tg mice treated with celecoxib revealed a reduction of peripheral levels of cytokines, while pro-inflammatory changes in the spinal cord were unaltered. Neuro-Cells-treated FUS-tg group displayed normalized CNS and plasma levels of pro-inflammatory markers. Among treated FUS-tg groups, Neuro-Cells-treated animals, but not riluzole- and celecoxib-treated mice showed marketable improvement of motor, emotional, cognitive behavior and basic physiological functions. Thus, new therapies such as stem cells (Neuro-cells) diminish neuro-inflammation during the ALS syndrome and thus, can be beneficial.

Replicative reprogramming in the context of physiological CNS aging and age-related neurodegeneration

Diane Penndorf

[1]Jena University Hospital, Hans Berger Department of Neurology, Am Klinikum 1, 07747 Jena, Germany
[2]Leibniz Institute on Aging – Fritz Lipmann Institute, 07745 Jena, Germany

Cellular senescence plays a crucial role in the aging process, particularly in proliferating cells, which respond with cell cycle arrest. However, aging mechanisms in post-mitotic neurons, where the paradigm of replicative senescence fails to a wide extent, are not well understood. In mature neurons, active cell cycle suppression appears mandatory to avoid atypical cell cycle re-itineration, a deregulation entailing neuronal dysfunction and apoptosis. The process of 'replicative reprogramming' and cell cycle-related neuronal death (CRND) is currently discussed broadly in neurodegeneration and substantiated for Alzheimer's disease. However, the importance for other age-related CNS pathologies and the physiological aging process of the central nervous system (CNS) itself is unknown.
In this study, parameters of aberrant cell cycle re-induction were assessed in the context of age-related amyotrophic lateral sclerosis (ALS), a lethal neurodegenerative disorder characterized by a progressive loss of motor neurons, and in physiological as compared to pathological CNS aging.
As model systems, the ventral spinal cord of ALS-mimicking hSOD1^{G93A} mice, and the neocortex of highly aged (27-30 months) C57BL/6 and progeroid Klotho mutants were investigated and compared with physiological control conditions. Our qPCR-based analyses illustrate broad alterations in the expression patterns of phase-specific cell cycle regulators both in ALS as well as in healthy aged and progeroid animals. In support, on the protein level we detected a strong increase of the G_1 phase cell cycle regulator CyclinD1 under disease-like conditions. Furthermore, an alteration in the subcellular location of different cell cycle regulators, e.g., the CNS-specific cell cycle inhibitor Cdk5 was observed. A nuclear Cdk5 loss, which is inductive for cell cycle re-initiation, was detected in hSOD1^{G93A} motor neurons already early in the disease process. Diminished nuclear Cdk5 was accompanied by reduction of its co-activator p35. Apart from immunofluorescence and western blot techniques, such down-regulation of Cdk5 was further confirmed using systematic mass spectrometry. In neurons, p35 is mandatory for the cell cycle-suppressive nuclear maintenance of Cdk5. This Cdk5/p35 interaction is disturbed by the calpain-dependent cleavage of p35. Using the quantitative Simple Western™ technique, we found an increased calpain activity along with a nuclear p35 degradation and Cdk5 depletion. As a putative so far undescribed upstream regulator for the induction of such a replicative reprogramming, we investigated the growth arrest-specific Gas2, which is involved in mediating replicative arrest, calpain inhibition and caspase 3-dependent apoptosis. Interestingly, we found a down-regulation of Gas2 under ALS-like and CNS aging conditions. According to these results, we hypothesize that the deregulation of Gas2-driven calpain activity triggers Cdk5/p35-dependent atypical cell cycle re-induction in the aging-related ALS pathology. Further analysis will prove if this axis is similarly affected under physiological aging conditions.

In summary, we introduce a novel molecular pathway as putative explanation for the phenomenon of replicative reprogramming and subsequent CRND in aging and ALS-related neurodegeneration.

Symposium

S20: Subcortico-cortical loops and their role in sensory processing and perception

Visual processing of feedforward and feedback signals in mouse dLGN

Laura Busse[1,2]

[1]LMU Munich, Department Biology II, Planegg-Martinsried, Germany

[2]Bernstein Center for Computational Neuroscience Munich, Planegg-Martinsried, Germany

Feed-forward sensory processing is a fundamental concept of how the brain mediates visual perception. Using a largely feed-forward architecture, artificial neural networks can now carry out robust and dynamic operations as to rival human perception.

So why then, in the brain, starting from nearly the earliest stages of sensory processing, is feedback such a prominent and ubiquitous motif? As a model for feedback effects on sensory processing, the cortico-thalamic (CT) circuit has, for over half a century, sparked much interest. Despite these efforts, however, how CT feedback influences the representation of visual information remains poorly understood.

Here, we revisited the fundamental question of cortical feedback's role in thalamic visual processing. We performed a series of experiments using optogenetic tools for circuit manipulations in awake mice.

We found that CT feedback during spontaneous activity enhanced firing rates and reduced bursting, and, during processing of natural movie clips, reduced sparseness of dLGN responses. Hence, CT feedback seems crucial for promoting tonic firing mode in dLGN, potentially allowing a more linear transmission of incoming visual information.

Furthermore, our results indicate that CT feedback shapes spatial processing. Measuring tuning for stimulus size, we found that dLGN RFs in conditions with intact CT feedback were smaller and showed stronger surround suppression. Finally, we demonstrate that these effects on spatial integration might, at least partially, be mediated by neurons in the visual part of TRN, via which CT feedback can exert suppressive effects.

Together, our findings suggest that a function of CT feedback is to enhance responses to local visual signals and shape contextual modulations.

Understanding the auditory hierarchy: modifications to auditory processing on the way to the cortex

Julio Hechavarria[1]

[1]Goethe University, Frankfurt am Main/ Institute for Cell Biology and Neuroscience, Neurobiology of Auditory Computations Group, Department for Neurobiology and Biosensors , Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

Understanding information processing along sensory hierarchies is key for unraveling the operational principles of the brain. In this talk, I will present data on the modifications that occur to auditory information processing along the colliculo-cortical axis. Located in the midbrain, the inferior colliculus is considered the “nexus” of the auditory system because of the large amount of synaptic inputs it receives, and for its role in the implementation of computational tuning types that are not created in the cochlea. Data collected in my group shows that the auditory cortex does not only inherit the tuning types that are implemented in the inferior colliculus, but that large modifications to basic response properties occur along the colliculo-cortical axis. These modifications include a decrease in the temporal precision of responses on the way to the cortex, an increase in response pattern variability across trials, and a decrease in the ability of “tracking” acoustic information over time. At least in bats, such modifications hinder the representation of natural vocalization sequences at the cortical level but allow cortical units to extract key acoustic cues embedded in acoustic sequences and to integrate information when listening in complex “cocktail-party” like scenarios.

Recurrent corticothalamic feedback in auditory cortex mediating salient auditory perception

Max F.K. Happel[1]

[1]Leibniz-Institute for Neurobiology, AG CortXplorer, Brenneckestr. 6, 39118 Magdeburg, Germany

In an ever-changing world, we need to detect, predict and behaviourally respond to important stimuli on short and longer time scales. Particularly in the vertebrate brain, neuronal circuit adaptations mainly generated by associative reinforcement learning are the fundament of remote memory formation and guided behavioural choices. For sensory learning, organisms have to extract and amplify a small number of sensory features with behavioural relevance to a particular situation.
In the auditory cortex (ACx), we found evidence that recurrent corticothalamic feedback promotes the salient representation of behaviourally relevant sensory information via prolonged local cortical input processing. We further demonstrated that this recurrent feedback is mediated by dopaminergic transmission in ACx and further leads to a subsequent recruitment of long-range cortical circuits integrating sensory associated bottom-up and relevant top-down information. Using direct microstimulation and laser-induced apoptosis, we could demonstrate that activating or lesioning the auditory corticothalamic feedback in Mongolian gerbils and ferrets enhanced or disturbed salient auditory perception, respectively.
We propose that the dopamine-mediated recurrent corticothalamic feedback thereby is a key circuit in order to integrate selected sensory information within a behavioural context.

Auditory midbrain coding of temporally sparse statistics

Livia de Hoz[1]

[1]Charité - Universitätsmedizin Berlin, Germany

Statistical learning of patterns in the sensory world is essential for selective attention and predictive coding. Statistical learning of relatively fast (seconds to tens of seconds) ongoing changes in a constant stimulus stream are known to occur through sensory adaptation of neuronal response-gain. Little is known, however, about statistical learning of temporally sparse patterns (across minutes/hours). Neurons in the auditory midbrain can encode temporally sparse context-sound associations and their predictability. Encoding is partially independent from corticofugal input and parallels changes in behavioural outcomes that suggest a role in decision making. The auditory midbrain plays, therefore, a significant role in the detection of statistical regularities that arise from temporally sparse interactions with the spatial context.

Cortical oscillations aid the representation of natural vocalization streams at multiple timescales

Francisco Garcia-Rosales, M. Jerome Beetz[1,2], Yuranny Cabral-Calderín[3,4], Manfred Kössl[1], Julio C. Hechavarría[1]

[1] Institut für Zellbiologie und Neurowissenschaften, Neurobiologie und Biosensorik, Max-von-Laue Str. 13, 60438 Frankfurt am Main, Germany

[2] Department of Zoology II, University of Würzburg, Am Hubland, Würzburg 97074, Germany

[3] MEG Labor, Brain Imaging Center, Goethe-Universität, 60528 Frankfurt/M., Germany

[4] German Resilience Center, University Medical Center Mainz, Germany

A prominent feature of the auditory cortex (AC) is its ability to represent, via neurophysiological oscillations, embedded temporal structures existent in complex yet well temporally-patterned sounds. In humans, for example, such multiscale temporal representation is deemed crucial for speech perception, considering that cortical oscillatory activity entrains simultaneously to the syllabic (~4 Hz) and the phonemic (> 30 Hz) rate of speech streams, thus allowing for an efficient parsing of the acoustic input. Although the relevance of multiscale temporal processing via oscillatory activity is well substantiated by empirical evidence, little is known about the representation of hierarchic temporal structures at a neuronal level. Here, we address this question presenting data from extracellular recordings of neuronal activity in the AC of the bat *Carollia perspicillata*, in response to conspecific distress vocalizations. Bat distress calls, which can elicit a variety of strong behavioural responses on the listener, have the interesting property of comprising embedded modulations at two main distinct timescales: a fast one (> 50 Hz), consistent with the syllabic rate of the vocalizations; and a slow one (< 15 Hz), consistent with the bout (i.e. group of syllables) rate of the vocalizations. Different neuronal subpopulations in *C. perspicillata*'s AC tracked either the fast (syllables) or the slow (bouts) temporal structure of the calls, and constitute a direct neuronal correlate of multiscale coding at a cortical level. Syllable- or bout-tracking (ST or BT) neurons synchronized differently to ongoing oscillations, in a way such that coherence between spikes and local-field potentials (LFPs) in BT units occurred mostly in the theta range, whereas spike-LFP coherence in ST units occurred typically at high frequencies which match the syllabic rate. Based on the above we argue that ST neurons directly represent thalamocortial inputs, whereas BT responses could be shaped by more complex mechanisms involving low frequency oscillations (mostly in the theta range). Theta-band spike-LFP coherence is a marker of phase coding (in response to periodic stimuli with rates < 20 Hz) in the AC, and since low frequency LFPs influence neuronal excitability, BT responses may benefit from low frequency spike-LFP phase synchrony during acoustic processing. Furthermore, because low frequency oscillations may be of modulatory nature, we argue that functional coupling between the frontal-auditory field (FAF, a region in the frontal cortex of *C. perspicillata* with complex response properties which receives inputs from the AC and "lower" auditory structures) and the AC, via low frequency LFPs, could be a mechanism that mediates (or benefits from) the processing of vocalizations at different time-scales. Altogether, our data show that cortical ST and BT responses provide independent information, and allow for the precise and non-redundant representation of natural vocalizations at a cortical level. We propose that their interaction could enhance sensory perception of relevant stimuli in the brain.

Dual-color imaging for isolating olfactory bulb output streams in mice

Kim Chi Le

[1]RWTH Aachen University, Dept. Chemosensation, AG Neuromodulation, Worringer Weg 3, 52074 Aachen, Germany

Mitral and tufted cells (MTCs) are the main output neurons of the olfactory bulb (OB) and project to different areas within the olfactory cortex (OC). Tufted cells predominantly send afferents to the anterior OC whereas mitral cells innervate the whole OC. It is known, that different brain areas can process segregated aspects of the sensory space as for example the “what & where” pathway in vision. Whether different regions in the OC receive different functional input from MTCs however, remains unknown.
Here, we established a dual-color imaging approach to visualize activity from anatomically defined MTC subpopulations. We performed AAV-mediated retrograde tracing from two regions of the OC (anterior olfactory nucleus (AON) & anterior piriform cortex (aPC)) to isolate MTC output streams based on their axonal targets. In order to separate these output streams we used green or red genetically encoded fluorescent calcium indicators; GCaMP6 and jRCaMP1a. Retrograde tracing from the AON or aPC yielded a strong expression in OB output neurons: labeled somata were mainly localized in the mitral cell and external plexiform layer.
Using widefield imaging we visualized AON or aPC traced MTC activity at the population level and compared their responses with PCD-GCaMP animals expressing GCaMP6 in olfactory sensory and OB output neurons. Odor presentation in PCD-GCaMP mice exhibited defined OB activity maps, consisting primarily of discrete glomerular foci, likely reflecting pre- and postsynaptic activity. In contrast, OB maps from AON or aPC traced MTCs showed a pronounced diffuse component. This was likely mediated by long range secondary MTC dendrites thereby confirming the specificity of our tracing approach.
Next, we compared target-defined OB output streams at a single-cell level using two-photon microscopy. So far, studies classifying mitral and tufted cells mainly based on somata position in the OB showed that TCs convey fast signals with short onset latency whereas MCs transmit late-onset signals. To our knowledge, here we present the first physiological data from MTC output streams isolated based on their axonal targets in the OC. Individual MTCs projecting to the AON showed a higher odor selectivity compared to MTCs innervating the aPC. Moreover, MTCs targeting the aPC displayed a broader range of onset latencies also containing slower responses. These data indicate that distinct regions in the OC may receive different odor information allowing for a parallel processing of olfactory information as already documented for other modalities.
Future dual-color imaging experiments from simultaneous traced MTC populations will directly compare the physiological properties of defined MTC output streams.

Symposium

S21: Behavioral decisions based on multimodal information

S21-1 As the craw flies and the beetle rolls:
Straight-line orientation from behaviour to neurons
Marie Dacke

S21-2 Desert ant navigation by olfactory and visual cues
Markus Knaden

S21-3 Compass Systems During Ant Learning Walks: The Role of Celestial Cues for Initial Compass Calibration in *Cataglyphis* Ants
Robin Grob, Pauline N. Fleischmann, Kornelia Grübel, Rüdiger Wehner, Wolfgang Rössler

S21-4 Multimodal odometry in navigating *Cataglyphis* desert ants
Matthias Wittlinger

S21-5 Timing, multimodal integration, and coordination in the neural control of agile flight in low light
Simon Sponberg

S21-6 Estimating body pitch from distributed proprioception: On the role of afferent number and distribution
Arne Gollin, Volker Dürr

As the craw flies and the beetle rolls: Straight-line orientation from behaviour to neurons

Marie Dacke[1]

[1]Lund University, Department of Biology, Sölvegatan 35, 223 62 Lund, Sweden

The seemingly simple act of walking in a straight line involves a complex interplay of various sensory modalities, the motor system, and cognition. This is obvious to anyone who have ever found themselves lost in the desert at night, or in a forest when the sun is high in the sky. A dung beetle released in the same unchartered territory does not move in circles, but holds a chosen bearing until it encounters a suitable spot to bury its ball of dung. The key to the beetle's success lies in their ability to detect and orient via a large repertoire of celestial compass cues, from the bright sun to the weak intensity differences of light provided by the Milky Way.

A beetle's drive to adhere to its set course is so strong that it sticks to it regardless of the costs; over stones, through bushes and grass or in an experimental arena. However, if a beetle is forced to make a new ball, the bearing information is reset in its brain and a new course is set. This unique and robust orientation behaviour, in combination with an accessible brain, make the dung beetle an ideal model system for understanding the fundamental visual and neural processes underlying straight-line orientation.

The presentation provides an overview of recent behavioural, anatomical and physiological results concerning how an insect brain is designed to facilitate straight-line orientation.

Desert ant navigation by olfactory and visual cues

Markus Knaden[1]

[1]Max Planck Institute for Chemical Ecology, Evolutionary Neuroethology, Hans Knoell Strasse 8, 07745 Jena, Germany

The desert ant *Cataglyphis fortis* inhabits the open salt pans of Tunisia, where individual foragers search for dead arthropods and return after far reaching foraging runs (>1km walking distance) to the inconspicuous nest entrance. During these foraging runs the ants are exposed to predators and heat. It is well established that the ants use path integration to always be informed about their relative position to the nest and follow visual landmarks they have learned when leaving the nest entrance. We could, however, show that the ants also take into account olfactory cues. They do not only localise their food based on olfaction, but also learn and remember olfactory landmarks along the route and finally pinpoint the nest entrance by following a nest plume. By taking into account all information available from different modalities, foraging *Cataglyphis* ants home quickly, and by that escape the risks they face while running on the salt pan.

Compass Systems During Ant Learning Walks: The Role of Celestial Cues for Initial Compass Calibration in *Cataglyphis* Ants

Robin Grob, Pauline N. Fleischmann[1], Kornelia Grübel[1], Rüdiger Wehner[2], Wolfgang Rössler[1]

[1]Biozentrum, University of Würzburg, Zoology II, Am Hubland, 97074 Wuerzburg, Germany
[2]Brain Research Institute, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

Cataglyphis desert ants are marvellous navigators. During long foraging excursions, the ants find their ways around the even most challenging environments. To find back home every time they leave their nest to search for food, they use an impressive navigational toolkit that allows home-vector integration using directional information from a celestial compass and distance information from a step counter. In addition, the ants use visual-landmark guidance whenever available. However, at the beginning of their foraging career, *Cataglyphis* is faced with the crucial challenge to learn the available landmark panorama and to calibrate its visual compass systems. To do so, naïve *Cataglyphis noda* ants perform learning walks – small loops around the nest entrance – during the transition phase between interior worker and outdoor forager. These loops around the nest entrance are repeatedly interrupted by small walked circles (voltes) and tight turns around the ants' body axes (pirouettes). During pirouettes, the ants stop to gaze back to the nest entrance with striking precision. By taking snapshots in this direction, ants learn the landmark panorama of the nest surroundings, which they can later use for their navigational tasks, especially homing. However, the ants need a reliable compass system to align their gaze direction. Experienced *Cataglyphis* foragers heavily rely on their celestial compass for directional information. To investigate whether celestial cues affect the pirouetting behaviour of naïve ants, we restricted skylight information received by the ants during learning walks in their natural habitat. To investigate neuronal consequences of learning walks, we quantified neuroanatomical changes in high-order sensory integration centres involved in learning and memory (mushroom bodies) as well as orientation (central complex) following manipulations of celestial information during learning walks. Surprisingly, even the exclusion of the skylight polarisation pattern in the UV part of the spectrum and blocking of the position of the sun did not alter the accuracy of the ants to look back to their nest entrance. At the neuroanatomical level, however, the presence of the natural skylight polarization pattern was essential for triggering synaptic plasticity in visual compartments of the mushroom bodies and to induce volume changes in the central complex. These changes exclusively occurred under a naturally moving skylight polarization pattern and were absent when the ants had performed learning walks under a stable polarization pattern. The results suggest that naïve ants perform learning walks not only to take nest-directed panoramic snapshots, but also to calibrate their visual celestial compass systems. Funded by DFG SFB 1047 (b6) and DFG RO1177/7-1, both to WR.

Multimodal odometry in navigating *Cataglyphis* desert ants

Matthias Wittlinger[1]

[1]University of Freiburg, Dept. for Animal Physiology, Neurobiology and Behavior, Hauptstrasse 1, 79104 Freiburg, Germany

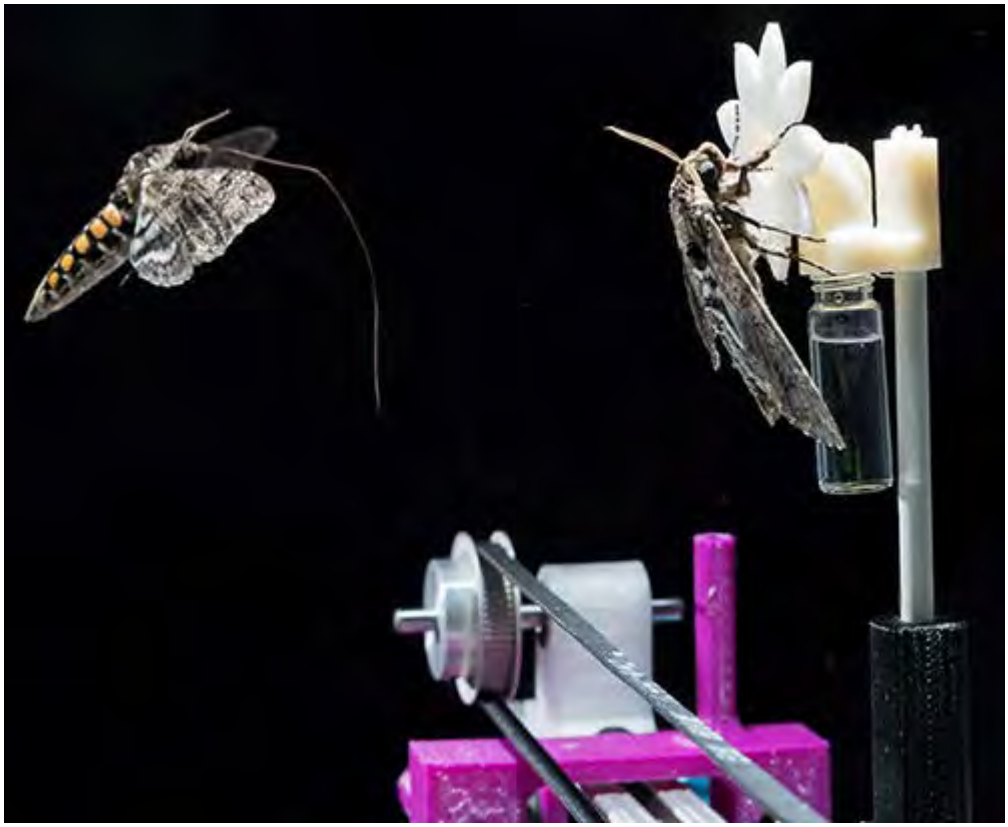
For a navigating animal the acquisition and integration of information of its travel direction and distance is a crucial prerequisite. *Cataglyphis* desert ants, the long-legged highspeed runners of North African hot and arid areas, master this with seemingly great ease while they roam through their harsh environment. Recent research has uncovered two different ways in which *Cataglyphis* ants use cues derived from their legs and from their eyes for odometry purposes. In other words ants possess a multimodal approach of estimating travel distance: a pedometer or stride integrator that receives distance information from the walking apparatus, and further, distance is gauged by integrating optic flow, the apparent motion of the visual world that is experienced *en route*.

Timing, multimodal integration, and coordination in the neural control of agile flight in low light

Simon Sponberg[1]

[1]Georgia Institute of Technology, Schools of Physics & Biological Sciences, 837 State St. NW, Atlanta, GA, 30332 USA

Organisms negotiate many complex environments, demonstrating remarkable stability, maneuverability, and multifunctionality. How do they integrate multiple sensory cues to enable this robust, agile movement? Here I will use techniques from control theory and information theory to disentangle multimodal control and its implementation and coordination in the motor control of flight. I will focus on flower foraging in hawkmoths, a behavior where moths must hover in mid air, cast back and forth up to 14 times a second to track flower movement, and do so in exceptionally dim light. Such behavior is a challenge from both a sensory and motor perspective. Using robotic flowers that the animal interacts with, we first showed that when moths visually track flowers in extremely low light levels, they slow their nervous systems to increase light sensitivity, but only to the point where they can keep up with the frequencies that natural flower blow in the wind. However moths also rely on mechanosensory cues, both fast and slow. We discovered that tactile sensors in their proboscis contribute to flower handling. Using a sensory conflict paradigm we reveal that the two modalities (vision and mechanosense) are each sufficient for behavior, but the animal linearly combines them as independent sensory streams. We can predict flight dynamics to ~95% even across individuals. Behavior can manifest “simple” dynamics, even if the implementation is potentially complex and the underlying neural and mechanical interactions are non-linear. These same dynamics generalize across different species with visual ecologies tuned to different times of day. Ecological context shapes neuromechanical control. Within the individual we find that the animal can detect and compensate for changes in mass due to feeding – moths can consume up to 50% of their body mass. Finally we examine the neurophysiological basis for these control strategies. Implementation of this motor behavior is accomplished with surprisingly constrained neural bandwidth. We record a comprehensive set of nearly every action potential that directly controls wing movement and find that the animal primarily uses timing codes rather than rate codes and that it controls flight with only about of 8 bits of mutual information per wingstroke shared between the motor program and the resulting torque. Informing our neurophysiological hypotheses with dynamic descriptions of behavior is starting to converge on neuromechanical principles of 1) bandwidth separation, 2) extensive and precise timing codes in motor control, and 3) robustness through redundancy.



Estimating body pitch from distributed proprioception: On the role of afferent number and distribution

Arne Gollin, Volker Dürr[1,2]

[1]Bielefeld University, Department of Biological Cybernetics, 33615 Bielefeld, Germany
[2]Cognitive Interaction Technology Center of Excellence, Bielefeld University, 33615 Bielefeld, Germany

Estimating higher-order motor parameters such as speed or inclination is important for flexible and adaptive control of legged locomotion. Especially during climbing behaviour, knowing about the own body orientation with respect to the substrate may be essential. A good reference frame to track relevant locomotion parameters such as absolute body inclination is provided by gravity, due to its absolute direction and constant strength. To monitor the gravity vector, most invertebrates rely on distributed proprioception, since they lack dedicated graviceptors, such as statocysts in crustaceans. Apart from graviception, distributed proprioception by means of hair fields, chordotonal organs and other proprioceptors, is also relevant for posture control during locomotion. Given the phasic-tonic response characteristics of these proprioceptor afferents, they could encode both the static posture and the change of posture.
To date, it is largely unknown what kind of information the central nervous system (CNS) of insects might use and which parameters are estimated for internal representations. Previously, we showed that absolute body pitch could be estimated from joint angle variations of the legs, by analysing whole-body kinematics of freely climbing stick insects in conjunction with simple spiking proprioceptor models and a feed forward artificial neural network (ANN). This showed, that leg pairs and joint combinations contribute differently towards the body inclination estimate [1]. In our current study we investigate the relevance of proprioceptor properties such as range fractionation and filter characteristics. Furthermore, we want to assess the benefit for the CNS to gate proprioceptive feedback according to their relevance to sensory estimates of higher-order parameters. As before, the goodness of the mapping of distributed proprioceptor activity on the estimate of body inclination was tested by means of a feed-forward ANN.
To this end we varied the number and arrangement of the sensory cells, equivalent to the granularity and overlap of range fractionation. We tested two arrangements: In the first arrangement the sensory cells were distributed equally within the working range of the joints. In the second one, we used an optimal arrangement where single cells were arranged according to the cumulative probability distribution of the joint angle monitored. We found that there is an optimal number of afferents per hair field which is similar to the number of sensory cells found in insect hair fields. Furthermore, the optimized arrangement using the empirical data considerably increased the estimation compared to the equally distributed arrangement. Additionally, we further investigated the contribution of the phasic and tonic activation characteristics. Finally, we investigated the particular relevance of swing- and stance phase. Although spikes during swing phases were inhibited, it was sufficient to estimate body pitch from leg angle time courses during stance phases only.

[1] Gollin A., Dürr V. (2018) Estimating Body Pitch from Distributed Proprioception in a Hexapod. In: Vouloutsi V. et al. (eds) Biomimetic and Biohybrid Systems. Living Machines 2018. Lecture Notes in Computer Science, vol 10928. Springer, Cham

Symposium

S22: The neuronal basis of tinnitus

Characteristics of auditory processing associated with tinnitus

Pim Van Dijk[1]

[1]University of Groningen, University Medical Center Groningen, Dept. of Otorhinolaryngology, POBox 30001, 9700 RB Groningen, Netherlands

Hearing loss is an important risk factor for developing tinnitus. Hearing loss results in changes in brain function. Tinnitus or 'Ringing in ears' is believed to be associated with maladaptive changes in the brain. This can be tested by comparing brain function in subjects with hearing loss and tinnitus to that in subjects with only hearing loss and no tinnitus. We measured sound-evoked fMRI responses in subjects with moderate sensorineural hearing loss. Sound stimuli were broad-band and presented monaurally with levels 30-90 dB SPL in quasi-random order at the left and right ear. A connectivity analysis revealed reduced functional connectivity between the inferior colliculus and auditory cortex. These results were confirmed in a second study, where subjects with near-normal hearing were included, and subjects with and without tinnitus were compared. In this confirmatory study, also a reduced connectivity was observed between the inferior colliculus and auditory cortex. In a third study, the relation between tinnitus loudness and brain activity was measured in subjects that can modulate their tinnitus loudness by eye-gaze. Here, eye-gaze resulted in (1) increase of activity in the inferior colliculus, (2) inhibition of activity in the medial geniculate body, and (3) reduction of inhibition in the auditory cortex. Together these results suggest a dissociation between the brainstem and auditory cortex, possibly caused by inhibited activity in the thalamus (medial geniculate body).

Tinnitus and comorbidities

Birgit Mazurek[1]

[1]Charité University Hospital Berlin, ENT / Tinnitus Center / TIN-ACT, Charitéplatz 1, 10117 Berlin, Germany

Tinnitus denotes the phantom perception of a sound in the absence of an external source.
Tinnitus research has focused on peripheral-central-auditory etiologies. For example, research investigating tinnitus in patients with concurrent hearing loss highlighted the roles of cochlea irritations and altered neural processing alongside the central auditory pathway. Hence, a growing body of evidence points towards complex interactions of central auditory and non-auditory processes across cortical and subcortical brain regions.

The identification of tinnitus-specific pathophysiological mechanisms is further complicated by its frequent co-occurrence with a wide range of psychological comorbidities such as depression, anxiety, somatoform disorders and insomnia. Increasingly, these comorbidities are conceptualized across transdiagnostic cognitive-emotional dimensions such as emotion regulation difficulties, experiential avoidance, repetitive negative thinking, intolerance of uncertainty or intolerance of negative affect.

On a phenomenological level, emotional difficulties can precede, exacerbate, or result from the tinnitus percept. For example, tinnitus may 1] be associated with pathological functions that also cause psychological distress, 2] temporally precede or 3] cause emotional difficulties in vulnerable individuals, 4] exacerbate existing psychological distress, 5] interact with psychological factors in increasing the risk of chronicity, i.e. altering the course of the percept, or 6] be influenced by third variables that account for both tinnitus and the development or maintenance of depressive or anxiety-related states.

Studies have highlighted the effect of acute stress on auditory processing via processes that have also been implicated in anxiety or depression such as changes in attention, changes in cortisol levels or limbic processes, suggesting distress as a shared risk factor for the development, exacerbation or maintenance of both tinnitus and emotional difficulties, particularly depression.

In summary, tinnitus is a common and distressing auditory disorder that is associated with substantial personal and economic burden. Whilst its exact pathophysiology remains unclear, recent research has begun to focus on interactions between central audiological, attentional and emotion-processing networks that partly overlap with neurological systems previously associated with psychological distress – particularly depressive mood.

Cortical Tonotopic Maps in Tinnitus and Hearing Loss

Elouise Alexandra Koops, Remco Renken[2], Cris Lanting[3], Pim van Dijk[1]

[1]Department of Otorhinolaryngology, University Medical Center Groningen, Hanzeplein 1, Postbus 30.001, 9700 RB Groningen, Netherlands

[2]Department of Neuroimaging, University Medical Center Groningen, Hanzeplein 1, Postbus 30.001, 9700 RB Groningen, Netherlands

[3]Department of Otorhinolaryngology, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen

Introduction

The development of tinnitus is highly associated with the presence of clinical hearing loss, which is associated with neural plasticity. The tonotopic organization is a striking feature of the auditory system, including the auditory cortex. Even though the specific pathophysiology involved in tinnitus remains elusive, a relation between hearing-loss-induced tonotopic reorganization and tinnitus is hypothesized. Participants with high-frequency sensorineural hearing loss, both with and without tinnitus were included in this large fMRI study. This allowed us to investigate to what extend reorganization is a consequence of hearing loss, and whether reorganization is specifically related to tinnitus.

Methods

To investigate the association between tonotopic reorganization, hearing loss and tinnitus functional magnetic resonance imaging (fMRI) was used. The changes in the tonotopic maps related to hearing loss and tinnitus were quantified, amongst other things by means of a principal component analysis (PCA), in order to assess any group differences. In 90 participants the bilateral cortical responses to sound stimulation were measured, split in to three groups: a hearing loss group with tinnitus, a hearing loss only group and a control group with normal hearing. The sound-evoked activation was triggered by means of loudness matched pure tone stimuli (0.25 – 8 kHz), presented in the MRI scanner while participants performed an unrelated visual task to control for attention effects.

Results

We found a considerable increase in activation level in response to stimulation with 8 kHz in both hearing loss groups, with and without tinnitus, compared to the control group. In addition to this, the tonotopic maps of the tinnitus and the hearing loss group appear very similar. Similarity in hearing thresholds in both hearing groups are consistent with this finding. However, the shift towards higher frequencies is less pronounced in the tinnitus group compared to the hearing loss group.

Conclusion

In high frequency sensorineural hearing loss, the tonotopic map shifts toward high frequency responsiveness. An increased responsiveness to high frequency stimuli is observed in both groups with high-frequency sensorineural hearing loss, in a loudness-matched protocol. The major framework of the cortical tonotopic map is not influenced by the presence of tinnitus, except for a less-pronounced shift to high-frequencies in tinnitus. The more pronounced shift in hearing loss without tinnitus compared to hearing loss with tinnitus suggests that in tinnitus a reduced cortical ability to adapt to the hearing loss might play a role.

The pathophysiology of tinnitus

Arnaud Jean Norena[1]

[1]cnrs, LNSC UMR CNRS 760, 3 place Victor Hugo, Université Aix-Marseille, Case B, 13003 Marseille, France

Tinnitus is a symptom that can result from many different causes. The tinnitus field is limited by the fact that it is unclear how much tinnitus sub-types exist and how to distinguish them. Nevertheless, tinnitus can be divided roughly into two broad sub-categories. Cochlear tinnitus are thought to result from an aberrant activity that is present as early as the cochlear nerve. This activity propagates all the way up to the auditory centers where it is associated to an auditory percept. Central tinnitus are supposed to result from the central plastic changes triggered by cochlear hearing loss. In this tinnitus sub-type, a reduced cochlear activity can be accompanied by central hyperactivity, i.e. the tinnitus-related neuronal activity. I will present a few possible tinnitus mechanisms and their clinical implications.

The fine-tuned brain: Better hearing in tinnitus patients due to stochastic resonance?

Holger Schulze[1]

[1]University of Erlangen-Nürnberg, Experimental Otolaryngology, Waldstrasse 1, 91054 Erlangen, Germany

Subjective tinnitus, the perception of sound in the absence of a physical sound source, is a frequent symptom with increasing prevalence, currently affecting 10 to 15% of the adult general population. Because the mechanisms leading to the condition are still not fully understood, a cure for subjective tinnitus is still not available. Common models of tinnitus development propose that damage to the peripheral auditory epithelium in cochlea leads to an imbalance of excitatory and inhibitory auditory circuits, thereby enhancing neuronal activity. Other models propose homeostatic plasticity within the central auditory system attempting to compensate for reduced cochlear input via an increased response gain.

Recently we have proposed an alternative model based on stochastic resonance (SR) which by adding internally generated neuronal noise serves to lift signals above noise trauma induced increased neuronal thresholds, thereby partly compensating for the hearing loss. We propose that this internally generated noise - which is crucial for SR to work - corresponds to neuronal hyperactivity which subsequently causes neuronal plasticity along the auditory pathway and finally may lead to the development of a phantom percept, i.e. subjective tinnitus. In that view, tinnitus would be a side product of the attempt of the auditory system to at least partially restore hearing thresholds after noise trauma, and indeed audiometric data from almost 40.000 patients from our ENT department are in favor of that view. In addition, we show evidence from our animal model, the Mongolian gerbil, that such tinnitus development is closely related to changes in neuronal activation patterns in auditory cortex that are associated with spontaneous activity, i.e. that are recorded during silence: these become similar to those evoked by pure tones matching the perceived tinnitus frequency.

Symposium

S23: Early information selection for robust vision

LACK OF ROBUSTNESS IN ARTIFICIAL NEURAL NETWORKS

Matthias Bethge

[1] Matthias Bethge, Tu¨bingen

Deep neural networks have become a ubiquitous tool in a broad range of AI applications. Resembling important aspects of rapid feed-forward visual processing in the ventral stream they can be trained to match human behavior on standardized pattern recognition tasks. Outside the training distribution, however, decision making of artificial neural networks exhibits large discrepancies to biological vision systems. I will give an overview on the lack of robustness in deep neural networks and present recent results of my lab to quantify and overcome these discrepancies.

Chromatic processing in the mouse retina

Katrin Franke[1]

[1]Bernstein Center for Computational Neuroscience Tübingen, Institute for Ophthalmic Research Tübingen

The basis for color vision emerges at the first stage of the visual system, the retina. Here, chromatic signals originating from different photoreceptor types sensitive to different wavelengths are locally compared by retinal circuits. Most mammals are dichromatic. They have two cone photoreceptor types, expressing the short- (S, blue) and medium-wavelength (M, green) sensitive opsin. Mice and a few other mammalian species, however, show opsin co-expression resulting in an asymmetric opsin distribution: While true S-cones exclusively expressing S-opsin are homogenously distributed across the retina (~5% of all cones), M-cones co-express S-opsin with increasing co-expression levels towards the ventral retina – resulting in a green-sensitive dorsal and a blue-sensitive ventral retina. Due to this uneven opsin distribution (especially in the ventral retina) it was unclear whether mice can extract chromatic information. However, recent behavioral studies demonstrated that mice can discriminate between light spots of different colors, at least in the upper visual field.

Here, we examine how the mouse retina extracts chromatic information across three consecutive processing levels along the vertical retinal pathway. For that, we use two-photon population imaging combined with visual stimulation to record light-evoked glutamate and calcium responses of photoreceptors, bipolar cells and retinal ganglion cells in the ex-vivo, whole-mounted retina, where long-range inhibitory connections are intact. For chromatic stimulation, we use a DLP projector with LEDs matched to the spectral sensitivity of mouse photoreceptors. Then, at every processing level, we use a similar set of chromatic stimuli including a sine-wave modulation and center-surround flicker stimulus of green and blue LED to analyze the chromatic preference of center-surround receptive fields of retinal neurons.

Our approach allows to systematically investigate how the chromatic information originating from photoreceptors is processed in downstream retinal circuits. In my presentation, I will summarize our results on chromatic processing in the mouse retina and relate them to previous behavioral studies demonstrating color discrimination in mice.

A vision for orienting in primate superior colliculus

Ziad M. Hafed[1]

[1]Tuebingen University, Werner Reichardt Centre for Integrative Neuroscience, Otfried-Mueller Str. 25, 72076 Tuebingen, Germany

Underlying the remarkable robustness of visual perception, visual brain areas exhibit tuning characteristics well suited for image statistics present in our natural environment. However, visual sensation itself is an active process, and if there are any brain areas that ought to be particularly in tune with natural scene statistics, it would be sensory-motor areas critical for guiding behavior. In this talk, I will describe how the primate superior colliculus, a structure instrumental for rapid visual exploration with saccadic eye movements, possesses visual processing machinery that make it ideal not only for rapid orienting with eye movements, but also for stabilizing percepts across the transient retinal-image shifts caused by such eye movements. Among the superior colliculus' visual processing capabilities, this structure's neurons detect low spatial frequencies, which are the most prevalent in natural scenes, much more rapidly than high spatial frequencies. Importantly, this accelerated detection happens independently of whether a neuron is more or less sensitive to low spatial frequencies to begin with, and it directly correlates with how rapidly saccadic eye movements can be triggered. At the population level, the superior colliculus additionally over-represents low spatial frequencies in neural response sensitivity, even at near-foveal eccentricities. Thus, the superior colliculus possesses both temporal and response gain mechanisms for efficient gaze realignment in low-spatial-frequency-dominated natural environments. More globally, the visual field representation of the primate superior colliculus also has non-uniform sampling resolution across the upper and lower visual fields, and this structure exhibits much higher visual sensitivity for the upper visual field. Once again, this visual-field asymmetry directly impacts properties of saccades directed with an upward or a downward component, and it likely reflects the consequences of peri-personal near space (predominantly in the lower visual field) and extra-personal far space (spanning also the upper visual field) on retinal image properties for objects that can be the targets of saccades. Thus, in the superior colliculus, the intricacies of motor control of saccades start at the very first visually-induced action potentials after visual stimulus onset. Critically, such visually-induced action potentials are under constant influence of ongoing oculomotor activity and are therefore continuously modulated by eye movements in a perpetual perception-action cycle. This means that the primate superior colliculus contains an entire spectrum of significant contributions to robust visual perception: from visual sensation to saccade commands, and all the way back again through trans-saccadic visual modulation.

Visual selection

Zhaoping Li[1]

[1]MPI for Biological Cybernetics, Sensory and Sensorimotor Systems, Max-Planck-Ring 8, 72076 Tübingen, Germany

No abstract available.

Mouse dLGN receives functional input from a diverse population of retinal ganglion cells with limited convergence

Yannik Bauer

[1]Centre for Integrative Neuroscience, University of Tübingen, 72076 Tübingen, Germany
[2]Institute for Ophthalmic Research, University Hospital Tübingen, 72076 Tübingen, Germany
[3]Division of Neurobiology, Department Biology II, LMU Munich, 82151 Munich, Germany
[4]Graduate School of Neural & Behavioural Sciences | International Max Planck Research School, University of Tübingen, 72074 Tübingen, Germany
[5]Graduate School of Systemic Neuroscience (GSN), LMU Munich, 82151 Munich, Germany
[6]ENB Elite Master of Science program in Neuroengineering, Technical University of Munich, 80333 Munich, Germany
[7]Bernstein Centre for Computational Neuroscience, 72076 Tübingen, Germany
[8]Bernstein Centre for Computational Neuroscience, 82151 Munich, Germany

In the mouse, the parallel output of more than 30 functional types of retinal ganglion cells (RGCs) serves as the basis for all further visual processing. Little is known about how the representation of visual information changes between the retina and the dorsolateral geniculate nucleus (dLGN) of the thalamus, the main relay station between the retina and cortex. Interest in these questions has been fueled by recent estimates of retinogeniculate convergence obtained by anatomical work, which far exceeded those obtained in electrophysiological recordings.

To get insights into the nature of retinal input to dLGN, we conditionally expressed the genetically encoded Ca2+ indicator GCaMP6f in dLGN-projecting (dLGN-p) RGCs, followed by in vitro retinal two-photon Ca2+ imaging of light-evoked responses. Using the same stimulus set as an earlier RGC classification by Baden et al. (Nature 2016), we compared the responses of each dLGN-p RGC to those of the previously described RGC types and identified the RGC population cluster with the best-matching response properties. We found that most functional RGC types seem to innervate dLGN, with certain types, such as ON- and OFF alpha cells or OFF contrast-suppressed cells, showing clear overrepresentations.

Using in vivo extracellular multi-electrode recordings in awake, head-fixed mice, we then recorded the responses of dLGN neurons to the same visual stimuli. We quantitatively assessed the degree of diversity in the dLGN responses by using cross-validated non-negative matrix factorization (NNMF), which decomposed the dLGN population response into a rich and highly diverse set of ca. 30 response components.

Finally, using a linear model to assess functional connectivity between RGC types and dLGN neurons, we found that the responses of dLGN neurons could be predicted as a linear combination of inputs from on average five RGC types, but only two of those had the strongest functional impact.

In summary, our study reveals that most mouse RGC types project to the dLGN, which yields an unexpectedly diverse representation that can be reconstructed by a feedforward model revealing limited RGC input convergence.

Symposium

S24: Form follows function? Rules and consequences of structural synaptic plasticity

Synaptic mechanisms for plasticity in the somatosensory cortex

Anthony Holtmaat[1]

[1]University of Geneva, Basic Neurosciences, 1 rue Michel Servet, 1211 Geneva, Switzerland

Sensory experience and perceptual learning changes the receptive field properties of cortical neurons. It is thought that this is mediated by a synaptic changes, varying from alterations in the strength of established synapses to the removal of old and the formation of new synapses. My laboratory is interested in the link between structural and functional synaptic plasticity in the adult mouse brain. We image cortical neurons in fluorescent transgenic mice in vivo, using high-resolution two-photon laser scanning microscopy through chronic cranial window implants. We track individual dendritic spines and axonal boutons, which are the structural proxies for synapses, over weeks to months upon new sensory experiences, after sensory stimulation, or during perceptual learning. In addition, we measure the molecular stability and dynamics of individual synapses by monitoring the transport and diffusion of synaptic proteins carrying a fluorescent tag. Together these studies will deepen our understanding of how the nervous system adapts to changes in sensory inputs or accommodates the animal's memory storage while the overall integrity of the neuronal network is preserved.

Structural dynamics following sensory deprivation in mouse visual cortex

Tara Keck[1], Samuel Barnes[1], Irene Jacobsen[1], Eleonora Franzoni[1]

[1]University College London, Neuroscience, Physiology and Pharmacology, 21 University Street, WC1E 6DE London, United Kingdom

Homeostatic synaptic scaling is thought to occur cell-wide, but recent evidence suggests this form of stabilizing plasticity can be implemented more locally in reduced preparations. To investigate the spatial scales of plasticity in vivo, we used repeated two-photon imaging in mouse visual cortex after sensory deprivation to measure TNF-α dependent increases in spine size as a proxy for synaptic scaling in vivo in both excitatory and inhibitory neurons. We found that after sensory deprivation, increases in spine size are restricted to a subset of dendritic branches. We found that the dendritic branches that had individual spines that increased in size following deprivation, also underwent a decrease in spine density. Within a given dendritic branch, the degree of spine size increases is proportional to recent spine loss within that branch. Using computational simulations, we show that this compartmentalized form of synaptic scaling better retained the previously established input-output relationship in the cell, while restoring activity levels. We then investigated the relationship between new spines that form after this spine loss and strengthening and find that their spatial positioning facilitates strengthening of maintained synapses.

The sequence of plasticity inducing events sets the lifetime of hippocampal synapses

Simon Wiegert

[1]J. Simon Wiegert, Hamburg

Synapses change their strength in response to specific activity patterns. This functional plasticity is assumed to be the brain's primary mechanism for information storage. We combine optogenetic and chemogenetic control of identified synapses in rat hippocampal slice cultures with calcium and glutamate imaging of synaptic transmission and long-term structural imaging. This approach enables us to perform all-optical quantal analysis of synaptic transmission, to induce long-term potentiation (LTP), long-term depression (LTD), or both forms of plasticity in sequence, to chronically manipulate activity and to follow the fate of individual synapses for 7 days. We ask how plasticity and activity are integrated at Schaffer collateral synapses over time. Our findings suggest that activity-dependent changes in the transmission strength of individual synapses are transient, but have long-lasting consequences for synaptic lifetime.

Memory linking through synapse clustering in active dendrites

Panayiota Poirazi[1]

[1]FORTH, IMBB, N. Plastira 100, Vassilika Vouton, 70013 Herakilon, Crete, Greece

Associative memories are believed to be stored in distributed neuronal assemblies through synaptic and intrinsic plasticity. The long-term plasticity of synapses involves the long-term potentiation/depression of synaptic responses, spine growth/elimination, protein synthesis and capture, homeostatic plasticity etc [1-2].

Based on experimental evidence, we developed a simplified computational model of plasticity that examines the role of dendrites and synaptic turnover dynamics during associative learning [5-6]. We use multi-scale modeling to model synaptic processes which span different temporal and spatial scales, such as calcium influx, protein synthesis and delivery, synaptic tagging and homeostasis to assess how memories are encoded in a population of neurons. Using the model, we show that memory storage increases the sparsity of population firing and that local protein synthesis promotes dendritic synapse clustering [3].

Moreover, our model suggests that memories learnt in close temporal proximity are stored in overlapping neuronal and dendritic populations. This overlap serves as the main mechanism for linking memories across time. These neuronal and dendritic overlaps underlie memory linking even in the absence of dendritic spikes, albeit at a very high cost of increased afferent connections, indicating that active dendrites serve as a means for resource savings. Finally, we propose that the same mechanisms can bind together sequential memories, creating memory episodes [3]. Our model also predicts that increased synaptic turnover facilitates the formation of synapse clusters within active dendrites, which in turn improves learning and maximizes the storage capacity of newly learnt memories [4].

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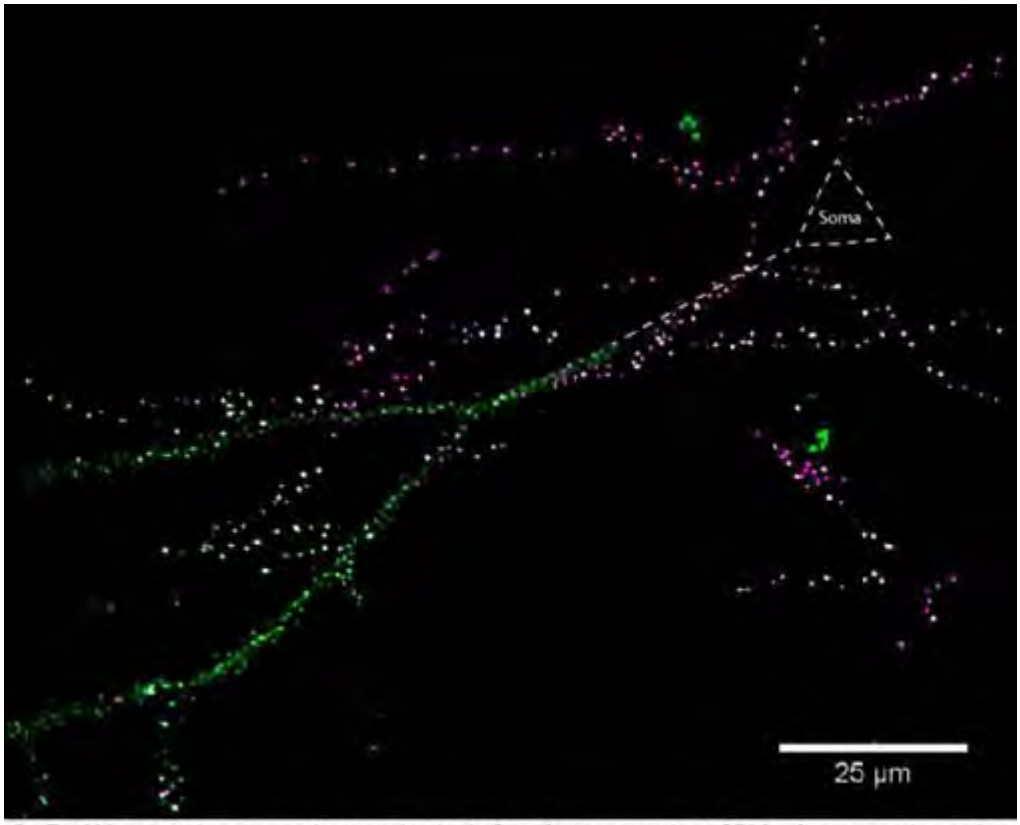
Mapping action potential back propagation using SynTagMA

Brenna C Fearey, Alberto Perez-Alvarez[1], Ryan OToole[2], Ignacio Arganda-Carreras[3], Eric R Schreiter[4], J. Simon Wiegert[5], Christian Schulze[1], Michael B. Hoppa[2], Christine E. Gee[1], Thomas G. Oertner[1]

[1]Center for Molecular Neurobiology, Institute for Synaptic Physiology, Falkenreid 94, 20251 Hamburg, Germany
[2]Dartmouth College, Dept. of Biological Sciences, Hanover, New Hampshire, USA
[3]Basque Foundation for Science, Ikerbasque, Bilbao, Spain
[4]HHMI, Janelia Research Campus, Ashburn, VA, USA
[5]Center for Molecular Neurobiology, RG Synaptic Wiring and Information Processing, Falkenreid 94, 20251 Hamburg, Germany

Advanced microscopy techniques have been used to great advantage to study subcellular calcium dynamics with high spatial and temporal resolution. At the level of single synapses, calcium and voltage imaging has given major insights into synaptic function and the mechanisms underlying synaptic strengthening and weakening. However, simultaneously measuring responses from thousands of synapses across a dendritic tree has not been possible. Here we present SynTagMA, a genetically encoded calcium integrator that marks active synapses by irreversible photoconversion from green-to-red fluorescence with violet illumination. We targeted the optogenetic tool, CaMPARI2.0 (F391W, L398V), to the pre-synapse by fusing it to the vesicular protein synaptophysin, and to the post-synapse by fusing it to an intrabody against PSD-95 (PSD-95 FingR). When expressed in cultured hippocampal neurons or pyramidal neurons in organotypic hippocampal slice cultures, the constructs were highly localized to the presynaptic or postsynaptic compartments, respectively. SynTagMA underwent photoconversion when intracellular calcium was high and 395nm light (PC light) was applied. We characterized SynTagMA properties and optimized conditions for photoconversion using field stimulation or back-propagating action potentials. We show that the precise timing and dosage of PC light is crucial for labelling synaptic events (see Perez-Alvarez et al). As our ultimate goal is to track synaptic activity maps over time, the relatively fast turnover of postsynaptic SynTagMA affords the opportunity to relabel synapses after about two hours. The strength of this tool is to capture a snapshot of network activity and find isolated active inputs across the entire dendritic arbor. At the same time, this strength creates the challenge of reliably finding and tracking thousands of individual synapses in 4D. To this end, we have developed a MATLAB-based software, Synapse Locator (see Schulze et al) which automatically aligns, transforms, detects and quantifies the fluorescence of SynTagMA expressing synapses. Interestingly, when evoking back-propagating action potentials in neurons expressing the postsynaptic SynTagMA, we have observed spine to spine and branch to branch differences in photoconversion, suggesting that calcium does not rise uniformly in all spines and branches. SynTagMA will open new doors into the investigation of synaptic circuits in vitro and potentially in intact animals during behavior.



SynTagMA expressing neuron (max projection) after photoconversion of 50 back-propogating action potentials. **Green**: unconverted SynTagMA ; **Magenta**: converted SynTagMA. Note difference in PC in proximal vs. distal branches

Symposium

S25: Go with the flow? Processing of sensory flows across modalitie

S25-1 An Eye towards Hovering: Species Differences in the Processing of Optic Flow in Birds in Relation to Flight Behaviour
Douglas R Wylie, Andrea H Gaede, Graham C Smyth, Douglas L Altshuler

S25-2 Optimal visual sensitivities: What the cichlid eye needs to tell the cichlid brain
Karen Carleton

S25-3 Echo flow patterns influence bat flight behavior and neural activity
Michaela Warnecke, Silvio Macias, Benjamin Falk, Cynthia F. Moss

S25-4 Close-loop control of active-sensing movements
Eric Scott Fortune, Noah Cowan

S25-5 Binocular processing and receptive fields of motion-sensitive neurons in the zebrafish pretectum and tectum
Kun Wang

S25-6 Natural stimuli reveal a spectrum of spatial encoding across the output channels of the retina
Dimokratis Karamanlis, Tim Gollisch

An Eye towards Hovering: Species Differences in the Processing of Optic Flow in Birds in Relation to Flight Behaviour

Douglas R Wylie[1], Andrea H Gaede[1,2], Graham C Smyth[2], Douglas L Altshuler[2]

[1]University of Alberta, Faculty of Science, P217 Biological Sciences Bldg, T6G 2E9 Edmonton, Canada
[2]University of British Columbia, Department of Zoology, Vancouver, Canada

The processing of optic flow is necessary to generate the optokinetic response to mediate gaze stabilization. In all vertebrate tetrapods, optic flow is initially processed by retinal recipient nuclei. In birds, these are the pretectal nucleus lentiformis mesencephali (LM) and the nucleus of the basal optic root (nBOR) of the accessory optic system. LM and nBOR are homologous to the mammalian nucleus of the optic tract (NOT) and the terminal nuclei of the AOS. Relying on similar data collected in wallabies and pigeons, Ibbotson and Price (2001) noted that responses in the LM/NOT were highly conserved in two respects. First, most neurons respond best to motion in the temporal-to-nasal direction. Second, with respect to stimulus speed, there were two clear groups: “fast” neurons preferred low spatial frequencies (SFs) and high temporal frequencies (TFs), whereas “slow” neurons preferred high SFs and low TFs. The speed preferences (TF/SF) of the fast and slow neurons was quite similar across species (fast 50^{o}/s, slow 1^{o}/s). Among birds, hummingbirds are remarkable for their stabilization ability. They are able to maintain sustained hovering as they feed from flowers, despite the fact that their wings are beating up to 100Hz. Iwaniuk and Wylie (2007) examined the brains of several dozen species of birds, and noted that the LM was 2-5X larger in hummingbirds. They speculated that this hypertrophy reflected the sophisticated optic flow processing needed to meet the demands of stabilization during hovering flight. Furthermore, they hypothesized that most LM neurons in hummingbirds would respond to extremely slow speeds, thus providing a very robust error signal. With respect to this hypothesis, and to no one’s surprise, Iwaniuk and Wylie could not have been more wrong. We will present data of recordings from LM in hummingbirds *(C. anna)* and zebra finches *(T. guttata)* to optic flow stimuli, and compare this with archival data from pigeons *(C. livia)*. The hummingbird LM is somewhat unique in two respects. First, a bias toward neurons preferring temporal-to-nasal motion was not apparent in the hummingbird LM, but was in the LM of both the finches and pigeons. Second, whereas LM neurons in pigeons and zebra finches were broadly tuned to stimulus speed, hummingbird neurons were narrowly tuned to higher speed stimuli. This was especially clear when we used sine wave gratings of varying SF and TF as stimuli: hummingbird LM neurons responded to a narrow range of SF/TF combinations compared to the finches and pigeons. Moreover, the preference for higher speeds in hummingbird LM appeared to be mainly due to a preference for lower SFs. We believe these differences are related to the fact that hummingbirds are hovering in the presence of very large visual stimuli, i.e. the flowers from which they are feeding. Finally, although the LM appears unique in hummingbirds, recordings from the nBOR in the three species suggest the visual response properties are highly conserved.

Optimal visual sensitivities: What the cichlid eye needs to tell the cichlid brain

Karen Carleton[1]

[1]University of Maryland, Biology, 4094 Campus Dr, 20742 College Park, USA

Animal survival depends on behavioral tasks from foraging to mating to predator avoidance. Behavioral responses are guided by sensory input with vision being a key sensory channel. In discriminating targets from backgrounds, the photoreceptors quantify and compare brightness and color. The optimal visual sensitivity for maximizing discrimination may require different visual sensitivities in different environments for different tasks. In fishes, we find that visual systems are actually quite variable since there are multiple opsin genes which can produce visual pigments sensitive across the spectral range from ultraviolet to red wavelengths. Through altering opsin gene expression, visual sensitivities can vary over times scales of a few days (plasticity) to months (development) to years (evolution). Variation can also occur within the retina of a single individual or between individuals in different locations. I will review some of our work on both temporal and spatial variation of opsin gene expression in the cichlid retina, its relationship to the light environment, and discuss why it might be important for cichlid behavior and ultimately for cichlid evolution.

Echo flow patterns influence bat flight behavior and neural activity

Michaela Warnecke[1], Silvio Macias[1], Benjamin Falk[1], Cynthia F. Moss[1]

[1]Johns Hopkins University, Dept. of Psychological and Brain Sciences, Comparative Neural Systems and Behavior Lab, 3400 N Charles St, Ames 123, 21218 Baltimore, USA

To navigate in the natural environment, animals must adapt their locomotion in response to environmental stimuli. The echolocating bat relies on auditory processing of echo returns to represent its surroundings. Recent studies have shown that echo flow patterns influence bat navigation, but the acoustic basis for flight path selection remains unknown. To investigate this problem, we released bats in a flight corridor with walls constructed of adjacent individual wooden poles, which returned cascades of echoes to the flying bat. We manipulated the spacing and echo strength of the poles comprising each corridor side, and predicted that bats would adapt their flight paths to deviate toward the corridor side returning weaker echo cascades. Our results show that the bat's trajectory through the corridor was not affected by the intensity of echo cascades. Instead, bats deviated toward the corridor wall with more sparsely spaced, highly reflective poles, suggesting that pole spacing, rather than echo intensity, influenced bat flight path selection. This result motivated investigation of the neural processing of echo cascades: We measured local evoked auditory responses in the bat inferior colliculus to echo playback recordings from corridor walls constructed of sparsely and densely spaced poles. We predicted that evoked neural responses would be discretely modulated by temporally distinct echoes recorded from the sparsely spaced pole corridor wall, but not by echoes from the more densely spaced corridor wall. The data confirm this prediction and suggest that the bat's temporal resolution of echo cascades may drive its flight behavior in the corridor.

Close-loop control of active-sensing movements

Eric Scott Fortune[1], Noah Cowan[2]

[2]Johns Hopkins University

Active sensing is the production of motor signals for sensing. The most common form of active sensing, found across animal taxa and behaviors, involves the generation of movements—e.g. whisking, touch, sniffing, and eye movements—that shape spatiotemporal patterns of feedback. Despite the fact that active-sensing movements profoundly affect the information carried by sensory feedback pathways, how such movements are regulated remains poorly understood. To investigate the control of active-sensing, we created an experimental apparatus for freely swimming weakly electric fish, Eigenmannia virescens, that modulates the gain of reafferent feedback by adjusting the position of a refuge based on real time videographic measurements of fish position. We discovered that fish robustly regulate sensory slip via closed-loop control of active-sensing movements. Specifically, as fish performed the task of maintaining position inside the refuge, they dramatically up- or down-regulated fore-aft active sensing movements in relation to a 4-fold change of experimentally modulated reafferent gain. These changes in swimming movements served to maintain a constant magnitude of sensory slip. The magnitude of sensory slip depended on the presence or absence of visual cues, but in each condition the respective magnitude was maintained across reafferent gains. These results indicate that fish use two control loops: an “inner loop” that controls the acquisition of information by regulating sensory slip, and an “outer loop” that maintains position in the refuge, a control topology that may be ubiquitous in animals.

Binocular processing and receptive fields of motion-sensitive neurons in the zebrafish pretectum and tectum

Kun Wang

[1]University of Tübingen, Institute of Neurobiology and Werner Reichardt Centre for Integrative Neuroscience, Germany
[2]Graduate Training Centre for Neuroscience, University of Tübingen, D-72076 Tübingen, Germany
[3]Current address: Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland
[4]Neurobiology and Behavior, Institute Biology 1, Faculty of Biology, University of Freiburg, D-79104 Freiburg, Germany

Optic flow processing by neurons in the diencephalic pretectum is essential for visually guided behaviors in vertebrates, such as the optokinetic and optomotor responses. Animals need to distinguish translational and rotational stimuli to actively stabilize both their gaze and position relative to their surroundings. Recently, pretectal neurons involved in this task have been identified. However, the underlying sensorimotor transformations in zebrafish are still unclear. To elucidate the mechanisms, we investigated the sensory representations in the larval zebrafish brain with calcium imaging. We find that the directional space of both pretectal and tectal neurons is represented by four preferred optic flow directions during monocular stimulation (roughly corresponding to Up, Down, Forward, and Backward motion). Similar numbers of direction-selective neurons are found for each of the four preferred directions. No anatomical segregation of direction-selective tectal neurons was found. Furthermore, we identified neurons responding to specific translational or rotational whole-field patterns by presenting all possible binocular combinations of the four (monocularly) preferred stimulus directions to both eyes of the fish. These binocular selective neurons could – in principle – directly instruct appropriate compensatory eye and tail movements during optokinetic and optomotor behavior respectively. Monocular receptive field mapping shows that the vast majority of motion-sensitive tectal neurons are tuned to motion in a small portion of the visual field, many of them with surrounding inhibition. In contrast, many pretectal neurons have large-sized receptive fields (> 60°x30°, azimuth and elevation). Compared to the receptive field centers of cells with large receptive fields, visual space of the small-sized tectal receptive fields is over-represented in the nasal-dorsal visual field. Our study characterizes fundamental features of optic flow processing in the zebrafish pretectum and tectum. Our results provide the basis for further investigations into the vertebrate sensorimotor circuits of visually guided behaviors and into the adaptations of the larval zebrafish brain to habitat and lifestyle.

Natural stimuli reveal a spectrum of spatial encoding across the output channels of the retina

Dimokratis Karamanlis[1,2,3], Tim Gollisch[1,2]

[1]Department of Ophthalmology, University Medical Center Göttingen, Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience Göttingen, Göttingen, Germany
[3]International Max Planck Research School for Neurosciences, Göttingen, Germany

The visual system routinely encounters natural scenes that contain fine spatial structure. Standard models of sensory processing assume that early visual neurons encode just the average light intensity in their receptive fields, ignoring any spatial detail. Such linear models may capture retinal ganglion cell responses to artificial stimuli, but often fail for natural stimuli. A potential reason is the nonlinear processing of light intensities in the receptive field. However, it has been challenging to link receptive field nonlinearities and linear model failure with natural stimuli, because of the diversity of functional properties between ganglion cell types (>30 in the mouse). To address this problem, we recorded the spiking activity of ganglion cells with multielectrode arrays from isolated mouse retinas and built linear receptive field models for hundreds of cells. We compared the predictions of such models to measured cell responses under natural image flashes. Linear models were able to completely capture responses for some cells (linear), but failed for a significant proportion of cells (nonlinear). When the receptive fields of nonlinear cells sampled image patches with rich spatial structure, the cells responded stronger than expected from a linear model. Additionally, we measured response changes when natural images were systematically altered by blurring. While the responses of linear cells barely changed, the responses of nonlinear cells diminished with a scale on the order of the receptive field size of their presynaptic neurons, bipolar cells. Using an artificial stimulus with two spatially separate light intensities, we showed that nonlinear cells strongly rectified their non-preferred intensities. Finally, we associated model performance with other cell-type specific properties, such as direction selectivity, and identified a known ganglion cell type that is particularly sensitive to spatially homogeneous stimuli. Our investigations establish the link between natural scene processing and receptive field nonlinearities in diverse populations of retinal ganglion cells. Thus, our approach may offer insights towards more complete encoding models and better understanding of cell type diversity in the context of natural stimuli.

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Symposium

S26: Neural mechanisms of social decision-making

S26-1 Neural mechanisms of social preferences in rats
Tobias Kalenscher

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Steve W. C. Chang

S26-3 Reciprocity and Punishment: Insights from Decision Neuroscience
Alan G. Sanfey

S26-4 How multiple motives affect the computation of social decisions in the human brain
Anne Christin Saulin

S26-5 The role of differential sensory input and attributional biases in social effort perception.
Caedyn Lachlan Stinson, Julia M. Rodriguez Buritica, Igor Kagan , Arezoo Pooresmaeili

Neural mechanisms of social preferences in rats

Tobias Kalenscher[1]

[1]Heinrich Heine University Düsseldorf, Comparative Psychology, Universitätsstr. 1, 40225 Düsseldorf, Germany

Mutual-reward preferences are revealed when decision-makers prefer alternatives yielding rewards to themselves and conspecifics over alternatives leaving conspecifics empty-handed. Mutual-reward preferences have been shown in humans and non-human primates, but it remains elusive if they evolved earlier in the phylogenetic history. In my talk, I will provide evidence that rats show mutual-reward preferences. I will argue that the rats' social preferences are the consequence of social reinforcement learning in which acoustic social signals emitted by the two interacting rats orchestrate their preferences for equal reward outcomes. I will present lesion and psychopharmacological data highlighting the importance of basolateral amygdala, and local serotonin action in amygdala, in developing mutual-reward preferences. Insights into the neural mechanism of mutual-reward preferences will help understanding disorders characterized by insensitivity to social signals and the well-being of others, such as psychopathy and antisocial personality disorder.

The coordinated interplay between prefrontal areas and amygdala in social gaze dynamics and decision-making

Steve W. C. Chang[1]

[1]Yale University, Psychology, Neuroscience, Kavli Institute for Neuroscience, 2 Hillhouse Ave., 06511 New Haven, USA

Gaze interaction is central to social behavior in humans and non-human primates, and is often used as a proxy for social attention. However, the neural mechanisms underlying contingent and spontaneous social gaze events as they unfold over time remain elusive. Recently, we have been using a real-life social gaze interaction paradigm to study neurophysiological mechanisms underlying spontaneous and contingent gaze dynamics occurring between pairs of rhesus macaques (*Macaca mulatta*). This naturalistic setting combined with high-resolution eye position monitoring of both animals allows for the necessary quantification of social gaze dynamics while increasing ecological validity. We have previously used this task to document that certain aspects of social gaze behaviors are not reliably captured using pictures and movies of conspecific monkeys (Dal Monte et al., 2016, J Neurophys), and also to report a combinatorial boost in social attention and contingent gaze dynamics when oxytocin processing is enhanced while opioid processing is concurrently attenuated using naloxone (Dal Monte et al., 2017, PNAS). Using this paradigm, here we investigated the contribution of the basolateral amygdala (BLA) and three prefrontal structures – the anterior cingulate gyrus (ACCg), the orbitofrontal cortex (OFC), and the dorsomedial prefrontal cortex (dmPFC) – in live social gaze interactions. Both spiking and local field potential activity were recorded from one of the prefrontal structures simultaneously with the BLA, allowing us to examine neural coordination patterns between each prefrontal region and the BLA in mediating social gaze behaviors. Neural data were aligned to diverse gaze events, such as looking at the partner's eyes (in the contexts of mutual gaze or exclusive gaze), other regions of conspecific's face, or non-social objects of interest. While neurons from all brain regions signaled various social gaze events, they displayed marked heterogeneities in the temporal dynamics of spiking activity when encoding these events. Across these brain regions, the proportion of cells that differentiated looking at the eyes from other parts of the face and those that exhibited face-selective signals also differed, suggesting distinct contributions of different brain regions. Furthermore, between the prefrontal regions and the BLA, divergent coupling patterns in the gamma band tracked social gaze events, depending on the specific prefrontal node being examined with respect to the BLA. For the ACCg-BLA pairing, we were also able to examine their interplay in a social decision-making context using a social reward allocation task, in which monkeys make decisions impacting the reward outcome of a conspecific monkey in the room, without affecting their own reward outcomes, in two separate decision contexts (Chang et al., 2013, Nat Neurosci). In this task, monkeys typically exhibit a prosocial preference in one context but an antisocial preference in the other context. The ACCg-BLA coherence patterns markedly differed across the gamma and beta bands between the two decision-making contexts, and these coherence patterns could be used to decode the prosocial preference of the animals. Our findings overall suggest that dynamic coordination between the prefrontal structures and the amygdala may play a central role in guiding social behavior.

Reciprocity and Punishment: Insights from Decision Neuroscience

Alan G. Sanfey[1]

[1]Donders Institute, Decision Neuroscience lab, P.O. Box 9101, 6500HB Nijmegen, Netherlands

Our lives consist of a constant stream of decisions and choices, from the mundane to the highly consequential. The standard approach to experimentally examining decision-making has been to examine choices with clearly defined probabilities and outcomes, however it is an open question as to whether decision models describing these situations can be extended to choices that must be made by assessing the intentions and preferences of both oneself and of another social partner. This class of social decision-making offers a useful approach to examine more complex forms of decisions, which may in fact better approximate many of our real-life choices. In particular, these social interactive scenarios reveal motivations other than economic gain that appear to guide our decisions in a systematic fashion. Here, we focus on the social motivation of reciprocity, and explore the conditions under which we either reciprocate or punish the actions of another. Data will be presented from several experiments where we use novel variants of economic games in conjunction with functional neuroimaging, pharmacological intervention, and computational modelling to observe how players decide in real, consequential, social contexts. I will also discuss how we can use these brain insights to build better models of human social preferences, incorporating both psychological and neurobiological constructs.

How multiple motives affect the computation of social decisions in the human brain

Anne Christin Saulin

[1]University of Würzburg, Clinic for Psychiatry and Psychosomatic, Würzburg, Germany
[2]University of Greifswald, Functional Imaging Unit, Institute of Diagnostic Radiology and Neuroradiology,Greifswald Germany.
[3]Goethe University, Institute of Medical Psychology, Faculty of Medicine, Frankfurt am Main, Germany.

Often, humans' social decisions are driven by a combination of different social motives, yet little is known at which stage of the decision process different motives interact, and how multi-motive interactions affect neural decision circuitries. To investigate this question, we analyzed prosocial decisions that were driven by either empathy (sharing the emotions of the other) or reciprocity (wish to return a favor), or a combination of empathy and reciprocity.

While undergoing functional magnetic resonance imaging (fMRI), participants performed a social decisions task in which they allocated points in favor of another person (prosocial decision), or in favor of themselves (egoistic decision). Before the social decision task, the empathy and the reciprocity motives were activated separately (single motive condition) or simultaneously (multi-motive condition).

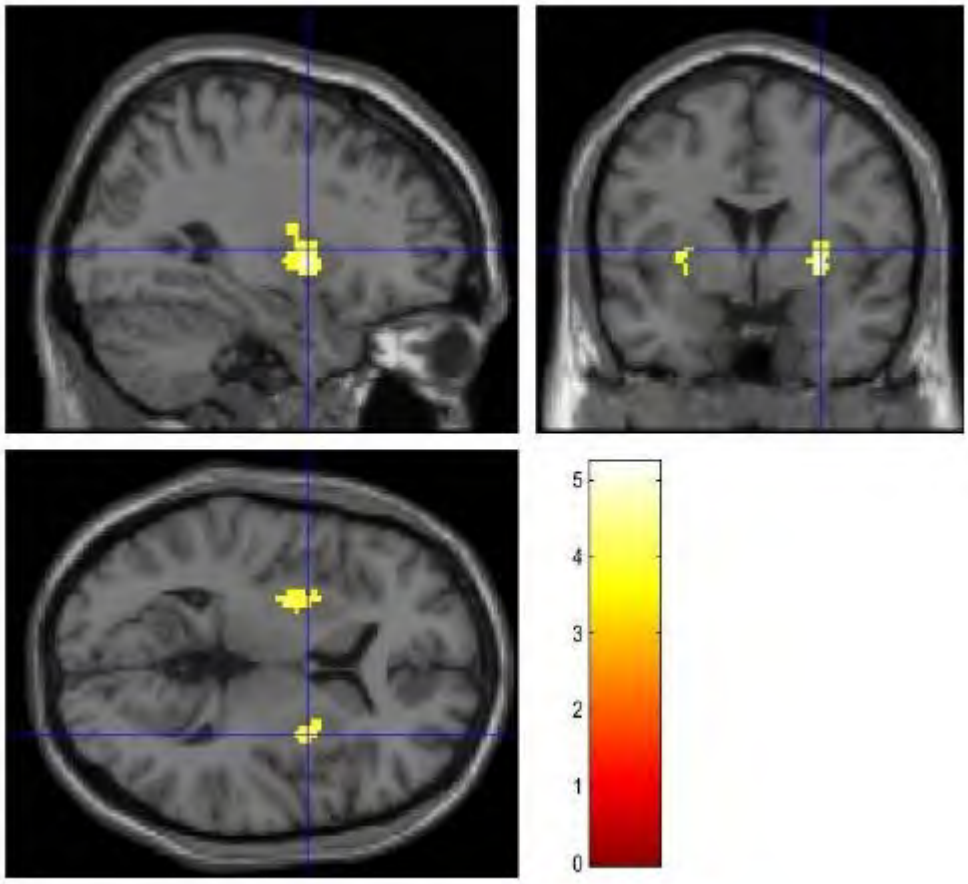
In line with previous findings, results yielded an increased frequency of prosocial decisions for the motive conditions compared to a baseline condition (no motive explicitly activated). Moreover, the combination of empathy and reciprocity resulted in significantly more prosocial decisions than reciprocity alone. These results indicate that the two motives interact, and that empathy enhances reciprocity.

To further investigate the mechanism underlying this enhancement, we conducted hierarchical drift diffusion modelling (HDDM). HDDM characterizes the decision process as a sequential sampling process in which information toward the response options is accumulated over time and a decision is made when a certain information threshold is reached. Based on this model, we assessed whether the single motives and their combination affect A) participants' a priori tendency to make a prosocial decision (z), or B) the speed of information uptake during the decision process (v).

Our results revealed significantly larger z parameters in the multi-motive compared to the reciprocity condition. This indicates that the simultaneous activation of empathy and reciprocity increases participants' a priori tendency to behave prosocially.

To investigate the neural circuitries that underly this increase, we contrasted neural activation during prosocial decisions in the multi-motive condition with activation in the reciprocity condition, and correlated this contrast with the individual increase in the z parameter. The results revealed a bilateral activation of the dorsal striatum (FWE cluster-level corrected $ps < 0.018$), i.e., a region that is known to be involved in reward-based decision making. The parameter v mirrored a generally increased speed of information uptake for the motive conditions in comparison to the baseline condition.

In sum, we show that prosocial motives and their combination differentially alter the initial tendency to behave in a prosocial manner, and that this shift in prosocial tendencies is tracked by striatal activation.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The role of differential sensory input and attributional biases in social effort perception.

Caedyn Lachlan Stinson, Julia M. Rodriguez Buritica[1], Igor Kagan [2,3], Arezoo Pooresmaeili [3,4]

[1]Freie Universität Berlin, Biological Psychology and Cognitive Neuroscience, Berlin, Germany
[2]Decision and Awareness Group, Cognitive Neuroscience Laboratory, German Primate Center (DPZ), Göttingen, Germany
[3]Leibniz ScienceCampus "Primate Cognition", Göttingen, Germany
[4]Perception and Cognition Group, European Neuroscience Institute, University Medical Center Göttingen, Göttingen, Germany

Our ability to perceive effort for both ourselves and others comprises a key social tool. Effort is a pervasive discounting factor in our decision-making processes, and an imbalance in effort distribution within a social group can lead to negative ramifications. However, our judgements of effort for ourselves and others can be subject to bias.

One proposed source of this bias is the inherent inequality in sensory input when estimating self and other effortful tasks, where sensory feedback is only received on tasks we perform ourselves. Self-serving biases also derive from attributional differences, where individuals categorically interpret their own experience differently to how they interpret others. While both sources of bias are well documented, it is unclear whether differential attribution is driven by the absence of direct sensory information when observing another's effort, or whether availability and attribution operate independently.

Our study investigates the role sensory availability and differential attribution have in biases when estimating the efforts of self and others. Specifically, we aim to investigate whether the bias resulting from being active or observing is differentiable and separate from the bias resulting from the designation of a task as self or other.

In a behavioral pilot experiment (n=5), participants performed an effortful task, where alternating left and right keyboard presses move a ball up a digital ramp against a simulated gravity of varying difficulty. The experiment comprised of three conditions: 1. Where participants physically perform the task (self+active) 2. Where participants observe their own pre-recorded tasks (self+observe) 3. Where participants observe their partners pre-recorded tasks (other+observe). Trials were presented as two interval forced choices (2IFC), where participants are instructed to identify the easier of the two trials. To observe bias, participants performed 2IFCs comparing tasks from different conditions. To measure accuracy, participants performed 2IFCs comparing trials from the same condition.

Our preliminary results are based on the comparison of point of subjective equality (PSE) across combinations of the three conditions. The PSE for self+active v other+observe 2IFCs, indicative of participant's overall bias, showed significant bias, although interestingly in a direction that underestimated self+active difficulty. The bias stemming from sensory availability and attribution was then dissociated by comparing this value for overall bias with the PSE for self+active v self+observe and self+active v self+observe respectively. This analysis suggested that greater sensory availability associated with the active condition resulted in participant's underrating task difficulty, while attribution of trials for self led to overrating. These results suggest the two biases are both present and dissociable. Accuracy of each condition was measured by calculating threshold values from the within condition 2IFCs, and indicate a significantly greater accuracy for active trials over observe trials. This project is currently ongoing, and the final results will be presented at the conference.

Symposium

S27: Neurodegenerative diseases: shaping neuronal circuits by membrane trafficking

S27-1 Loss of functional huntingtin causes activity-dependent presynaptic defects in Huntington's disease
Michael Alan Cousin, Robyn McAdam, Sarah Gordon, Andrew Morton, Elizabeth Davenport, Julia Alterman, Anastasia Khvorova, Karen Smillie

S27-2 One-way ticket for a ride: how endocytic proteins prevent neurodegeneration in the brain
Natalia Kononenko, Sujoy Bera, Albert Negrete, Soraia Martins, James Adjaye, Elena Calleja Barca, Julia Racho, Christoph Wittich, Nina Ellrich

S27-3 Endocytosis and autophagy dysfunction in neurodegeneration
Ira Milosevic, Christine Rostosky, Amandeep Arora, Sindhuja Gowrisankaran, Nuno Raimundo

S27-4 Protective modifiers unveiled impaired endocytosis in Spinal Muscular Atrophy and opened new therapeutic options
Brunhilde Wirth

S27-5 Decreased filopodial dynamics at autophagy-deficient photoreceptor axon terminals lead to ectopic synapse formation and neuronal miswiring
Ferdi Ridvan Kiral, Gerit Arne Linneweber, Bassem Hassan, Peter Robin Hiesinger

Loss of functional huntingtin causes activity-dependent presynaptic defects in Huntington's disease

Michael Alan Cousin[1], Robyn McAdam[1], Sarah Gordon[1], Andrew Morton[1], Elizabeth Davenport[1], Julia Alterman[2], Anastasia Khvorova[2], Karen Smillie[1]

[1]University of Edinbrugh, Centre for Discovery Brain Sciences, George Square, EH8 9XD Edinburgh, United Kingdom [2]RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, Massachusetts, USA

Huntington's disease (HD) is caused by CAG repeat expansion within the HTT gene, with synaptic atrophy prevalent in striatal medium spiny neurons. Since medium spiny neurons are highly active, we hypothesised this vulnerability originates from an inability to sustain presynaptic performance during intense neuronal activity. Primary cultures of either hippocampal or striatal neurons were prepared from either wild-type mice or a knock-in HD mouse model which contains 140 poly-glutamine repeats in the huntingtin protein ($htt^{Q140/Q140}$). Two distinct signatures of presynaptic dysfunction were discovered in $htt^{Q140/Q140}$neurons, both of which were only revealed during elevated neuronal activity. First, the number of nerve terminals in which activity-dependent bulk endocytosis was triggered was increased in both hippocampal and striatal $htt^{Q140/Q140}$ cultures. Second, the rate of clathrin-mediated endocytosis was retarded only in $htt^{Q140/Q140}$ striatal neurons. Both aspects of dysfunction occurred in neurons that were heterozygous for the mutant HTT allele, reflecting the prevalent genetics in humans. Depletion of endogenous huntingtin recapitulated both of these defects in wild-type neurons, whereas depletion of mutant huntingtin had no effect in $htt^{Q140/Q140}$ neurons. Importantly, both disease signatures were corrected by overexpression of wild-type huntingtin in homozygous $htt^{Q140/Q140}$ neurons. Therefore we have identified two activity-dependent signatures of presynaptic dysfunction in neurons derived from pre-symptomatic HD mice which are due to loss of wild-type huntingtin function. This suggests that an intrinsic presynaptic susceptibility of specific HD neurons to elevated neuronal activity may render them vulnerable to physiological firing patterns, potentially resulting in synapse failure and degeneration in later life.

One-way ticket for a ride: how endocytic proteins prevent neurodegeneration in the brain

Natalia Kononenko[1], Sujoy Bera[1], Albert Negrete[1], Soraia Martins[2], James Adjaye[2], Elena Calleja Barca[1], Julia Racho[1], Christoph Wittich[1], Nina Ellrich[1]

[1]University of Cologne, CECAD, Joseph-Stelzmann str. 26, 50931 Cologne, Germany

[2]Institute for Stem Cell Research and Regenerative Medicine, Medical Faculty, Heinrich Heine University

Cleavage of amyloid precursor protein (APP) by BACE-1 (beta-site APP cleaving enzyme-1) is the rate-limiting step in amyloid-beta (Abeta) production and a neuropathological hallmark of Alzheimer's disease (AD). Despite decades of research, molecular and cellular mechanisms of BACE1 trafficking and APP cleavage remain highly controversial. Here we show that in neurons amyloidogenic processing of APP is controlled by the adaptor protein complex-2 (AP-2), a heterotetramer previously thought to function exclusively in clathrin-mediated endocytosis. AP-2 prevents amyloidogenesis via endocytosis-independent regulation of BACE1 intracellular trafficking. AP-2 is decreased in iPSCs-derived neurons from patients with late-onset AD, while conditional neuronal-confined AP-2 knock-out (KO) mice suffer from increased Abeta generation, resulting from intracellular accumulation of BACE1 within the late endosomes and autophagosomes. Deletion of BACE1 decreases amyloidogenic processing of APP and mitigates synapse loss in neurons lacking AP-2. Taken together, these data suggest a mechanism for BACE1 intracellular trafficking and degradation via an endocytosis-independent function of AP-2 and reveal a crucial role of endocytic proteins in the prevention of AD.

Endocytosis and autophagy dysfunction in neurodegeneration

Ira Milosevic[1,2], Christine Rostosky[1], Amandeep Arora[1], Sindhuja Gowrisankaran[1], Nuno Raimundo[3]

[1]European Neuroscience Institute (ENI), Synaptic Vesicle Recycling, Grisebachstr 5, 37077 Göttingen, Germany

[2]UniverstitätsMedizin, Robert-Koch-Str 40, 37075 Göttigen, Germany

[3]UniverstitätsMedizin, Zellbiochemie, Humboldtalle 23, 37073 Göttingen, Germany

Neurodegenerative diseases impose a significant burden on patients, families and society, and will become of greater concern as life expectancy increases, and the world population continues to age. These disorders are characterized by the accumulation of aggregated proteins, and by a series of cellular pathologies including altered proteostasis, protein degradation and trafficking defects. Nevertheless, the precise molecular underpinnings of these disorders are still elusive. Defective endocytic and protein degradation pathways have been linked to disorders such as Parkinson's disease, Alzheimer's disease, ataxias, etc.. However, the connection between impaired endocytosis/protein homeostasis and the pathological characteristics of these diseases is not well understood. Conflicting studies suggest that these defects can be either cause, or consequence of the neurodegenerative process.
We use cellular and animal models of defective endocytosis and autophagy to identify early signs and mechanisms of neurodegeneration. We have previously reported that endophilin deficiency induces the Foxo3a-Fbxo32 network in the brain and causes dysregulation of autophagy and the ubiquitin-proteasome system (UPS). We have now identified new players in this network, and found out that the interplay between Foxo1-Foxo3a/Fbxo32-endophilinA-CtBP1 proteins can link neurotransmission and synaptic vesicle recycling to protein homeostasis (through UPS and autophagy), neuronal degeneration and survival. Interestingly, poor motor coordination and neurodegenerative defects observed in the mice with altered endophilin function could be ameliorated by altering Fbxo32 protein levels, showing that this network is also important on the organismal level. Our data further show that the manipulation of this signalling network can be a target for drugs that would slow down neurodegenerative changes.

Protective modifiers unveiled impaired endocytosis in Spinal Muscular Atrophy and opened new therapeutic options

Brunhilde Wirth[1]

[1]Institute of Human Genetics, University of Cologne, Kerpener Str 34, 50931 Cologne, Germany

Spinal muscular atrophy (SMA), a devastating neuromuscular disorder, affects around 1:6000 people, every 1:35 is carrier in Europe and it is the most frequent genetic cause of infant death. Recently, the first SMA therapy based on antisense oligonucleotides, namely Nusinersen, has been FDA- and EMA-approved. Nusinersen restores the suboptimal full-length SMN2 transcript expression and elevates SMN protein level. SMN is crucial for all cells but particularly for motor neurons and neuromuscular junctions. In the most severe type I form, which accounts for ~60% of SMA patients, who usually carry only two SMN2 copies, the elevated SMN level may be still insufficient to restore motor neuron function lifelong. We show that genetic SMA protective modifiers might provide additional independent functional support for motor neuron function.

Here I will talk about two SMA protective modifier, identified in asymptomatic SMN1-deleted individuals carrying either 3 or 4 SMN2 copies. Plastin 3 (PLS3), an F-actin binding and bundling protein, which rescues SMA by overexpression and Neurocalcin delta (NCALD), a neuronal calcium sensor protein, which counteracts SMA by suppression. We found that both, PLS3 overexpression or NCALD suppression protect against SMA across species including zebrafish and mice. Moreover, both modifiers show a rescuing effect using combinatorial therapies – low dose Nusinersen and PLS3 overexpression or NCALD suppression - in severely-affected SMA mice. Lastly, both modifiers hinted us towards the main cellular mechanism in SMA, which we believe is impaired endocytosis, and which is restored by both modifiers. Recently, we identified a third protective modifier, calcineurin EF-hand protein 1 (CHP1), that interacts with PLS3 but is a calcium sensor like NCALD and protects SMA by downregulation. Most importantly, CHP1 reduction restores impaired endocytosis in SMA, by inhibiting calcineurin an important phosphatase that dephosphorylates all major proteins involved in endocytosis.

These three protective SMA modifiers not only unveiled the most likely disturbed pathway in SMA but also opened new avenues for therapy.

Decreased filopodial dynamics at autophagy-deficient photoreceptor axon terminals lead to ectopic synapse formation and neuronal miswiring

Ferdi Ridvan Kiral, Gerit Arne Linneweber[1,2], Bassem Hassan[1,2], Peter Robin Hiesinger[1]

[1]Freie Universität Berlin, Department of Neurobiology, Königin-Luise-Str. 1-3, 14195 Berlin, Germany

[2]Institut du Cerveau et de la Moelle Epinière (ICM) – Hôpital Pitié-Salpêtrière, Sorbonne Université, Inserm, CNRS, Paris, France

Neuronal circuit assembly requires synapse formation between correct pre- and post-synaptic partners. Autophagy is a major degradation mechanism with specific roles in neurons, including presynaptic function[1]. However, neurons lacking autophagy develop relatively normal and little is known about potential roles of autophagy in neural circuit assembly. Here we show that loss of autophagy in Drosophila R7 photoreceptors leads to increased synapse formation with aberrant synaptic partners. While overall R7 terminal structure and function appear largely normal, axon terminals develop an increased number of stable, ectopic active zones in layers where R7 neurons do not normally form synapses. Using the recently developed 'trans-tango' technique to label postsynaptically connected neurons[2], we found that autophagy-deficient R7s indeed form connections with incorrect interneurons that specifically have dendrites in layers with ectopic R7 active zones. Autophagy-dependent neuronal miswiring in the visual system further leads to increased visual attention as a behavioral output. To identify the primary role of autophagy in the formation of correct and ectopic synapses, we performed live imaging of filopodial dynamics [3] and autophagic cargo degradation in R7 axon terminals during synapse formation [4]. Loss of autophagy leads to increased stability of filopodia. Autophagosomes are localized at the tips of such filopodia and their dynamics are correlated with filopodial retraction. Live and fixed analyses of axon terminals further suggest selective degradation of early, but not late, presynaptic active zone assembly factors in the filopodial tips. We conclude that a lack of local autophagy-dependent degradation of nascent synaptic building material leads to increased filopodial stability and aberrant synapse formation. These findings support a model whereby the presynaptic terminals have the capacity to form synapses with incorrect partners, but are normally prevented from doing so by fast kinetics of filopodia and synaptic building material as a limited resource.

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Symposium

S28: Modulatory circuits of central pain processing

S28-1 Oxytocin Acts on Astrocytes in the Central Amygdala to Promote Comfort
Alexandre Charlet, Damien Kerspern, Jérôme Wahis, Ferdinand Althammer, Stéphanie Goyon, Daisuke Hagiwara, Ron Stoop, Pierrick Poisbeau, Valery Grinevich

S28-2 Brain rhythms of pain
Markus Ploner

S28-3 Somatosensory modulation of oxytocin neurons drives social communication
Valery Grinevich

S28-4 Cortical control of thalamic pain processing
Alexander Groh, Sailaja Goda, Emilio Isaías-Camacho, Sanjeev Kaushalya, Rohini Kuner, Thomas Kuner, Rebecca Mease

S28-5 Expression profile of tight junction proteins in a model of diabetic neuropathy
Carla Norwig, Reine-Solange Sauer, Adel Ben-Kraiem, Robert Blum, Heike L. Rittner

S28-6 The cellular basis of volumetric brain changes during chronic pain –
a novel approach to correlate voxel-based morphometry with *in vivo* microscopy.
Livia Asan, Wolfgang Weber-Fahr, Claudia Falfán-Melgoza, Carlo Antonio Beretta, Rainer Spanagel, Thomas Kuner, Johannes Knabbe

Oxytocin Acts on Astrocytes in the Central Amygdala to Promote Comfort

Alexandre Charlet[1], Damien Kerspern[1], Jérôme Wahis[1], Ferdinand Althammer[2], Stéphanie Goyon[1], Daisuke Hagiwara[2], Ron Stoop[3], Pierrick Poisbeau[1], Valery Grinevich[2]

[1]CNRS, UPR3212 INCI, 5 rue Blaise Pascal, 67000 Strasbourg, France

[2]Schaller Research Group on Neuropeptides at German Cancer Research Center (DKFZ) and CellNetwork Cluster of Excellence at the University of Heidelberg, Heidelberg, Germany

[3]Center for Psychiatric Neurosciences, Hôpital de Cery, Lausanne University Hospital (CHUV), Lausanne, Switzerland

Oxytocin orchestrates social and emotional behaviors through modulation of neural circuits in brain structures such as the central amygdala (CeA). The long-standing dogma is that oxytocin signaling in the central nervous system occurs exclusively via direct actions on neurons. However, several findings over the last decades showed that astrocytes can actively participate in the modulation of neuronal networks. Here, we investigate the degree of astrocytes' involvement in oxytocin functions. Using a multidisciplinary approach we show that CeA astrocytes not only respond to oxytocin, but are actually necessary to its effects on neuronal networks. Remarkably, optogenetic stimulation of CeA astrocytes induced both anxiolysis and place preference behaviors, depicting their active involvement as a cellular substrate underlying the promotion of comfort. These results prove that astrocytes are key regulators of neuronal circuits by responding to specific inputs, and opens up new perspectives to understand how neuromodulators, such as oxytocin, gate brain function.

Brain rhythms of pain

Markus Ploner[1]

[1]Technical University of Munich, Department of Neurology, Ismaninger Str. 22, 81675 Munich, Germany

The presentation will provide an overview of how neuronal oscillations and synchrony subserve the experience of pain. I will particularly summarize findings from electroencephalography studies in humans, which have investigated how oscillations at different frequencies and locations serve experimental and clinical pain on different timescales. I will discuss these findings in light of their implications for the diagnosis and therapy of chronic pain.

Somatosensory modulation of oxytocin neurons drives social communication

Valery Grinevich[1]

[1]DKFZ (Deutsches Krebsforschungszentrum), Neuropeptides, Im Neuenheimer Feld 581, 69120 Heidelberg, Germany

The hypothalamic neuropeptide oxytocin (OT) promotes social communication via its central release in the mammalian brain. However, how social interaction affects electrical activity of OT neurons remains unknown. To address this question, we used cell-type specific viral vectors in combination with optoelectrode-based techniques. We performed the in vivo single-unit recording of optogenetically identified OT neurons in the paraventricular nucleus (PVN) of adult female rats during their social interactions with unfamiliar female conspecifics. Simultaneously, we monitored behavior and recorded ultrasonic vocalizations. Our results showed that active social interaction induced an increase in PVN theta rhythmicity, as well as in the firing rate of individual PVN OT neurons. The spikes of simultaneously recorded OT neurons were synchronized and phase-locked with the PVN theta rhythm precisely at the time of social interactions, but not during non-social exploratory behavior. To decipher which sensory stimuli trigger OT neuron activity, we performed experiments with total or partial derivation of socially-relevant visual, olfactory and somatosensory signals. We found that direct physical contact between rats, or even gentle skin stimulation, led to a profound increase in OT firing rates. In contrast, visual, auditory and olfactory signals did not significantly alter OT neuron activity. Given that OT system is composed by magno- and parvocellular OT neurons and the latter are critically involved in nociception (Eliava et al., Neuron, 2016), next we explored the role of parvocellular OT neurons during social interactions. Based on the ex vivo electrophysiological results that parvocellular OT neurons terminate on magnocellular OT neurons within the PVN, we manipulated them and subsequently monitored social behavior. We found that chemogenetic silencing of parvocellular OT neurons reduces the time spent for social interaction, suggesting that this type of neurons represents “master cells” driving the activation of entire OT system during social behavior. Altogether, our results indicate that non-nociceptive stimulation is essential to activate OT neuron ensembles and, hence, can induce central neuropeptide release in socially interacting female rats. This opens perspectives for studying functional and anatomical connectivity between the somatosensory and OT systems in normal and psychopathological conditions.

Cortical control of thalamic pain processing

Alexander Groh[1], Sailaja Goda [1], Emilio Isaías-Camacho [1], Sanjeev Kaushalya[2], Rohini Kuner[2], Thomas Kuner[3], Rebecca Mease[1]

[1]Institute of Physiology and Pathophysiology, INF 307
[2]Institute of Pharmacology, INF 366
[3]Institute of Anatomy, INF 307
[4]Heidelberg University, 69120 Heidelberg, Germany

Pain is a complex sensory and emotional experience that arises from neuronal interactions between the thalamus and the cortex. The activity patterns of thalamocortical neurons that lead to the experience of pain are largely unknown. Furthermore, corticothalamic pathways are known to regulate these thalamic activity patterns, however the role of this massive feedback system has not been studied in the context of pain. In mouse models of acute and inflammatory pain we investigated (1.) the representation of pain in the spiking activity in the ventral posterior lateral thalamus (VPL) and (2.) the effect of corticothalamic feedback from cortical layer 6 (L6) on pain responses in VPL. We found that in response to noxious stimulation of the hind paw, VPL spiking activity is enhanced in a subset of neurons and these pain responses in VPL are suppressed by optogenetic activation of cortical L6. We are currently testing the hypothesis that cortical suppression of thalamic pain responses is mediated by di-synaptic feed-forward inhibition via the thalamic reticular nucleus.

Expression profile of tight junction proteins in a model of diabetic neuropathy

Carla Norwig, Reine-Solange Sauer[1], Adel Ben-Kraiem[1], Robert Blum[2], Heike L. Rittner[1]

[1]Department of Anaesthesiology and Critical Care, University Hospital of Würzburg, Oberdürrbacher Str. 6, 97080 Würzburg, Germany [2]Institute of Clinical Neurobiology, University Hospital of Würzburg, Versbacherstr. 5, 97078 Würzburg Würzburg, Germany

Neuropathic pain (NP) affects up to 21% of patients with diabetic neuropathy. Although several pathophysiological mechanisms (e.g. modification of sodium channels by glucose metabolite methylglyoxal [1]) have been proposed, many questions regarding the development of NP in diabetic neuropathy remain unanswered. Microangiopathic changes including basement membrane thickening and endothelial dysfunction are present in diabetic neuropathy, but their relevance has been discussed controversially.

NP in traumatic models such as partial sciatic nerve injury is associated with an opening of the blood nerve barrier (BNB) [2] and also with an opening of the blood spinal cord barrier in corresponding segments [3]. The BNB consists of the perineurium surrounding the peripheral nerve and the endoneurial vessels. An intact BNB protects nerves from toxins and creates an immune-privileged space inside the endoneurial space. Build up by tight junction complexes sealing endoneurial endothelial and perineurial cells, the BNB's most important components are tight junction proteins such as claudin (cldn)1, cldn5, cldn12, cldn19, tricellulin, junctional adhesion molecule (JAM) C, tricellulin, occludin and the tight-junction associated protein zona occludens (ZO) 1. Barrier function in diabetes is impaired as seen in an altered morphology of tight junctions in the BNB in freeze-fracture sections of human sural nerve biopsies [4] as well as a breakdown of the retinal barrier [5]. In this study, we hypothesize that painful diabetic neuropathy is accompanied by an opening of the BNB caused by downregulation of one or more tight junction proteins.

All animal experiments were approved by the Regierung von Unterfranken and were conducted according to ARRIVE guidelines. Diabetes was induced by intravenous injection of 40 mg streptozocin (STZ) to Wistar rats. Pain behavior (mechanical allodynia and thermal hypersensitivity) was tested with the von-Frey test and Hargreaves test, respectively. To analyze sealing properties of the BNB, intravenous injection of a large molecular tracer dye (Evans blue, 69 kDa) and a small molecular tracer dye (sodium fluorescein, 376 Da) to STZ-diabetic rats was undertaken. Cryosections of sciatic nerves were analyzed for leakage of fluorescent dyes into the endoneurial space and the presence of CD68+ macrophages. Tissue was analyzed 4 and 8 w after STZ injection for tight junction protein expression.

STZ treatment elicited high blood glucose levels, polyuria and mechanical as well as thermal hyperalgesia. Quantitative real-time PCR of whole sciatic nerve and the dorsal root ganglion showed no significant changes in the expression of the above-mentioned tight junction proteins. No macrophage infiltration was observed. However, laser-assisted microdissection of endoneurial capillaries or in perineurial cells and permeability pointed towards an altered tight junction protein mRNA expression profile.

Results of this investigation will improve our understanding of the function or dysfunction of endothelial cells in diabetic neuropathy and barrier dysfunction. Resealing of a defect BNB could be a future approach to treat painful diabetic neuropathy.

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The cellular basis of volumetric brain changes during chronic pain – a novel approach to correlate voxel-based morphometry with *in vivo* microscopy.

Livia Asan, Wolfgang Weber-Fahr[2], Claudia Falfán-Melgoza[2], Carlo Antonio Beretta[3], Rainer Spanagel[4], Thomas Kuner[1], Johannes Knabbe[1,5]

[1]Institute for Anatomy and Cell Biology, Department of Functional Neuroanatomy, Heidelberg University, Heidelberg, Germany.
[2]Central Institute of Mental Health, Research Group Translational Imaging, Heidelberg University- Medical Faculty Mannheim, Mannheim, Germany.
[3]CellNetworks & Nikon Imaging Center, Math Clinic, Heidelberg University, Heidelberg, Germany.
[4]Central Institute of Mental Health, Institute of Psychopharmacology, Heidelberg University- Medical Faculty Mannheim-Mannheim, Germany.
[5]Center for Psychosocial Medicine, Department of General Psychiatry, Heidelberg University Hospital, Heidelberg, Germany.

Our ability to experience pain relies on highly complex signal processing and integration from the periphery up to higher order brain cortices. Increasing evidence suggests that during pathologic states of chronic pain, neuronal circuits are altered on multiple levels. In addition to these observations of functional changes, numerous neuroimaging studies using **voxel-based morphometry (VBM) in MRI** also described striking **structural differences** in certain brain areas of chronic pain patients; the anterior- and midcingulate cortex (ACC and MCC) for example exhibit smaller volumes as compared to healthy controls. The mechanistic **cellular basis** of these gross volumetric brain changes, which possibly characterize a long-lasting structural consolidation of maladaptive pain processing, yet needs to be identified.

In order to back-translate human neuroimaging findings to a model organism in which we additionally are able to investigate histologic adaptation longitudinally, we studied mice before and after induction of chronic neuropathic pain by spared-nerve injury or sham surgery. Up to nine months after surgery we repeatedly measured brain morphology with VBM in MRI, whereas microscopic brain changes were captured with **two-photon *in vivo* laser scanning microscopy (2PLSM)** of large cortical volumes within the ACC and MCC.

In my talk I will present our strategy to get unbiased, comprehensive readouts of **cortical cytoarchitecture** by imaging all cell nuclei in the cortex with 2PLSM and using novel artificial intelligence tools to automatedly detect and classify cell nuclei. We monitor local microscopic tissue volume to validate VBM, and correlate VBM signal changes to cytoarchitectonic metrics such as changes in cell density, spatial organization or cell-type composition.

To understand how chronic pain develops, how it is maintained, and how we can effectively treat or prevent it, it will be crucial to link all dimensions of structure as well as function that are associated with the normal and the diseased state of this complex and interconnected sensory system of pain. Here we achieved the first steps in linking volumetric brain changes during chronic pain observed at the level of the whole brain to the cellular level.

Symposium

S29: Orexin beyond sleep

Orexin regulation of fear learning and extinction

Fernando Berrendero[1,2], Rocío Saravia[2], Marc Ten-Blanco[1], África Flores[3]

[1]Faculty of Experimental Sciences, Universidad Francisco de Vitoria,Carretera Pozuelo a Majadahonda, Km 1.800, 28223 Pozuelo de Alarcón, Madrid [2]Neuropharmacology laboratory, Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona Biomedical Research Park, 08003 Barcelona[3]Institute of Neuroscience, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona

An understanding of the neurobiological mechanisms involved in the regulation of fear is essential for the development of new treatments for anxiety disorders, such as phobias, panic disorder, and posttraumatic stress disorder. Orexins, also known as hypocretins, are neuropeptides located exclusively in hypothalamic neurons initially involved in the regulation of feeding behaviour. Orexins have emerged as a key regulator of aversive memory, and several recent reports support their role in this process at diverse levels. Thus, orexin receptor-1 (OX1R) signaling promotes the expression of learned fear by enhancing both the acquisition and consolidation phases of fear memory formation, a process in which orexins may modulate noradrenergic activity from the locus coeruleus. On the other hand, orexins also delay the ability to extinguish these fear memories through OX1R transmission. In this case, orexins might be regulating the communication between the basolateral amygdala and the medial prefrontal cortex. However, our knowledge about how the orexinergic system modulates emotional behaviour is scarce and further research is needed. Importantly, human studies have reported that orexin dysfunction causes deficits in aversive learning, and increased activity of the orexin system is associated with acute anxiety states. These findings underline the potential of orexin receptor antagonists as promising opportunities for treating diseases characterized by abnormal fear persistence.

Role of orexin deficiency in panic-like anxiety

Nadine Faesel[1,2], Michael Koch[2], Markus Fendt[1,3]

[1]Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Germany
[2]Department of Neuropharmacology, Brain Research Institute, University of Bremen, Germany
[3]Center for Behavioral Brain Sciences, Otto-von-Guericke University Magdeburg, Germany

The neuropeptide orexin is known for its crucial role in regulating the sleep/wake cycle and feeding behavior, but it is also important for a variety of other processes. Here, we focus on the involvement of orexin in panic-like anxiety. For that, wild-type and orexin-deficient mice received intracerebroventricular injections of the panicogenic substance cholecystokinin-4 (CCK-4) prior to testing them in the elevated plus maze. In wild-type mice, CCK-4 injections induced an increase of anxiety behavior in the elevated plus maze and of plasma corticosterone levels. These behavioral effects of CCK-4 injections were absent in female orexin-deficient mice, whereas male orexin-deficient mice still appeared to respond to this panicogenic treatment. Currently, we analyze CCK-4 induced c-Fos immunoreactivity as a marker of neural activity with the aim to elucidate the neural pathways involved in panic-like anxiety. Taken together, our data indicate an important and gender-dependent role of orexin in panic-like anxiety, thus emphasizing orexin and its receptors as potential targets for future pharmacological therapies of human panic disorders.

Role of orexin in cognitive flexibility

Archana Durairaja, Markus Fendt[1,2]

[1]Otto von Guericke university, Institute for pharmacology and Toxicology, Leipziger strasse 44, 39120 Magdeburg, Germany
[2]Otto von Guericke university, Center for Behavioural Brain Sciences, Magdeburg, Germany

Neuropsychiatric disorders are often associated with cognitive impairments, especially deficits in cognitive flexibility. Cognitive flexibility is the ability to switch behavioural responses by inhibiting previously learned rules and/or adapting these rules to new situations. A well established paradigm to investigate cognitive flexibility in mice is the Attentional Set Shifting Task (ASST). In the ASST, mice have to learn compound discrimination to find a reward. Then, cognitive flexibility is tested by challenging the mice to intradimensional shifts (IDS) and extradimensional shifts (EDS) within and between perceptual dimensions, as well as to reversal learning tasks. Frontal cortex regulates cognitive flexibility and it has been demonstrated that different subareas of the frontal cortex are involved for the different shifts and reversals of the ASST. Although several neurotransmitter systems are suggested to be involved, the neuropharmacological mechanisms regulating these cognitive processes are not yet clear. We investigated the role of orexin neuropeptides in regulating cognitive flexibility. Orexins are mainly known to regulate sleep/wake cycle, reward seeking behaviour, feeding behaviour and learning processes. It has been previously shown that blocking orexin receptors in basal forebrain impair reversal learning performance whereas orexin administration in this area improved learning performance suggesting an important role of the orexins in regulating cognitive flexibility, possibly by attenuating cholinergic signaling from basal forebrain to prefrontal cortex. In the present study, we investigated orexin-deficient mice in the ASST to investigate the role of orexin in the different phases of ASST that require cognitive flexibility. Our results clearly indicate deficits in intradimentional set shifting in orexin knock out mice. We hypothesize that orexinergic projections into frontal and/or anterior cingulate cortex mediate the deficits. This hypothesis is currently tested by local injections of orexin receptor antagonists.

Neurochemical investigation of impulse control in a rat model of binge eating disorder

Julia Sabine Schuller, Michael Koch[1]

[1]University Bremen, Dept. of Neuropharmacology, Center for Cognitive Sciences, 330440, 28334 Bremen, Germany

Binge eating disorder (BED) is an eating disorder based on deficient impulse control. Besides eating binges, the hedonic properties of food are reduced.

Aim of the current study is to investigate the neuronal basis of BED using an animal model of the disorder, and to find possible correlations to impulsivity in rats. Basis of the experimental set-up is the hypothesis that eating disorders including BED result from disturbances in neural circuits underlying impulse control and reward processing.

The involvement of the nucleus accumbens (NAc) in impulse control and in pathological feeding behavior have been studied extensively both in animals and humans. However, the neurochemical basis of BED needs to be further elucidated.
Several neuropeptides (e.g. the group of the orexins or cocaine- and amphetamine-regulated transcript (CART)) and neurotransmitters (e.g. dopamine or γ-aminobutyric acid (GABA)) which are involved in the natural feeding behavior are candidates to play an important role in BED.

We here investigate the role of two neuropeptides in impulse control related to BED. For this purpose, rats are screened for impulsivity using a simple impulse control paradigm (5-choice serial reaction time task) and subsequently tested in a rat model paradigm for BED.
Microinfusion into the NAc shell of an orexin 1-receptor antagonist and a CART-antibody are performed to reveal their possible roles in BED.

Hypothalamic network oscillations and regulation of feeding behaviour

Marta Carus-Cadavieco[1], Maria Gorbati[2], Li Ye[3,4], Franziska Bender[2], Suzanne van der Veldt[2], Christin Kosse[5], Soo Yeun Lee[3], Charu Ramakrishnan[3], Yubin Hu[2], Natalia Denisova[2], Franziska Ramm[2], Denis Burdakov[5], Karl Deisseroth[3,4,6], Tatiana Korotkova[1], Alexey Ponomarenko[7]

[1]Max Planck Institute for Metabolism Research, Neuronal Circuits and Behavior Group, Gleueler Str. 50, 50931 Köln, Germany

[2]Behavioural Neurodynamics Group, Leibniz Institute for Molecular Pharmacology (FMP)/ NeuroCure Cluster of Excellence, Berlin, Germany

[3]Department of Bioengineering, Stanford University, Stanford, CA 94305, USA

[4]Howard Hughes Medical Institute; Stanford University, Stanford, CA 94305, USA

[5]Department of Health Sciences and Technology; ETH Zürich, Switzerland

[6]Department of Psychiatry and Behavioral Sciences, W080 Clark Center, 318 Campus Drive West, Stanford University, Stanford, CA 94305, USA

[7]Institut of Clinical Neuroscience and Medical Psychology, Heinrich Heine University,40225, Düsseldorf

Feeding is largely regulated by hypothalamic neuronal circuits, yet little is known about neuronal coding of different feeding-related behaviours by Vgat, MCH, orexin and other cell types in the lateral hypothalamus (LH) and its extrahypothalamic inputs. Top-down forebrain innervation of LH is provided, to a large extent, by inhibitory inputs from the lateral septum (LS), a key region for governing innate behaviors according to environmental context, connected, in turn, with cortical networks. How do forebrain regions influence activity of LH neurons remains elusive. To investigate coordination between LS and LH, we combined optogenetics with electrophysiological recordings in behaving mice during spontaneous behavior in the free-access feeding paradigm. We found that coordinated gamma (30-90 Hz) oscillations in the LH and upstream brain regions organize food-seeking behavior. When mice engaged in approach to the food zone, the power of gamma oscillations in LS and LH matched the time required to reach the food zone. Gamma-rhythmic input to LH from somatostatin-positive LS cells evoked food approach without affecting food intake. It also increased probability of entering the food zone prior to food-free zones, located in other corners of the enclosure. Identified Vgat LH cells responded to intracellular injections of gamma oscillatory currents in brain slices with higher magnitude of membrane potential oscillations than and melanin-concentrating hormone (MCH). Optogenetically tagged presumed LH Vgat cells in behaving mice also showed rhythmic gamma-frequency activity. The gamma-rhythmic input enabled fine-time scale separation of LH cells, in particular Vgat neurons, according to their feeding-related activity: a subset of LH neurons, active at the food location ("food-match" cells), prominently reduced their firing during the gamma oscillation trough. In contrast, LH cells, which were preferentially active distantly from the food zone ("food-mismatch" cells), showed high excitability at the gamma trough. Using CLARITY, in vivo electrophysiology and computational modeling, we identified medial prefrontal cortex projections providing gamma-rhythmic inputs to LS, leading to improved performance in a food-rewarded learning task. Overall, our study identifies a novel top-down pathway, which utilizes gamma synchronization to guide activity of LH neurons and to regulate feeding behavior by dynamic reorganization of functional cell groups in hypothalamus.

Symposium

S30: Inhibitory synapse diversity in health and disease

S30-1 Neuronal $GABA_A$ receptor trafficking and turnover underlying synaptic transmission and cognitive function
Matthias Kneussel, Frank F Heisler, Torben J Hausrat, Mary Muhia

S30-2 Altered prefrontal pyramidal-GABAergic interneuron circuit architecture in a genetic mouse model of psychiatric illness.
Jonas-Frederic Sauer, Marlene Bartos

S30-3 Amygdala intercalated neurons form an interconnected and functionally heterogeneous network
Martin Zeller

S30-4 Proteo-connectomics to Discover Novel Mechanisms of Inhibition In Vivo
Scott Haydn Soderling

S30-5 The cell adhesion molecule IgSF9b regulates inhibitory synapse function in the amygdala anxiety circuitry
Dilja Krueger-Burg, Olga Babaev, Hugo Cruces-Solis, Nils Brose

Neuronal $GABA_A$ receptor trafficking and turnover underlying synaptic transmission and cognitive function

Matthias Kneussel[1], Frank F Heisler[1], Torben J Hausrat[1], Mary Muhia[1]

[1]University Medical Center Hamburg Eppendorf, ZMNH, Molecular Neurogenetics, Falkenried 94, 20251 Hamburg, Germany

Controlling the number of $GABA_A$ receptors ($GABA_A$Rs) is critical for the regulation of synaptic transmission and plasticity at central inhibitory synapses. $GABA_A$Rs are dynamically exchanged at postsynaptic sites, depending on intracellular transport mechanisms and cell surface diffusion events. While the factors that traffic $GABA_A$Rs remain incompletely understood, we characterized two $GABA_A$R-binding proteins that participate in synaptic receptor turnover. The ERM protein radixin provides an extrasynaptic anchor for alpha-5 subunit-containing plasma membrane $GABA_A$Rs. Depending on an activity-dependent phosphorylation switch, these extrasynaptic clusters release surface $GABA_A$Rs for synaptic integration, thereby affecting hippocampal-dependent learning and memory. In contrast, muskelin interacts with internalized $GABA_A$Rs at intracellular vesicles and transports $GABA_A$Rs via myosin VI and dynein-dependent mechanisms towards the lysosome. Depletion of muskelin affects learning and memory, however the trafficking factor plays additional roles in neuronal transport and neurodegeneration. The role of $GABA_A$R trafficking in the context of cytoskeleton transport and its impact to cognitive performance is subject to our investigations.

Altered prefrontal pyramidal-GABAergic interneuron circuit architecture in a genetic mouse model of psychiatric illness.

Jonas-Frederic Sauer[1], Marlene Bartos[1]

[1]University of Freiburg, Institute of Physiology I, Hermann-Herder-Straße 7, 79104 Freiburg, Germany

Psychiatric disorders greatly impact life quality of affected individuals while treatment options remain limited. Both environmental and genetic risk factors contribute to psychiatric conditions but the molecular and network defects leading to psychiatric disease states are insufficiently understood. We have investigated the prefrontal cortex network of genetic and environmental mouse models resembling key aspects of major depressive disorder (MDD). First, we analyzed mice lacking functional Disrupted-in-Schizophrenia 1 (Disc1), which resemble an ultra-rare mutation with high predisposition to MDD and schizophrenia in humans. Prefrontal glutamatergic neurons show a mild reduction in spike rate in awake Disc1-mutant mice. In contrast, putative GABAergic neurons display strongly reduced spike rates, resulting in an increased excitation/inhibition balance. To get mechanistic insight, we interrogated the prefrontal circuit architecture of Disc1-mutant mice in vivo. Using cross-correlation techniques of spike trains we find reduced connectivity and synaptic efficacy of pyramidal cell-GABAergic interneuron connections, thus revealing a causal role of Disc1 in hardwiring and maintenance of synapse function in the prefrontal cortex. Second, we investigated a stress-induced model of MDD-related behaviour induced by repeated forced-swimming. This procedure induced a lasting phenotype of chronic behavioural despair. In contrast to the Disc1-mutant model, chronically despaired mice show enhanced activity of prefrontal pyramidal neurons while GABAergic neuron firing rates appear unaffected in vivo. Our data thus suggest that genetic and environmental lesions predisposing for MDD-related behaviours result in an elevated prefrontal excitation/inhibition ratio via distinct mechanisms.

Amygdala intercalated neurons form an interconnected and functionally heterogeneous network

Martin Zeller

[1]Hertie Institute for Clinical Brain Research and Centre for Integrative Neuroscience, University of Tübingen, Tübingen, Germany

[2]Department of Neurobiology, IBBS, University of Stuttgart, Stuttgart, Germany

[3]Laboratory of Behavioral and Genomic Neuroscience, NIAAA, NIH, Bethesda, MD, USA

[4]Friedrich Miescher Institute, Basel, Switzerland

The intercalated cells (ITCs) constitute densely packed clusters of inhibitory neurons in the amygdala, a brain structure involved in emotional behaviour. In particular, the amygdala is critical for associative fear learning. It is the site of long-term storage for associative fear memories, and neural activity in the central amygdala (CeA) mediates fear responses such as freezing. In a process called fear extinction, learned fear responses can be suppressed in a context-specific manner, which requires activity in the medial prefrontal cortex (mPFC).

Within the circuitry of learned fear, the ITCs have been conceptualized in the past mainly as an inhibitory relay of the mPFC and basolateral amygdala (BLA) to CeA, making them the top-down “off-switch” for the CeA during the extinction of learned fear. Recent studies additionally suggest close reciprocal interactions between ITC clusters and functional heterogeneity between clusters, whereby the dorsomedial cluster (dmITC) is activated during fear and the ventromedial cluster (vmITC) during extinction. To explore how the wiring of the ITC circuit could support such functional heterogeneity, we expressed channelrhodopsin-2 in single ITC clusters using a Cre-dependent viral strategy, and measured optogenetically evoked postsynaptic currents in acute brain slices of mice. By combining whole-cell voltage clamp recordings with pharmacology, we provide evidence that the dmITCs and vmITCs mutually inhibit each other via $GABA_A$ receptors, which could support opposing activity patterns during different behavioural states. This circuit architecture also provides a mechanistic explanation for ITC activity patterns observed with *in vivo* calcium imaging, and for the differential effects of pharmacogenetic interventions in dmITCs and vmITCs on fear expression. To further dissect the functional basis of heterogeneity among ITCs, we have begun to investigate projection specificity of single clusters.

Our data raise the intriguing possibility that ITCs do not only serve as an inhibitory relay for top-down control of amygdala output, but also form a functionally heterogeneous and interconnected network that is likely involved in multiple emotional processes.

Proteo-connectomics to Discover Novel Mechanisms of Inhibition In Vivo

Scott Haydn Soderling[1]

[1]Duke University , Cell Biology, 320 Research Drive, 27710 Durham, USA

I will discuss work on identifying novel proteins at GABAergic synapses using in vivo proximity biotinylation and high resolution quantitative mass spectrometry. I will first present data on the diverse proteomes of synapses within multiple neuron types mediating inhibition in vivo. This will be followed by data on the synaptic and behavioral analysis of knockout mice for a newly discovered GABAergic postsynaptic protein, InSyn1.

The cell adhesion molecule IgSF9b regulates inhibitory synapse function in the amygdala anxiety circuitry

Dilja Krueger-Burg[1], Olga Babaev[1], Hugo Cruces-Solis[1], Nils Brose[1]

[1]Max Planck Institute of Experimental Medicine, Molecular Neurobiology, Hermann-Rein-Str. 3, 37075 Göttingen, Germany

Abnormalities in inhibitory synaptic transmission are intricately linked to the pathophysiology of psychiatric disorders, but the underlying mechanisms are poorly understood. While synaptic inhibition is crucial throughout the brain, a number of key nodes exist that are particularly heavily controlled by inhibitory inputs, and one of these is the centromedial amygdala (CeM). The CeM represents the major output nucleus of the amygdala complex, through which anxiety responses and other psychiatrically relevant behaviors are processed. CeM neurons receive numerous inhibitory inputs from both afferent projections and local interneurons, which play a pivotal role in gating CeM projections and hence amygdala output. Understanding the biology of these inhibitory synapses is therefore essential in evaluating their vulnerability to pathogenic mutations and their potential as therapeutic targets. In the present study, we investigate the role of two psychiatrically relevant components of the inhibitory postsynapse, the synaptic adhesion molecules Neuroligin 2 (Nlgn2) and IgSF9b, in the CeM anxiety circuitry in mice. Using WT, Nlgn2 KO, IgSF9b KO and double KO mice, we show that deletion of IgSF9b normalizes aberrant anxiety-related behaviors in the open field test and elevated plus maze in Nlgn2 KO mice. This behavioral effect was accompanied by a normalization of neuronal activation in the CeM as assessed using cFos immediate early gene assays and local field potential recordings during exposure to an open field apparatus. Using stereotaxic injection of AAV-IgSF9b-shRNA into the CeM, we show that local knockdown of IgSF9b has prominent anxiolytic consequences in Nlgn2 KO mice. To investigate the mechanism behind the anxiolytic effect of IgSF9b deletion in the CeM, we investigated inhibitory synaptic transmission and inhibitory synapse number in acute slices. We find that deletion of IgSF9b results in increased inhibitory synaptic transmission and an increase in the number of inhibitory synapses in the CeM. These findings support a model in which the anxiety-related CeM overactivation observed in Nlgn2 KO mice is counteracted by the increased inhibition resulting from additional deletion of IgSF9b. Together, our data provide the first description of IgSF9b function in vivo and uncover a novel role for IgSF9b in anxiety-related behavior and amygdala inhibitory synapses. Moreover, our findings highlight that IgSF9b-expressing neurons in the CeM may represent an important common target for anxiolytic treatments that is independent of individual upstream mutations.

Symposium

S31: The tripartite synapse in health and disease

Perisynaptic astrocyte structure dynamically shapes hippocampal glutamate signalling

Christian Henneberger[1,2,3]

[1]University of Bonn Medical School, Institute of Cellular Neurosciences, Sigmund-Freud-Str. 25, 53127 Bonn, Germany
[2]University College London, Institute of Neurology, WCN1 3BG, London, United Kingdom
[3]Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Bonn, Germany

Uptake of glutamate by perisynaptic astrocyte processes limits glutamate escape into extrasynaptic space and thus determines the spatial precision of synaptic transmission. Importantly, the coverage of synapses by perisynaptic astrocyte processes can vary strongly between nearby synapses. The factors that determine local astrocytic coverage of synapses and the functional significance of variable coverage remain largely unknown.

Interestingly, expansion microscopy of dendritic spines of pyramidal cells in the hippocampal CA1 region and adjacent astrocyte processes revealed a negative correlation between the spine size and the abundance of perisynaptic glutamate transporters. Functional tests using two-photon excitation imaging of extracellular glutamate and intracellular Ca2+ provided evidence for more efficient glutamate uptake at smaller spines. These results indicate that spine size may determine the efficiency of local glutamate uptake and thus the spatial precision of synaptic transmission. They also suggest that astrocytic coverage of spines is affected by plasticity-related changes of spine size. The latter is a common observation after induction of synaptic long-term potentiation (LTP). We provide evidence that LTP induction indeed withdraws astrocyte processes from synapses. On the functional level, this withdrawal led to increased escape of glutamate into extrasynaptic space (detected by optical glutamate sensors). In addition, increased glutamate spill-over onto high-affinity N-methyl-D-aspartate receptors at inactive synapses was observed after LTP induction.

Our observations indicate that spine size and synaptic plasticity dynamically determine the spatial configuration of synapses and perisynaptic astrocyte processes. As a consequence, glutamate uptake is less efficient at larger postsynaptic spines and after LTP induction, which increases the probability of glutamate to escape from active synapses and to invade neighbourin

Sodium loading in metabolically compromised cortex

Niklas J. Gerkau[1], Cordula Rakers[2], Gabor C. Petzold[2,3], Christine R. Rose[1]

[1]Heinrich Heine University Düsseldorf, Institute of Neurobiology, Universitätsstr. 1, 40225 Düsseldorf, Germany
[2]German Center for Neurodegenerative Diseases (DZNE), Sigmund-Freud-Str. 27, 53127 Bonn, Germany
[3]Department of Neurology, University Hospital Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany

The brain is the organ with the highest energy need relative to its mass. Transport processes that maintain cellular ion gradients are responsible for about 75% of its energy consumption. A particularly relevant metabolic challenge is given by influx of sodium ions (Na^+) during action potentials and excitatory synaptic transmission. Recovery from resulting changes in intracellular Na^+ concentrations ($[Na^+]_i$) is ensured by the Na^+/K^+-ATPase (NKA), which is the main energy consumer. Moreover, the inward Na^+ gradient set by the NKA provides the energy for a plethora of secondary active transport processes. These include the sodium/calcium exchanger (NCX), which couples Na^+ regulation to Ca^{2+} homeostasis and signalling. Because ATP is mainly generated by oxidative phosphorylation, the brain depends on a permanent oxygen supply. Interruption of oxygen and blood glucose delivery for only a few minutes results in breakdown of energy production, which is the main reason for neuronal death during the acute phase of stroke. Increased activation of glutamate receptors and subsequent excessive influx of Ca^{2+} through NMDA receptors and/or voltage-activated Ca^{2+} channels are important mediators of cell damage under pathological conditions, a phenomenon termed “excitotoxicity”. In the tissue surrounding the ischemic core, the so-called “penumbra”, metabolism is impaired, but cell death can be prevented by timely reperfusion, allowing for some functional recovery. Recovery is, however, jeopardized by waves of spreading depolarization, invading the penumbra from the core tissue. These “peri-infarct depolarizations” (PIDs) impose additional ionic loads and metabolic stress on the cells, accelerating damage and aggravating stroke-induced death. PIDs are accompanied by extracellular accumulation of glutamate and potassium resulting in Ca^{2+} overload of neurons. In addition, astrocytes show considerable Ca^{2+} waves during PIDs to which IP_3-mediated Ca^{2+} release from internal stores as well as influx through TRPV4 channels contribute significantly. In addition, reverse operation of the NCX might play a role in the generation of Ca^{2+} influx and Ca^{2+}-related cell damage. Many studies, however, report neuroprotective effects of NCX activity during metabolic failure, which seems contradictory to this view. It has been hypothesized that NCX reversal is driven by concurrent increases in the $[Na^+]_i$, but up to now, no information on PID-related increases in $[Na^+]_i$ *in vivo* and their relation to NCX activity is available. In my talk, I will present first data on PID-related Na^+ loading of neurons and astrocytes in the mouse brain *in vivo*. Moreover, cellular mechanisms for the influx of Na^+ will be presented. Finally, the talk will demonstrate that PID-related Na^+ loads drive reversal of NCX, mediating substantial Ca^{2+} influx into as well as promoting Na^+ export from both cell types. Reported neuro-protective effects of NCX activity in stroke models might thus be related to its dampening of ischemia-induced sodium loading.

Astroglial chloride-homeostasis in health and disease

Verena Untiet[1]

[1]University of Copenhagen, Center for Translational Neuromedicine - Division of Glial Disease and Therapeutics, Blegdamsvej 3B, 2200 Copenhagen N, Denmark

More than 20 years of research have shown that anions can regulate neural activity, but little attention has been paid to their role in inhibitory synaptic transmission. Studies in cultured astrocytes show that they actively accumulate cytosolic chloride ($[Cl^-]_i$), leading to Cl^- efflux upon Cl^- channel opening (Kettenmann et al. Brain research (1987) 404, 1-9; Kimelberg Biochim biophys acta (1981) 1, 179-184; Bekar Glia (2002) 3, 207-216). It is known that cultured astrocytes express high levels of the Cl^- accumulating transporter sodium-potassium-chloride cotransporter 1 (NKCC1). However, NKCC1 is not expressed by astrocytes *in vivo* (Clayton Brain Res Dev Brain Res (1998) 109, 281-292; Plotkin Am J Physiol (1997) 272, C173-183) and the actual $[Cl^-]_i$ in astrocytes *in vivo* is not known. Determination of $[Cl^-]_i$ in acute cerebellar slices using the technique of fluorescence lifetime imaging microscopy (FLIM) and the Cl^- sensitive fluorescent dye MQAE showed glial $[Cl^-]_i$ to be in a dynamic equilibrium. Bergmann glial cells exhibit age-dependent changes in $[Cl^-]_i$ with adult levels of around 35 mM (Untiet et al. (2017) Glia 65, 388-400). Utilizing FLIM *in vivo* investigation of astroglial chloride will be possible.

Astroglial $[Cl^-]_i$ regulate regulatory volume changes; determine the driving force for secondary active transporter as well as GABA and glycine mediated chloride currents. Similar to inhibitory neurons, astrocytes express $GABA_A$ receptors (Riquelme J Neurosci (2002) 22, 10720-10730), which are ligand gated chloride channel. Since neurons have low $[Cl^-]_i$, GABA triggers Cl^- influx generating inhibitory postsynaptic potentials (IPSPs). If the astrocyte covering the synapse has high $[Cl^-]_i$, GABA will trigger Cl^- efflux, maintaining $[Cl^-]_o$ and fuel IPSPs as hypothesized by Kettenmann et al. (Kettenmann et al. Brain research (1987) 404, 1-9). Furthermore, glutamate release upon synaptic activity reduces astroglial $[Cl^-]_i$ concentrations by activating EAAT1 and EAAT2 anion channels (Untiet et al. (2017) Glia 65, 388-400). Astrocytic $[Cl^-]_i$ appears to be involved in different signaling pathways of the central nervous system. Therefore, $[Cl^-]_i$ is crucial for astroglial neuronal signaling understanding chloride homeostasis will lead to unraveling pathomachanisms of neurological disorders in which signaling is impaired. As for example episodic ataxia 6, a human genetic disorder that is associated with an increased anion conductance of EAAT1 (Jen Neurology (2005) 65, 529-534; Winter Brain (2012) 135, 3416-3425). Or gliomas which have extremely high $[Cl^-]_i$ that facilitates their migration and tissue infiltration (Habela J Neurophysiol (2009) 101,750-757; Ransom J Neurosci (2001) 21, 7674-7683).

Role of astroglial calcium changes in Alzheimer's disease and stroke

Gabor Petzold[1]

[1]German Center for Neurodegenerative DIseases (DZNE), DZNE, Sigmund-Freud-Str. 27, 53127 Bonn, Germany

Glia are the most important safeguards of neuronal health. Astrocytes, in particular, support synaptic function and plasticity, provide energy substrates and contribute to the regulation of regional cerebral blood flow. In response to acute or chronic injury, astrocytes show strong and sustained changes in calcium activity. However, the molecular pathways governing these changes, and the consequences for neuronal function and survival in neurological diseases remain incompletely understood. Using mouse models of Alzheimer's disease (AD) or acute stroke, we have addressed these questions using a combination of in vivo multiphoton imaging, behavioral analysis, transgenic manipulation, electrophysiology and biochemical analysis. In a mouse model of AD, we have found that astrocytes in the vicinity of amyloid plaques display a hyperactive phenotype, as measured by spontaneous calcium elevations, that is largely mediated by metabotropic purinoreceptor signaling. Importantly, chronic pharmacological or transgenic reduction of this hyperactivity normalized astroglial and neuronal network dysfunction, augmented structural synaptic integrity, preserved hippocampal long-term potentiation, and alleviated the decline of spatial learning and memory. Moreover, in a separate set of studies using a mouse model of acute stroke, we have shown that astrocytic calcium changes are important contributors to detrimental spreading depolarization waves in peri-lesional cortex. Specifically, we found that during these depolarization waves, astrocytes show strong calcium elevations that are mediated by inositol triphosphate receptor type 2–dependent (IP3R2-dependent) release from internal stores. Importantly, mice deficient in Ip3r2 displayed a reduction of spreading depolarization frequency and burden, and showed increased neuronal survival after stroke. Moreover, we showed that the release and extracellular accumulation of glutamate during these depolarization waves is strongly curtailed in Ip3r2-deficient mice, resulting in ameliorated calcium overload in neurons and astrocytes. Together, these data implicate astroglial calcium pathways as important novel treatment targets in AD and stroke.

Unravelling potential mechanisms causing astrocytic death during early epileptogensis

Zhou Wu, Tushar Deshpande[1], Peter Bedner[1], Christian Steinhäuser[1]

[1]Institute of cellular Neuroscience, Medical Faculty, University of Bonn, Sigmund-Freud-Straße 25, 53127 Bonn, Germany

Epilepsy is a disorder of the brain characterised by unprovoked, recurrent seizures and affects about 1% of the population worldwide. A deeper understanding of the cellular mechanisms leading to epilepsy are essential for the identification of novel targets for therapeutic intervention. Growing evidence suggests that dysfunctional astrocytes are crucial players in the development of temporal lobe epilepsy (TLE). In a mouse model of TLE with hippocampal sclerosis (HS) we found a transient but significant reduction in the number of GFAP-positive astrocytes in the CA1 stratum radiatum (SR) of the ipsilateral hippocampus, starting 4 hours after kainate-induced status epilepticus. The goal of the present study was to elucidate molecular mechanism responsible for the astrocytic loss. For this purpose, we used immunohistochemical staining and semiquantitative RT-PCR analysis to identify marker of different cellular death mechanism 4 hours after epilepsy induction. We did not find any cleaved-caspase 3 or TUNEL positive astrocytes in the epileptic tissue, ruling out the involvement of apoptotic death. A contribution of autophagic cell death could also be excluded, since we observed only low/negligible expression of autophagy-related genes and proteins (*lc3a, b; lamp2, becn1*, LC3B). However, we found a significant increase of receptor interacting protein kinase 3 (RIPK3) and mixed lineage kinase domain-like protein (MLKL) positive astrocytes as well as enhanced expression of the corresponding necroptosis-related genes (*ripk3 and mlkl)* in the ipsilateral CA1 SR of kainate-injected mice. Moreover, using phospho-specific antibodies ipsilaterally we observed phosphorylation of MLKL (pMLKL) and the formation of necrosome complexes between RIPK3 and pMLKL in kainate-injected animals. Co-localization analysis showed translocation of pMLKL to the nucleus and the plasma membrane in astrocytes of the ipsilateral hippocampus. Taken together, the present study suggests that a considerable proportion of hippocampal astrocytes undergo necroptotic cell death during early epileptogenesis.

Astroglial MHC class II molecules are associated with fusion of larger vesicles.

Mico Bozic, Matjaz Stenovec[1,2], Robert Zorec[1,2]

[1]Faculty of Medicine, University of Ljubljana, Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Zaloška 4, 1000 Ljubljana, Slovenia.

[2]Celica BIOMEDICAL, Tehnološki park 24, 1000 Ljubljana, Slovenia.

Astrocytes maintain homeostasis and contribute to defensives responses in the central nervous system (CNS). Inflammation that accompanies virtually all CNS insults represents a protective pathophysiological response, which can also exacerbate damage if exaggerated or inappropriate. Released inflammatory cytokines alter the expression of numerous genes and induce distinct morphological and functional alterations of astrocytes collectively termed reactive (astro)gliosis. Upon exposure to the cytokine interferon γ (IFNγ), astrocytes acquire a (re)active phenotype characterized by the increased expression of major histocompatibility complex class II (MHCII) molecules and enhanced mobility of endolysosomes. The exact nature of astrocytic vesicles delivering MHCII molecules to the plasmalemma and their interactions with it are less well understood. Here we activated cultured rat astrocytes with IFNγ (600 U/ml, 48 h) to examine the subcellular localization of MHCII by confocal and structured illumination microscopy (SIM), and studied single vesicle interactions with the plasmalemma by high-resolution membrane capacitance measurements. Cell activation with IFNγ augmented the expression of MHCII molecules. In IFNγ-activated cells, MHCII-positive vesicles co-localized strongly with lysosomal marker LAMP1-EGFP, modestly with immunolabeled Rab7, and weakly with Rab4A, EEA1, TPC1, diverse markers of early endosomes. Treatment with glycyl-L-phenylalanine-β-naphthylamide, a cathepsin C substrate that causes osmotic lysis of lysosomes, diminished the number and surface area of MHCII-positive vesicles in IFNγ-activated cells, indicating lysosomal localization of MHCII. As revealed by SIM, the diameter of MHCII-positive vesicles was larger in IFNγ-activated cells than in controls, and the MHCII-positive vesicles were more numerous in the peri-plasmalemmal space of IFNγ-activated cells. In the later, reversible fusion of vesicles with larger diameters was observed when compared to controls. IFNγ treatment also decreased the frequency of full vesicle fissions when compared to controls. Cell stimulation with ATP (100 μM) increased the frequency of reversible and full vesicle fusions, and decreased the frequency of full vesicle fissions in both IFNγ-activated cells and controls. In conclusion, activation of astrocytes with IFNγ induces expression of MHCII molecules that predominately localize into lysosomes and traffic towards the cell surface. The altered dynamics of vesicle interactions with plasmalemma of IFNγ-activated astrocytes indicates heightened lysosomal fusion and inhibition of endocytosis.

Symposium

S32: Hearing system adaptation for diverse lifestyles across the animal kingdom

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Jan Clemens

S32-2 Talk to me darling – neuronal adaptations for intraspecific communication in the bushcricket ear
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S32-6 Adaptations in an identified honeybee auditory interneuron responsive to waggle dance vibration signals
Ajayrama Kumaraswamy, Hiroyuki Ai, Kazuki Kai, Hidetoshi Ikeno, Thomas Wachtler

Acoustic communication in the wild – a shared song feature detector drives male and female responses to song in Drosophila

Jan Clemens[1]

[1]European Neuroscience Institute, Neural Computation and Behavior, Grisebachstr. 5, 37077 Göttingen, Germany

Acoustic communication during courtship is ideal for understanding the neural computations underlying the transformation from sensory information into behavior in an ecologically relevant behavioral context. Drosophila melanogaster is particularly suited as a model organism since genetic tools grant unprecedented access to the brain for identifying the neural substrates of acoustic communication.
During the courtship, male flies chase females and produce song while females base their mating decision on the properties of the song. Song consists of two modes: sine song corresponds to a sinusoidal oscillation at a carrier frequency of 150Hz while pulse song consists of trains of pulses with a carrier frequency of 250Hz and an interval of ~40ms. During social interactions, both sexes rapidly change their speed when hearing song, thereby providing a highly-resolved behavioral readout of song responses: Females decelerate in response to conspecific male song, allowing the male to interact with her; males accelerate in search of a courtship target.
However, the song features and neural circuits driving this sensorimotor transformation are largely unknown. We developed a novel automated, single-fly playback assay that yields a readout of male and female changes in walking speed with sub-second resolution. Using this assay, we provide the first detailed description of the locomotor tuning for song features in Drosophila and use this information to interrogate of the auditory system.
Responses to sound playback are surprisingly complex and feature selective. Speed changes in males and females for pulse song are similar in their feature tuning but differ in sign, with males accelerating and females decelerating. This suggests that sexually monomorphic pathways underlie the feature tuning for pulse song, while the sex-specific locomotor response is generated downstream. By contrast, responses to sine song are relatively similar in both sexes, suggesting that sine song is processed by an independent pathway.
We then test these hypotheses by examining the responses of higher-order auditory neurons in the fly brain called pC2. Using genetically-encoded calcium indicators we find that pC2 responds strongly to pulse but not to sine song. The neuronal tuning for pulses song matches behavioral tuning, is similar in both sexes and does not display the difference in sign between sexes. This places pC2 as the feature detector for pulse song downstream of which sex-specific locomotor responses are generated. This is confirmed by manipulations of pC2 activity: Optogenetic activation does indeed drive the sex-specific behaviors observed in response to song, and suppression of pC2's synaptic output reveals that pC2 is necessary for normal song responses during courtship in females. Our behavioral and imaging results constitute important first steps and provide powerful constraints for further elucidating the neural basis of acoustic communication in Drosophila.

Talk to me darling – neuronal adaptations for intraspecific communication in the bushcricket ear

Manuela Nowotny[1], Jan Scherberich[1], Stefan Schöneich[2]

[1]Goethe University Frankfurt, Institute for Cell Biology and Neuroscience , Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

[2]University Leipzig, Institute for Biology, Talstrasse 33, 04103 Leipzig, Germany

Successful acoustic communication requires sender (sound production) and receiver (sound perception) to be attuned. With more than 7000 species, bushcrickets are a highly diverse group with a remarkable variability in their acoustic behaviours that often show asymmetrical signalling between males and females. In our comparative studies we examined sound production and sound perception in different bushcricket species to investigate if sex-specific signalling differences are also reflected in the morphological structures and physiological responses of the hearing organs. For mate finding, bushcrickets produce sound with their forewings, a processes that is called stridulation. In *Mecopoda elongata* (Tettigoniidae: Mecopodinae), the males send acoustic cues to attract females, which use the acoustic information for their phonotactic orientation. In *Ancylecha fenestrata* (Tettigoniidae: Phaneropterinae), both sexes form an acoustic duet. The male starts to call and females respond. In contrast to *M. elongata*, males of *A. fenestrata* also take over the task of the phonotactic approach. In regard to the morphology of the receiver structure, we found pronounced differences between both groups in the auditory tuning of the ears. The male ears of *A. fenestrata* have a remarkably long sensory organ with a significantly higher number of auditory receptor cells (about 115) compared to females. These additional receptors in the male ear are sharply tuned to the sound frequency of the female acoustic response and form an auditory fovea. Indeed, the Q_{10dB} value, which is a quality factor of tuning sharpness, is significantly higher in the fovea region compared to the other receptors. In *M. elongata*, only about 45 auditory receptors, which is more typical in Tettigoniidae, send axonal projections to the prothoracic ganglion without any frequency overrepresentation. Our anatomical, biomechanical and neurophysiological data revealed pronounced and behaviourally relevant differences in the sender and receiver structures in the two different species. Furthermore, we discovered a sex-specific auditory fovea in the ears of male *Ancylecha fenestrata* that is tuned to the dominant frequency of the female call. Population coding by similarly tuned afferent projections from the ears may provide hyperacute temporal signal information, which is currently under further investigation. (Supported by the DFG: NO 841/8-1, 10-1)

Auditory adaptations for detecting echolocating predators in moths and katydids

Hannah Marie ter Hofstede[1]

[1]Dartmouth College, Department of Biological Sciences, 78 College Street, 03755 Hanover, USA

Bats use ultrasonic echolocation calls to orient in space and locate insect prey in flight. Many nocturnal insects have ultrasound-sensitive ears to detect and avoid these voracious predators. Auditory adaptations in insects for detecting and avoiding bats have been most intensively studied in the Lepidoptera (moths) and Orthoptera (crickets and katydids). Moths have the simplest ears in nature, with only 1-4 auditory receptor cells per ear depending on the family. Ears evolved in moths for the purpose of detecting bats, and in most cases this is still their only function. In general, the ears of moths are broadly tuned to ultrasonic frequencies, but the sensitivity and tuning of their ears can be influenced by a variety of ecological factors. Moth species that are larger, and therefore generate stronger echoes, and fly more at night have more sensitive ears than other moths, presumably due to the increased risk from echolocating bats. Bat species within an ecological community use different sound frequencies for echolocation, and the ears of moths are tuned to the sympatric community of bat predators. The shape of the tuning curve allows moths to initiate escape responses at approximately the same safety margin across all the bat species in their community that use different frequency sounds for echolocation. Hearing in the Orthoptera first evolved for intraspecific communication and later evolved to detect bat echolocation calls as well. Cricket and katydid ears are broadly tuned to both low and high frequencies, whereas sensory interneurons within the central nervous system are more narrowly tuned to specific frequency ranges of interest. In katydids, one sensory interneuron, called TN-1 or the T-cell, has several properties that make it well-adapted to encoding bat echolocation calls. It is broadly tuned to high-frequency sounds encompassing all the frequencies of bat echolocation calls in a community. It also readily adapts to background noise, such as a cricket chorus, but will be activated to fire action potentials if sounds of a different frequency are detected, such as ultrasonic bat echolocation calls. Although there is not the same relationship between size and sensitivity for ultrasound hearing in katydids as there is in moths, there is a relationship between the activity of the T-cell and flight ability in Neotropical katydids that corresponds with risk of bat predation.

Death on silent wings – adaptations for sound localization in the barn owl

Christine Köppl[1]

[1]Department of Neuroscience, and Research Center Neurosensory Science, Carl von Ossietzky Universität, Oldenburg, Germany

[2]Cluster of Excellence "Hearing4all"

Only barn owls that listen and localize precisely will eat. Consequently, the barn owl's auditory system has been shaped at all levels by its nocturnal hunting habits. This talk will give an overview and focus specifically on the inner ear (basilar papilla) with its auditory fovea and the associated massive neural overrepresentation of a behaviourally salient frequency band.
The barn owl's characteristic facial disk is not just a pretty face: it amplifies and filters sound. In addition, it is subtly asymmetric, creating unique interaural differences for sounds originating at different elevations relative to the animal. As a result, interaural level differences (ILD) indicate the elevation, and interaural time differences (ITD) indicate the azimuth of a sound source – different to most other animals where both cues are correlates of azimuth. For physical reasons, these cues are most informative to the owl for localizing the high-frequency components of natural sounds, in the upper range of the barn owl's hearing, approximately 4 – 10 kHz. Consistent with its behavioural relevance, this frequency range is greatly overrepresented at the level of the inner ear. More than half the length of the tonotopically organized basilar papilla is devoted to transducing frequencies >4 kHz and also about half of all auditory nerve fibres carry information from this frequency band to the central auditory system – compared, e.g., to about 10% in the chicken. In analogy to visual foveae, this overrepresentation was termed an auditory fovea. The owl's auditory fovea similarly correlates with a high auditory acuity, however, unlike in the visual system, this is not related to the spatial density of receptor cells. Also, unlike in bat cochleae with an auditory fovea, more space does not equate to higher frequency resolution in the barn owl. Auditory nerve fibres in the barn owl are unique in their ability to code the temporal fine structure of sound via phase-locking up to very high frequencies near 10 kHz. The cellular mechanisms enabling this are still unclear and probably not directly related to the foveal overrepresentation. However, the convergence of many such temporally precise inputs onto individual neurons of the binaural brainstem nucleus laminaris is the basis for uniquely precise detection and discrimination of ITDs. It is thus the highly redundant neural input that is the important contribution of the auditory fovea to ITD computation, and thus also to the owl's unique localization acuity. The same principle probably applies to other auditory brainstem centres in which the overrepresentation of high frequencies continues, but its role is less well understood.

Understanding sound encoding: correlation of response properties of afferent inner hair cell synapses at near physiological conditions

Lina Maria Jaime Tobon, Tobias Moser[1,2,3]

[1]Synaptic Nanophysiology Group, Max Planck for Biophysical Chemistry, Göttingen, Germany; [2]Institute for Auditory Neuroscience and InnerEarLab, University Medical Center Göttingen, Göttingen, Germany; [3]Collaborative Research Center 889, University of Göttingen, Göttingen, Germany; [4]IMPRS MSc/PhD Neuroscience Program, International Max-Planck Research School, Göttingen, Germany

Inner hair cells (IHC) are the gateway of sound stimuli to the auditory pathway. They are responsible for transforming mechanical sound-borne vibrations into electrical signals and conveying this information to the afferent spiral ganglion neurons. Upon stimulation, the receptor potential triggers the opening of voltage-gated Ca^{2+} channels, mediating the fusion of vesicles and the consequent release of neurotransmitter from the presynaptic active zone to the postsynaptic bouton (for review, see Moser & Vogl, 2016).

Since the first *in vivo* recording of single auditory nerve fibers, their distinctive response properties have been a major focus of research for understanding cochlear function. Differences in the spontaneous firing rate, spike rate adaptation and operating range of individual fibers have been attributed to both presynaptic and postsynaptic mechanisms, including presynaptic heterogeneity and diverse postsynaptic molecular composition (for a comprehensive overview, see Rutherford and Moser, 2015). Despite efforts to dissect the underlying mechanisms of these response properties, the prevalent techniques only provide information from either one of the synaptic players, i.e. IHC or spiral ganglion neuron, creating a gap between *ex vivo* and *in vivo* analysis of sound encoding.

Here, we used paired pre- and postsynaptic recordings in near physiological conditions in an attempt to bridge this gap. In order to mimic the physiological state, we performed perforated patch-clamp recordings from IHCs held at a physiological resting potential (-58 mV), under physiological temperature (34-37°C) and $[Ca^{2+}]e$ (1.3 mM). We studied synaptic transmission at the single synapse level by relating the depolarization-evoked IHC Ca^{2+} current with the concomitant excitatory postsynaptic current (EPSC) at the postsynaptic bouton. The response properties of 14 paired recordings from hearing mice were analyzed, including spontaneous EPSC rates, voltage threshold of release, synaptic depression and recovery, and voltage operating ranges for Ca^{2+} influx and EPSCs. The *ex vivo* comparison between these pre- and postsynaptic response properties contributes to a better understating of the *in vivo* diversity of auditory nerve fibers responses and of auditory sound encoding in general.

Adaptations in an identified honeybee auditory interneuron responsive to waggle dance vibration signals

Ajayrama Kumaraswamy, Hiroyuki Ai[2], Kazuki Kai[2], Hidetoshi Ikeno[3], Thomas Wachtler[1]

[1]Department of Biology II, Ludwig-Maximilians-Universität München, 82152 Planegg-Martinsried, Germany
[2]Department of Earth System Science, Fukuoka University, 814-0180 Fukuoka, Japan
[3]School of Human Science and Environment, University of Hyogo, 670-0092 Himeji, Japan

Honeybees are social insects, and individual bees take on different social roles as they mature, performing a multitude of tasks that involve multi-modal sensory integration. Specifically, once they become foragers, honeybees follow the waggle dance, in which fellow foragers communicate the direction and distance of food sources to their hive mates, and which involves sensing air vibrations through the antennae.

While the waggle dance communication has been studied extensively, little is known about the neural mechanisms underlying the processing and encoding of waggle dance signals. Here we focus on the identified interneuron DL-Int-1 [1] in the primary auditory center of the honeybee Apis mellifera, which is responsive to air vibration stimuli as produced during the waggle dance [2].

DL-Int-1 neurons are GABAergic and have their main arborizations in the dorsal lobe, the dorsal subesophageal ganglion, and the posterior protocerebral lobe, close to afferents from Johnston's organ [1]. They are spontaneously active and respond to antennal vibration with phasic excitation to stimulus onset as well as to stimulus offset, and show inhibition during continuous stimulation [1]. DL-Int-1 are thought to play a central role in a disinhibitory network encoding the duration of waggle, which indicates the distance of the advertised food source from the hive [2].

We investigated changes in morphology and electrophysiological properties of DL-Int-1 during maturation by comparing properties of neurons from young, newly emerged and mature, forager honeybees. Comparison of morphological reconstructions of the neurons revealed minor changes in gross dendritic features and specific, region dependent and spatially localized changes in dendritic density, consistent with outgrowth in distal dendritic regions and pruning in proximal regions. Specifically, quantifying dendritic length in voxels of 20 µm revealed increases in dendritic length, by up to 52 µm per voxel for distal regions of the arborization in dorsal lobe, while several proximal regions showed reductions in dendritic length by up to 43 µm per voxel. Comparison of electrophysiological properties showed a significant increase in spontaneous activity by 39.6% on average, a correspondingly stronger relative inhibition during vibration stimuli, and enhanced postinhibitory rebound after stimulus offset.

The observed differences in DL-Int-1 neurons of foragers as compared to newly emerged honeybees indicate improved signal collection and propagation, and suggest enhancement of response features important for downstream processing of air vibration signals relevant in the waggle-dance communication of honeybees.

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Symposium

S33: Pro-survival versus toxic NMDA receptor signaling and the fight against neurodegenerative disorders

S33-1 The NMDA receptor paradox: pro-survival versus death signaling.
Hilmar Bading

S33-2 Role for extrasynaptic NMDA receptors in prodromal Huntington disease: Mechanisms and therapeutic implications
Lynn A Raymond, Wissam Nassrallah, James MacKay

S33-3 Probing the roles of GluN2 C-terminal domain signalling in health and disease
Giles E. Hardingham

S33-4 The Novel NMDAR Antagonist NitroSynapsin As Therapy for hiPSC- and Mouse-Models of Human Autism Spectrum Disorder
Stuart A. Lipton, Dorit Trudler, Swagata Ghatak, Nima Dolatabadi, Maria Talantova, Nobuki Nakanishi, Nicholas Schork, Daniel H. Geschwind, Rajesh Ambasudhan

S33-5 Specific Mutations in Presenilin 1 have a Differential Role on Mitochondrial Phenotype and Function
Liliana Rojas-Charry

The NMDA receptor paradox: pro-survival versus death signaling.

Hilmar Bading[1]

[1]Heidelberg University, Neurobiology, INF 366, 69120 Heidelberg, Germany

The dialogue between the synapse and the nucleus controls activity-driven gene transcription and is vital for virtually all adaptive responses in the nervous system including the build-up of a neuroprotective shield, the formation of memories, but also unwanted adaptations such a chronic pain or addiction. Calcium signals generated by synaptic activity and the opening of synaptic NMDA receptors and voltage-gated calcium channels serve as initiators of this communication pathway. They also mediate the propagation along the synapse-to-nucleus axis, although additional protein-based transport processes, such as the ERK-MAP kinase cascade, play a role. Nuclear calcium transients represent an important signaling endpoint in synapse-to-nucleus communication and function as master switch for adaptations-associated transcription. Blockade of nuclear calcium signaling in hippocampal neurons eliminates 'acquired neuroprotection', an activity-driven form of adaptation in which neurons that have been electrically activated are more resistant to harmful, cell death-inducing conditions. Similarly, the consolidation of memories and their extinction, as well as the development of chronic pain in mice is critically dependent on nuclear calcium signaling. In my presentation I will outline the role of synaptic NMDA receptors in synapse-to-nucleus communication, discuss genomic targets, and summarize how in neurodegenerative conditions this transcription-promoting axis is being antagonized by a cell death promoting signaling pathway activated by extrasynaptic NMDA receptors.

Role for extrasynaptic NMDA receptors in prodromal Huntington disease: Mechanisms and therapeutic implications

Lynn A Raymond[1], Wissam Nassrallah[1,2], James MacKay[1]

[1]University of British Columbia, Psychiatry, Djavad Mowafaghian Centre for Brain Health, 4834-2255 Wesbrook Mall, V6T 1Z3 Vancouver, Canada; [2]Graduate Program in Neuroscience, University of British Columbia

Huntington disease (HD) is an inherited neurodegenerative disorder caused by expansion of a CAG triplet repeat in the Huntingtin gene. Disease onset is typically in middle age, and neurodegeneration predominantly affects striatum and cortex. The clinical diagnosis is based on a characteristic movement disorder, but subtle motor abnormalities and cognitive changes – especially impaired mental flexibility and skilled motor learning – often precede the diagnosis by 5 - 10 years. Using HD mouse models, we found that increased expression and function of extrasynaptic NMDA-type glutamate receptors in striatal neurons occurs before an overt motor phenotype. These changes lead to altered balance of synaptic/extrasynaptic NMDAR signaling that contribute to impaired motor learning and increased vulnerability to cell death. Previous work by the Bading lab indicates that Activin A, whose expression is upregulated by pro-survival nuclear calcium signaling, reduces levels of extrasynaptic NMDARs. A role for Activin A in altered NMDAR trafficking in HD striatal neurons is under investigation. A link between this mechanism and previously reported early impairment in endoplasmic reticulum calcium signaling will also be explored. Results may lead to novel approaches to target extrasynaptic NMDAR signaling with the goal of delaying onset and slowing progression of HD.

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Probing the roles of GluN2 C-terminal domain signalling in health and disease

Giles E. Hardingham[1]

[1]University of Edinburgh, UK Dementia Research Institute, Chancellor's Building, EH16 4SB Edinburgh, United Kingdom

The GluN2 subtype (2A vs. 2B) determines key biophysical properties of forebrain NMDA receptors. During development, GluN2A becomes incorporated into previously Glu2NB-dominated NMDARs, but both are highly expressed in the adult forebrain. In addition to controlling channel properties, GluN2A and GluN2B have large and highly divergent cytoplasmic C-terminal domains. Using genetically modified mice with targeted mutation of exchange of GluN2 C-terminal domains, we are investigating their role in development and disease. Key questions include their role in directing the switch in NMDA receptor subunit composition, and in pro-death signaling in acute and chronic neurological conditions.

References: Martel et al (2012) Neuron; Hardingham & Do (2016) Nat. Rev. Neuro; McQueen et al (2017) ELife; McKay et al (2018) Cell Rep

The Novel NMDAR Antagonist NitroSynapsin As Therapy for hiPSC- and Mouse-Models of Human Autism Spectrum Disorder

Stuart A. Lipton[1], Dorit Trudler[1], Swagata Ghatak[1], Nima Dolatabadi[1], Maria Talantova[1], Nobuki Nakanishi[2], Nicholas Schork[3], Daniel H. Geschwind[4], Rajesh Ambasudhan[1,2]

[1]Molecular Medicine and Neuroscience Translational Center, The Scripps Research Institute and UC San Diego, La Jolla, CA USA.

[2]Neurodegenerative Disease Center, Scintillon Inst, , San Diego, CA USA.

[3]Dept of Human Biology, J Craig Venter Inst, La Jolla, CA, and Dept. of Quantitative Medicine, TGen, Phoenix, AZ USA.

[4]Center for Autism Research and Treatment (CART), University of California, Los Angeles, CA USA

We and our colleagues discovered that transcription factor MEF2C regulates multiple genes linked to autism spectrum disorder (ASD). Moreover, human MEF2C haploinsufficiency results in ASD, intellectual disability, and epilepsy. However, molecular mechanisms underlying MEF2C haploinsufficiency syndrome remain poorly understood. Here we report that Mef2c+/- (Mef2c-het) mice exhibit behavioral deficits resembling those of human patients. Gene expression analyses on brains from these mice show changes in genes associated with neurogenesis, synapse formation and neuronal cell death. Accordingly, Mef2c-het mice exhibit decreased neurogenesis, enhanced neuronal apoptosis, and an increased ratio of excitatory to inhibitory (E/I) neurotransmission. Additionally, we developed an ASD "disease-in-a-dish" model, using human induced pluripotent stem cells (hiPSCs) generated from three ASD patients who carry heterozygous mutations in MEF2C (1 microdeletion and 2 point mutations), as well as CRISPR/Cas9 isogenic control. Like the mouse, hiPSC-derived cerebrocortical neurons, in both 2D cultures and in cerebral organoids, displayed aberrant synaptic connectivity, abnormal bursting behavior, and E/I imbalance. In the mouse model, neurobehavioral deficits, E/I imbalance, and histological damage were all ameliorated by treatment of juvenile mice with NitroSynapsin, a new dual-action compound representing a vastly improved version of the EMA- and FDA-approved drug memantine, an uncompetitive/fast off-rate antagonist of NMDA-type glutamate receptors (NMDARs). NitroSynapsin also improved electrical deficits, behavior and histological parameters in mouse models of other forms of ASD, including TSC (Tuberous Sclerosis Complex) and Rett Syndrome (MeCP2 knock out). Additionally, Nitrosynapsin corrected electrical abnormalities in the hiPSC-based cultures. These results suggest that MEF2C haploinsufficiency and other forms of ASD lead to abnormal brain development, E/I imbalance, and neurobehavioral dysfunction, which may be mitigated by pharmacological intervention.

Specific Mutations in Presenilin 1 have a Differential Role on Mitochondrial Phenotype and Function

Liliana Rojas-Charry

[1]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2]Institute for Clinical Chemistry, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Mitochondria are well known for its role as the “power center” of the cells. But they hold more functions such as cellular homeostasis, apoptosis, iron processing, calcium buffering and steroid synthesis, to name a few. The importance of mitochondria in cellular homeostasis is unquestionable, and they cannot be set aside when promoting healthy aging. In fact, the relationship between mitochondria and neurodegenerative diseases is widely known and has been discussed extensively. Presenilin 1 (PS1), one of the proteins whose mutations cause Familial Alzheimer´s Disease, has been found in different cellular locations, including mitochondrial associated membranes, but its possible role in mitochondrial function is not well understood.

Objective: The aim of this study is to characterize the role of mutant PS1 overexpression in mitochondrial morphology and function.

Methods: Transgenic mice overexpressing human PS1 mutations G384A and E280A were used as models. Primary neurons were grown in co-culture with astrocytes for immunofluorescence experiments, evaluation of mitochondrial morphology and for respiration assays using the Seahorse system to evaluate oxygen consumption under different stimuli. Whole brains were dissected from male mice at different age groups (1, 4, 6, 9 and 12 months) to isolate intact mitochondria in a Percoll gradient. The mitochondrial fraction was prepared for proteomics using LC-MS/MS analysis.

Results: Significant differences were found in mitochondrial morphology in primary neurons carrying the hPS1E280A mutation, in contrast to hPS1G384A and control mitochondria. PS1 colocalized more with the mitochondrial marker Tom20 in cortical neurons, not in hippocampal. Proteomic analysis showed variation in the expression of proteins especially related to respiration and mitochondrial ribosomal function in pure mitochondria extracted from hPS1E280A mouse brains. Finally, mitochondrial oxygen consumption showed functional impairment in primary neurons from hPS1E280A mutants, but not in the hPS1G384A mutants, confirming a differential mitochondrial phenotype.

Conclusions: Until now it is not known if mitochondrial dysfunction is a cause or a consequence in Alzheimer’s neurodegeneration, but increasing evidence has shown its relevance in cellular processes directly related to neurodegeneration. Our results demonstrate that PS1 overexpression of specific mutations modulate mitochondrial morphology, oxygen consumption and the expression of key ribosomal and respiratory mitochondrial proteins. It is remarkable that the mitochondrial phenotype was different between mutations, being more dramatic in the hPS1E280A mutation, which generates similar concentrations of Aβ40 and AB42, contrary to what it is observed in the mutation hPS1G384A which tends to increase the Aβ42/ Aβ40 ratio, suggesting a mutation-specific mitochondrial pathology in familial Alzheimer’s disease.

Symposium

S34: The dentate gyrus - from microcircuit function to control of behavior

Two-Photon Imaging of Dentate Granule Cells and CA3 Pyramidal Cells in Mouse Hippocampus

Fritjof Helmchen[1]

[1]University of Zurich, Brain Research Institute, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Recently it has become possible to extend two-photon imaging of neuronal populations in the hippocampus of awake, behaving mice to regions beyond the CA1 region. Here, I will highlight new opportunities opened by this development. We used combinations of transgenic Cre driver lines and injection of Cre-dependent viral constructs to label either granule cells in the dentate gyrus (DG) or pyramidal cells in the CA3 region with a sensitive calcium indicator (R-CaMP1.07 or GCaMP6). We measured calcium transients in the neuronal populations of DG and CA3 during anesthesia and in awake mice during resting and running. In DG granule cells the rate of calcium transients was extremely low during anesthesia whereas it was slightly increased, albeit still sparse, during wakefulness (Pilz et al., J Neurosci 2016, 36:7407). Neurons displayed heterogeneous activation preference for resting versus running state, which also changed over days. In contrast, CA3 pyramidal neurons displayed a completely different activity pattern during anesthesia, with a large fraction of neurons showing high rates of activity. In the awake state CA3 populations displayed heterogeneous activities which also varied across days. Further experiments will be required to investigate the modulation of spatiotemporal activity in DG and CA3 in distinct brain states. As another exciting new option, I will also present the results from our collaboration with the Jessberger group at the University of Zurich, in which we performed live imaging of adult neurogenesis in the DG, tracking the proliferation and differentiation of neural stem cells over 2 months (Pilz, Bottes, et al. Science 2018, 359:658).

Mechanisms of sparse coding in the dentate gyrus

Heinz Beck[1]

[1]Institute for Experimental Epileptology and Cognition Research, Life and Brain Center, Sigmund-Freud Str. 25, 53127 Bonn, Germany

The dentate gyrus is the most important relay station that transfers polysensory information from the entorhinal cortex into the hippocampus proper. Theoretical and experimental evidence suggests that sparse coding of granule cells is critical for the ability to discriminate similar sensory percepts. The talk will explore mechanisms governing input-output transformations in the dentate gyrus ranging from cell-intrinsic dendritic integrative properties of granule cells to the control of information transfer by modulatory and inhibitory motifs.

Probing cellular mechanisms of pattern separation in the dentate gyrus

Christoph Schmidt-Hieber[1], Matthias Christenson[2], Huayi Wei[2], Michael Hausser[3]

[1]Institut Pasteur, Neuroscience, 25 Rue du Dr Roux, 75015 Paris, France
[2]Columbia University, New York, NY 10027, USA
[3]University College London, London WC1E 6AE, United Kingdom

The hippocampus must form distinct neuronal representations of memory items in order to differentiate efficiently between similar events. Granule cells of the dentate gyrus, the first stage of the hippocampal circuit, have been proposed to perform this task in a process termed pattern separation. It is unclear how synaptic input patterns are decorrelated and converted into sparse action potential output during this process. To address this question, we performed whole-cell patch-clamp recordings from hippocampal neurons in head-restrained mice navigating in a virtual-reality environment. While most granule cells were silent throughout the recording duration, spatial modulation of firing could be induced by near-threshold sustained current injections in a subset of granule cells. Spatial specificity was sensitive to the depolarisation amplitude, suggesting the presence of multiple proximal dendritic nonlinearities. Modelling revealed that such proximal nonlinearities can promote the separation of overlapping synaptic input patterns. Thus, our results show how nonlinear properties of individual neurons can computationally contribute to pattern separation.

In vivo imaging of stable and dynamic memory engrams in the rodent hippocampus

Marlene Bartos[1], Thomas Hainmüller[1]

[1]Institute for Physiology, Department I, Hermann-Herder-Straße 7, 79104 Freiburg, Germany

During our daily life, we depend on memories of past experiences to plan future behaviour. These memories are represented by the activity of specific neuronal groups or ‘engrams'. Neuronal engrams are assembled during learning by synaptic modification, and engram reactivation represents the memorized experience. In the dentate gyrus of the hippocampus, granule cells transform rich inputs from the entorhinal cortex into a sparse output, which is forwarded to the highly interconnected pyramidal cell network in hippocampal area CA3. This process is thought to support pattern separation. CA3 pyramidal neurons project to CA1, the hippocampal output region. Consistent with the idea of transient memory storage in the hippocampus, engrams in CA1 and CA2 do not stabilize over time. Nevertheless, reactivation of engrams in the dentate gyrus can induce recall of artificial memories even after weeks. Reconciliation of this apparent paradox will require recordings from dentate gyrus granule cells throughout learning, which has so far not been performed for more than a single day. We use chronic two-photon calcium imaging in head-fixed mice performing a multiple-day spatial memory task in a virtual environment to record neuronal activity in all major hippocampal subfields. Whereas pyramidal neurons in CA1–CA3 show precise and highly context-specific, but continuously changing, representations of the learned spatial sceneries in our behavioural paradigm, granule cells in the dentate gyrus have a spatial code that is stable over many days, with low place- or context-specificity. Our results suggest that synaptic weights along the hippocampal trisynaptic loop are constantly reassigned to support the formation of dynamic representations in downstream hippocampal areas based on a stable code provided by the dentate gyrus.

Imaging the dentate gyrus circuitry during virtual navigation

Thomas Hainmueller, Marlene Bartos[1]

[1]Institute for Physiology I, Hermann-Herder Str. 7 [2]Spemann-School of Biology and Medicine (SGBM) [3]Faculty of Biology, University of Freiburg, 79104 Freiburg i.Br., Germany

The hippocampus is essential for the storage and recall of conscious memories. Each of its major subfields hosts neuronal ensembles (engrams) which form abstract representations of memory-specific contents, such as places, sounds, time or individuals (O'Keefe and Dostrovsky, 1971; Quiroga et al., 2005; Aronov et al., 2017). Artificial activation of these neuronal engrams, e.g. in the hippocampal denate gyrus, can be used to manipulate existing memories in the mouse brain or even create new ones (Ramirez et al., 2013). Despite their importance as a correlate of episodic memories in the brain, the circuit mechanisms by which hippocampal memory engrams evolve during different forms of learning are still poorly understood (Bittner et al., 2017; Sheffield and Dombeck, 2017).

To study the emergence and activity of memory engrams in the dentate gyrus of head-fixed, behaving mice, we use two-photon calcium imaging with (sub-) cellular resolution. During the imaging sessions, the mice perform different behaviours in a virtual environment displayed on screens around them (Hainmüller and Bartos, 2018). We investigate, how the cellular elements of the denate gyrus circuitry represent the individual elements of episodic memories (places, objects, time etc.) and how these representations develop with learning. We also study the local circuit mechanisms underlying the formation of memory-bearing neuronal ensembles (Sheffield and Dombeck, 2017). By these means, we are trying to decipher how neuronal circuits in the hippocampal formation store and retrieve the contents of complex episodic memories.

Symposium

S35: The presynaptic active zone: converging and diverging mechanisms across species

S35-1 Molecular machinery required for synaptic organization and release
Janet Elizabeth Richmond

S35-2 From compost to the clinic: using C. elegans to study psychiatric disorders
Joshua M. Kaplan, Stephen J. Nurrish, Xia-Jing Tong, Edward Pym, Zhitao Hu, Luna Gao, Daniel Nedelcu

S35-3 Active zone physiology in the context of olfactory information processing in *Drosophila*
Nadine Ehmann

S35-4 *thin* promotes presynaptic homeostatic plasticity at the *Drosophila* neuromuscular junction
Martin Baccino-Calace, Martin Mueller

S35-5 Dissecting release site architecture for fast neurotransmitters and for neuromodulators
Pascal Kaeser

S35-6 Resolving the Ultrastructural Organization of Synaptic Vesicle Pools at Hippocampal Mossy Fiber and Schaffer Collateral Synapses
Lydia S. B. Maus

Molecular machinery required for synaptic organization and release

Janet Elizabeth Richmond[1]

University of Illinois at Chicago, Biological Sciences, 950 S. Halsted St, 60607 Chicago, USA

Synaptic release occurs at specialized active zones within synaptic terminals that coordinate the docking and priming of synaptic vesicles in close proximity to calcium channels. An electron dense cytomatrix within the active zone (CAZ) is critical for the organization and function of synapses. Many proteins associated with the CAZ domain have conserved roles in the synaptic vesicle cycle across species, allowing general principles underlying synaptic function to be gleaned from genetically tractable model organisms such as C. elegans.
In a recent genetic screen we identified a novel CAZ protein, clarinet (CLA-1) named for its homology to the piccolo, bassoon, fife family of proteins1.
In an initial characterization of this protein, we demonstrated that CLA-1 regulates synapse number, CAZ ultrastructure and synaptic vesicle localization. Consequently, cla-1 mutants exhibit defects in both spontaneous and evoked synaptic release. CLA-1 encodes three isoforms of varying length that share common C-terminal PDZ and C2 domains. Both long and short CLA-1 isoforms colocallize within the CAZ domain via a C-terminal interaction with unidentified binding partners. We are currently addressing this anchoring mechanism through the analysis of genetic crosses with candidate CAZ proteins. CLA-1 isoforms appear to serve different synaptic functions. We are using a combination of electrophysiology, superresolution imaging and electron microscopy to further explore these distinct roles.
Both synapse development and function are activity-dependent processes. We will present new findings in an established calcium-dependent developmental synaptic remodeling pathway, building on a previous study implicating the transcriptional regulation of the underlying molecular machinery2. We will demonstrate additional roles for calcium signaling in the regulation of key synaptic proteins that control synapse strength.

1. Clarinet (CLA-1), a novel active zone protein required for synaptic vesicle
clustering and release. Zhao Xuan, Laura Manning, Jessica Nelson, Janet E Richmond, Daniel A Colon-Ramos, Kang Shen, Peri T Kurshan
Elife. 2017 e29276.

2. The DEG/ENaC Cation Channel Protein UNC-8 Drives Activity-Dependent Synapse Removal in Remodeling GABAergic Neurons. Tyne W. Miller-Fleming, Sarah C. Petersen, Laura Manning, Cristina Matthewman, Megan Gornet, Allison Beers, Sayaka Hori, Shohei Mitani, Laura Bianchi, Janet Richmond, David M. Miller, III. Elife 2016 e14599

From compost to the clinic: using C. elegans to study psychiatric disorders

Joshua M. Kaplan[1], Stephen J. Nurrish[1], Xia-Jing Tong[1,2], Edward Pym[1], Zhitao Hu[1,3], Luna Gao[1], Daniel Nedelcu[1]

[1]MASSACHUSETTS GENERAL HOSPITAL, MOLECULAR BIOLOGY, SIMCHES 7, 185 CAMBRIDGE ST., 02114 BOSTON, USA; DEPARTMENT OF NEUROBIOLOGY, HARVARD MEDICAL SCHOOL, BOSTON, USA

[2]SHANGHAI TECHNICAL UNIVERSITY, SHANGHAI, CHINA

[3]QUEENSLAND BRAIN INSTITUTE, BRISBANE, AUSTRALIA

Recent human genetic studies suggest that mutations in ~500 genes are linked to autism spectrum disorder. Although many of these genes encode proteins that are localized at synapses, the sites of connection and communication between neurons, relatively little is known about how these mutations alter brain function or development. An important goal for the field is to identify specific cellular defects caused by mutations linked to Autism and to determine how (and if) these defects contribute to the cognitive and developmental deficits found in Autism. My lab uses a simple model organism (Caenorhabditis elegans) as a genetic platform to investigate the impact of Autism-linked genes on brain development and function.

Our studies suggest that mutations linked to Autism in humans alter neurotransmitter release, the strength of inhibitory synapses, and activity-induced gene expression in worms. We propose that these cellular defects play an important role in the pathophysiology of Autism.

Active zone physiology in the context of olfactory information processing in *Drosophila*

Nadine Ehmann[1]

[1]Leipzig University, Animal Physiology, Talstraße 33, 04103 Leipzig, Germany

Structural, functional and molecular features of synaptic active zones (AZs) can differ significantly between organisms, neuronal systems, within one and the same neuron and at an individual site over time. Work at the *Drosophila* neuromuscular junction has helped to clarify basic molecular mechanisms of AZ function. The present study aims to apply this knowledge to the fly's central nervous system to investigate how the molecular physiology of AZs controls olfactory signal processing.

thin promotes presynaptic homeostatic plasticity at the *Drosophila* neuromuscular junction

Martin Baccino-Calace, Martin Mueller[1]

[1] Institute of Molecular Life Sciences, University of Zurich, CH-8057 Zurich, Switzerland

Synaptic transmission is actively stabilized by homeostatic mechanisms. At various synapses in different species, neurotransmitter receptor perturbation results in enhanced neurotransmitter release. At the Drosophila neuromuscular junction (NMJ), pharmacological or genetic manipulations that reduce glutamate receptor activity trigger a retrograde signal that enhances presynaptic release to precisely compensate for this perturbation. Due to the 'precise' restoration of baseline function, this form of synaptic modulation has been termed 'presynaptic homeostatic plasticity' (PHP). Electrophysiology-based genetic screens at this synapse have identified several genes that are required for PHP. However, it has remained largely elusive how proteins are regulated during PHP. PHP can be induced in the presence of protein translation inhibitors, implying that PHP induction does not require new protein synthesis. Interestingly, recent data implicated the ubiquitin-proteasome system (UPS), a major protein degradation pathway, in PHP. Specifically, it was found that presynaptic proteasome function is necessary for PHP. E3 ubiquitin ligases confer target specificity to protein ubiquitination. Nevertheless, little is known about the role of E3 ligases in the regulation of synaptic function.

We therefore systematically investigated E3 ligases in the context of synaptic transmission and PHP. Specifically, we assayed spontaneous miniature neurotransmission and action potential (AP)-evoked synaptic transmission after pharmacological glutamate receptor perturbation at the Drosophila NMJ employing presynaptic expression of RNAis targeting 200 evolutionarily conserved E3 ligase-encoding genes. This genetic screen identified *thin* (*tn*), an ortholog of human tripartite motif-containing 32 (TRIM32), a gene associated with human limb-girdle muscular dystrophy. Presynaptic tn^{RNAi} expression (elavc155 > UAS-tn^{RNAi}) induced an increase in baseline synaptic transmission. In previously published flies harboring a *tn* deletion (tn^{dA}; LaBeau-DiMenna et al., 2012), homeostatic increase in quantal content (AP-evoked EPSC amplitude/mEPSC amplitude) normally induced by pharmacological glutamate receptor perturbation (PhTX-433 for 10') was blocked, suggesting that *tn* functions presynaptically during PHP.

Previous work revealed that TRIM32 ubiquitinates the dystrophin-associated complex member Dysbindin (Kudryashova et al., 2005). *dysbindin* (*dysb*) has been linked to schizophrenia in humans (Mullin et al., 2011) and PHP in *Drosophila* (Dickman and Davis, 2009). tn^{dA} mutants displayed an increased quantal content under baseline conditions, i.e. in the absence of glutamate receptor impairment, indicating that *tn* negatively regulates presynaptic release. Interestingly, a similar phenotype was seen after presynaptic ^{dysb} overexpression (elavc155 > UAS-*dysb*; Dickman and Davis, 2009). These genetic data indicate a potential link between *tn* and *dysb* in the context of neurotransmitter release modulation, which we are currently further exploring. Together, our data implicate the E3 ligase *thin* in the regulation of neurotransmitter release under baseline conditions and during presynaptic homeostatic plasticity.

Dissecting release site architecture for fast neurotransmitters and for neuromodulators

Pascal Kaeser[1]

[1]Harvard Medical School, Department of Neurobiology, 210 Longwood Ave, Armenise 305, 02115 Boston, USA

Proper function of synaptic circuits relies on the precise control of synaptic transmission. Our major research focus is to understand molecular mechanisms that underlie speed, regulation and diversity in secretory pathways of neurons. Active zones are highly organized sites in a nerve terminal that set neurotransmitter release apart from other pathways for regulated exocytosis. Our laboratory pursues three major questions: 1. Assembly mechanisms of the active zone are not well understood. We identify molecular mechanisms that control release site assembly. 2. Neurotransmitter release is remarkably fast and functionally plastic. The laboratory dissects the molecular mechanisms that underlie the speed and plasticity of neurotransmitter release. 3. Secretory pathways in neurons are extraordinarily functionally diverse, but current molecular models don't account for this diversity. We determine the molecular architecture and function of the secretory apparatus for the neuromodulator dopamine. This project reveals dopamine release mechanisms and will likely lead to a better understanding of secretory diversity.

Resolving the Ultrastructural Organization of Synaptic Vesicle Pools at Hippocampal Mossy Fiber and Schaffer Collateral Synapses

Lydia S. B. Maus

[1]Max Planck Institute for Experimental Medicine, Molecular Neurobiology, Hermann-Rein-Str. 3, 37075 Goettingen, Germany
[2]Department of Pharmacology, University of Maryland School of Medicine, 670 West Baltimore St., Baltimore, MD 21201, USA

Synaptic neurotransmission occurs with high spatiotemporal precision at presynaptic active zones (AZs) to rapidly propagate information between neurons in the CNS. Molecular components at the AZ are involved in docking and priming synaptic vesicles (SVs) at the presynaptic membrane to generate a pool of fusion-competent, readily releasable (RRP) vesicles that can rapidly fuse and release their contents into the synaptic cleft upon calcium influx. Despite similarities in the composition of the molecular release machinery at AZ release sites, synapses can exhibit strikingly different morphological and functional properties. Paradigmatic and well-characterized examples of this type of functional heterogeneity are the Schaffer collateral (SC) and mossy fiber (MF) synapses of the mammalian hippocampus. Although both synapse types use glutamate as their neurotransmitter, they differ drastically in their ultrastructural architecture, transmitter release properties, short- and long-term plasticity characteristics, and in the mechanisms that modulate synaptic efficacy. Despite being functionally well characterized, MF ultrastructure has not previously been scrutinized at a level of resolution permitting the accurate discrimination of morphologically and functionally distinct SV pools at AZ release sites. Consequently, the extent to which such differences in structural organization contribute to or reflect distinct functional properties of SC and MF synapses remains unclear.
We have addressed this by using a combination of hippocampal organotypic slice culture, high-pressure freezing, freeze substitution, and 3D-electron tomography to preserve synaptic ultrastructure in a near-native state and resolve the spatial organization of SV pools with nanoscale precision. This experimental approach, which permits a direct comparative analysis of SC and MF synapses in the same organotypic slice, revealed that at 14 days *in vitro* MF synapses harbored fewer morphologically docked SVs per AZ area than SC synapses. However, in more mature slices frozen at 28 days *in vitro*, the spatial density of docked SVs at MF and SC synapses was highly comparable indicating that the different transmitter release characteristics of these two synapse types are likely not due to docked SV availability. Consistent with our previous work on SC synapses, we found the number of morphologically docked SVs in MF synapses to be in close agreement with RRP estimates from electrophysiological studies. Interestingly, we found that MF synapses exhibit considerable heterogeneity in docked SV size and that they possess a distinct pool of non-docked but possibly tethered membrane proximal SVs that may play a role in rapidly refilling SV release sites during sustained activity, thereby contributing to MF short-term facilitation properties. Our analysis also revealed that in contrast to SC synapses, MFs frequently harbored dense-core vesicles (DCVs) docked at AZ release sites, indicating that DCVs may fuse at AZs in this synapse type. Both SV and DCV docking was completely abolished in Munc13-deficient MF synapses, demonstrating that both vesicle types require Munc13-priming factors for fusion at AZ release sites. Our data provides novel insight into how differences in the ultrastructural architecture of MF and SC synapses at individual presynaptic AZs could contribute to their distinct functional properties and corresponding short- and long-term plasticity characteristics.

Symposium

S36: Beyond expression of fear: mechanisms and circuits of the extended amygdala

S36-1 Targeting the bed nucleus of the stria terminalis to reduce anxiety in rats and patients
Laura Luyten, Kelly Luyck, Bart Nuttin

S36-2 The way forward is backward: BNST mediates fear to ambiguous threats
Stephen Maren, Travis D. Goode, Reed L. Ressler

S36-3 Endocannabinoids Impact on Responses to Predictable and Unpredictable Threat via CRH neurons
Maren Denise Lange, Thomas Seidenbecher

S36-4 Mechanisms underlying stress-enhanced fear
Ki Ann Goosens

S36-5 The transcription factor MEF-2A mediates the anxiogenic effect of chronic oxytocin
Julia Winter, Magdalena Meyer, Inga Neumann, Benjamin Jurek

S36-6 *The watchdog won't stop barking!*
Top-down control of the amygdala by medial prefrontal cortex in major depression: The role of medication, genetic liability and childhood maltreatment.
Roman Kessler, Andreas Jansen

Targeting the bed nucleus of the stria terminalis to reduce anxiety in rats and patients

Laura Luyten[1,2], Kelly Luyck[2], Bart Nuttin[2]

[1]KU Leuven, Psychology of Learning and Experimental Psychopathology, Tiensestraat 102 PB 3712, 3000 Leuven, Belgium[2] KU Leuven, Experimental Neurosurgery and Neuroanatomy, Herestraat 49 PB 1036, 3000 Leuven, Belgium

Conscientious fundamental research has implicated the bed nucleus of the stria terminalis (BST or BNST, also referred to as the extended amygdala) in fear, anxiety, stress and addiction, and has greatly enhanced our understanding of this complex brain region at the level of neurons and circuits. Here, we present the multidisciplinary approach that we have been using to translate some of this knowledge into clinical practice, and back. We recently showed that deep brain stimulation (DBS) in the BST reduces obsessions and anxiety symptoms in patients suffering from severe, treatment-refractory obsessive-compulsive disorder, in a double-blind randomized crossover trial. Moreover, we demonstrated that continuous, high-frequency electrical stimulation in the BST reduced contextual fear in a rat model, although not to the same extent as electrolytic lesions. In this model, freezing and startle responses of animals are measured as indices of anxiety, in a context that was previously paired with unpredictable foot shocks, resulting in anticipatory anxiety upon re-exposure to this context. We are currently investigating these findings in more detail, both on a behavioral and neurocircuitry level. Our data underline the therapeutic potential of DBS in the BST for disorders that are hallmarked by pathological anxiety.

The way forward is backward: BNST mediates fear to ambiguous threats

Stephen Maren[1], Travis D. Goode[1], Reed L. Ressler[1]

[1]Texas A&M University, Psychological and Brain Sciences, 301 Old Main Dr,, 77843-3474 College Station, USA

The bed nucleus of the stria terminalis (BNST) has been implicated in fear and anxiety, but the specific factors that engage the BNST in defensive behavior are unclear. Here we explore the possibility that ambiguous threats recruit the BNST during Pavlovian fear conditioning in rats. We arranged a conditioned stimulus (CS) to either precede or follow an aversive unconditioned stimulus (US), a procedure that established reliable (forward) or ambiguous (backward) signals for US onset. After conditioning, reversible inactivation of the BNST selectively reduced freezing to the backward CS; BNST inactivation did not affect freezing to the forward CS even when that CS predicted a variable magnitude US. Backward CSs increased Fos in the ventral BNST and in BNST-projecting neurons in the infralimbic cortex, but not the hippocampus or amygdala. These data reveal that BNST circuits process ambiguous threat signals central to the etiology and expression of anxiety.

Endocannabinoids Impact on Responses to Predictable and Unpredictable Threat via CRH neurons

Maren Denise Lange[1], Thomas Seidenbecher[1]

[1]Institute of Physiology I, Westfälische Wilhelms-University, Robert-Koch Str. 27a, 48149 Münster, Germany

The response to a threat can shift from a rapid phasic state of fear to a more sustained anxious apprehension, particularly in face of diffuse cues with unpredictable environmental contingencies. Unpredictability, in turn, is considered an important variable contributing to anxiety disorders.
We have recently demonstrated that cannabinoid type 1 receptors (CB1) on distinct amygdala inputs to neurons in the anterolateral BNST (alBNST) are causal for a shift to sustained fear. However, subpopulations of neurons driving the CB1 effect remain elusive. To identify specific cell populations, which are critically involved in regulating the fear profile, we combined retrograde tracer studies with immunohistochemistry, optogenetic and electrophysiological approaches in CRH- and PKCδ;-reporter mice. Further, we performed bilateral local application of a CRH1-receptor agonist (Stressin I) in freely behaving mice before fear memory retrieval, 24h after predictable CS-US training, which induced sustained fear (maintained freezing). In addition, local application of a CRH1-receptor antagonist (CP 154526) before fear memory retrieval, 24h after unpredictable CS-US training, blocked sustained fear. Supplementary, CRH-cre mice were crossed with floxed-CB1 or floxed-STOP-CB1 mice and tested in the equal Pavlovian-conditioning paradigm with unpredictable CS-US occurrence concerning CB1-CRH interaction. Here, behavioral results indicated that specific rescue of CB1 in CRH neurons resulted in reconstitution of the sustained fear phenotype in close resemblance to wildtype behavior. First evidence obtained from optogenetic and electrophysiological approaches corroborated these findings in showing that projections from centrolateral amygdala (CeL) to alBNST are devoid of PKCδ; and that CRH is expressed in a large portion of this projection. Further, CRH-positive inputs from CeL to alBNST seem to reside CB1 receptors which thereby regiment the CRH-effect. These results suggest a causal role for CB1-CRH-interaction in circuits of the extended amygdala which seems crucial for the development of a sustained state of anxious apprehension as a response to prior experienced unpredictable environmental influences.

Mechanisms underlying stress-enhanced fear

Ki Ann Goosens[1]

[1]Icahn School of Medicine at Mount Sinai, Psychiatry, One Gustave Levy Place, Box 1230, 10029 New York, USA

Prolonged stress exposure dramatically increases the development and symptoms of psychiatric disorders, including post-traumatic stress disorder (PTSD). This can be examined in a rodent model of PTSD in which chronic stress exposure greatly enhances the strength of subsequently encoded fear memories. I will discuss recent work from my laboratory examining the underpinnings of this effect. Several of these studies from my laboratory have provided a novel link between the hormone ghrelin and multiple maladaptive health consequences following prolonged stress exposure. Ghrelin is a hormone made by endocrine cells in the stomach and released into the bloodstream, where it can cross the blood-brain barrier. My team established that the prolonged elevation of endogenous ghrelin that follows chronic stress exposure enhances fear memory by facilitating fear memory consolidation. We have also found that repeated stress enhances fear memory by recruiting a serotonergic memory consolidation process between the dorsal raphe and amygdala that is not present in unstressed animals. These results show that the excessive fear memories that are observed in chronically stressed animals do not arise simply from enhanced mnemonic processes that occur in unstressed animals.

The transcription factor MEF-2A mediates the anxiogenic effect of chronic oxytocin

Julia Winter, Magdalena Meyer[1], Inga Neumann[1], Benjamin Jurek[1]

[1]University of Regensburg, Behavioral and Molecular Neurobiology, Universitätsstrasse 31, 93053 Regensburg, Germany

In recent years, oxytocin (OT) has gained interest as potential treatment for anxiety disorders, though, the underlying molecular mechanism downstream of OT receptor (OTR) activation remains poorly understood. In our study, we focus on chronically applied OT via osmotic mini pumps in male rats. The pumps were implanted subcutaneously and connected to an icv cannula via a silicone tubing, delivering two concentrations of OT (1ng/h or 10ng/h) for 14 days. As previously shown in mice, chronic OTR activation at a high dose of OT (10 ng/h) for 14 days leads to enhanced anxiety-related behavior, in contrast to the anxiolytic effect of an acute OT bolus.

One downstream transcription factor that we were able to link to OTR signaling is the myocyte enhancer factor 2 (MEF-2). This factor is known to be a central regulator of neuronal differentiation, its activity reduces dendritic outgrowth, and its dysfunction has been associated with autism spectrum disorder. Intriguingly, MEF-2 can be regulated by MAP kinase signaling and calcium signals, both being pathways that are coupled to the OTR.

Unlike acute OT administration, chronic OT treatment leads to increased protein levels of MEF-2A, altered MEF-2A phosphorylation (Thr312/319, S408) via the ERK1/2 pathway, and increased binding to its responsive element in relevant target genes, such as neuroplasticity-regulating genes and anxiety-relevant genes (CRFR2, CRF-BP).

The anxiety-related genes are especially interesting, as we could directly link their downregulation to the anxiogenic phenotype of chronic OT. In more detail, not only the decreased expression of the corticotropin releasing factor receptor 2 (CRFR2) and of the CRF binding protein (CRF-BP) play an important role, but also a shift in alternative splicing of the CRFR2 towards a soluble version, short sCRFR2. Additionally, we confirmed the connection between OTR activation and MEF-2A using OTR and MEF-2A knockout cell lines that were created by means of CRISPR/Cas9.

In conclusion, our data shows that chronically applied OT activates MEF-2A, leads to altered gene transcription, and in consequence, increased anxiety in male rats.

The watchdog won't stop barking! Top-down control of the amygdala by medial prefrontal cortex in major depression: The role of medication, genetic liability and childhood maltreatment.

Roman Kessler, Andreas Jansen[1,2]

[1]University of Marburg, Laboratory for Multimodal Neuroimaging, Department of Psychiatry, Rudolf-Bultmann-Str. 8, 35039 Marburg, Germany

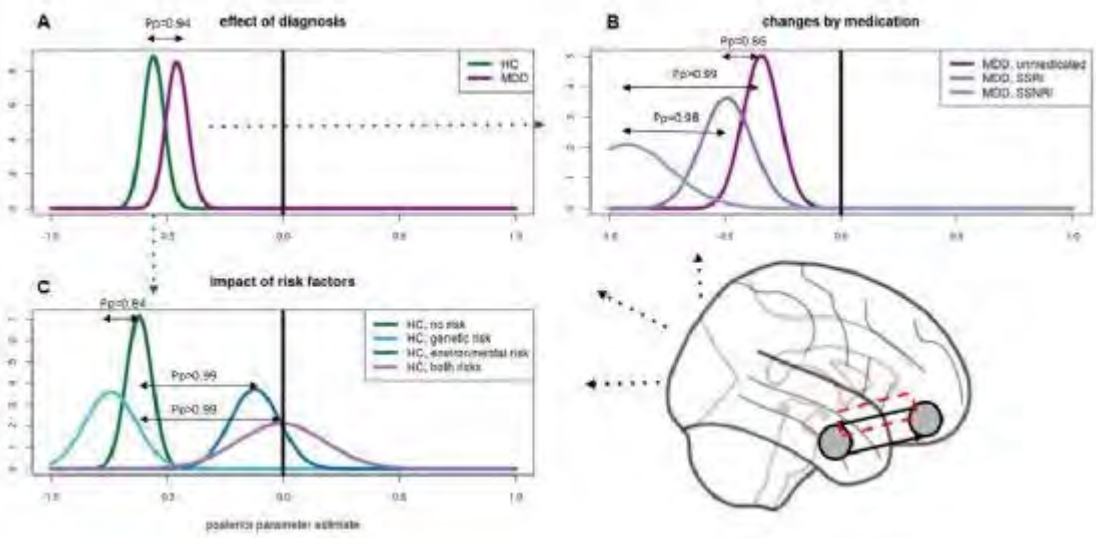
[2]University of Marburg, Core-Unit Brainimaging, Department of Psychiatry, Rudolf-Bultmann-Str. 8, 35039 Marburg, Germany

Patients suffering from Major Depressive Disorder (MDD) typically show an amygdala-hyperactivation as response to emotional stimuli. This hyperactivation is reduced with serotonergic medication. Likewise, healthy controls with risk for depression (i.e. a family history or childhood maltreatment) show similar hyperactivation. Complementary models of limbic-cortical dysfunction proposed a reduced inhibition of medial prefrontal cortex (mPFC) over the amygdala in MDD. We examined this inhibition using Dynamic Causal Modeling (DCM) in a large cohort of MDD patients and controls.

We included a total of 666 participants (314 MDD patients, 352 controls) from the Marburg Münster Affective Cohort Study (www.for2107.de), an ongoing multi-center study. An emotional face matching task was used to identify relevant brain areas (mPFC, right amygdala). Different DCMs were constructed, and Bayesian Model Averages were conducted within each group. We then compared amygdala inhibition during emotion processing between groups.

Inhibition was present in all subgroups. However, MDD patients had a reduced amygdala inhibition, but it was stronger for patients under serotonergic medication (SSRI and SSNRI) than for unmedicated patients. At last, childhood maltreatment but not genetic liability was associated with reduced amygdala inhibition by mPFC in healthy controls.

In this work, we highlighted deficits in amygdala inhibition by mPFC in MDD, and proposed a mechanistic explanation of frequently reported amygdala hyperactivity in MDD and in healthy subjects that experienced childhood maltreatment. The same model was further able to explain the normalization of amygdala activation via serotonergic medication.


A
effect of diagnosis
Pp=0.94
HC
MDD
B
changes by medication
Pp=0.66
Pp=0.99
Pp=0.98
MDD, unmedicated
MDD, SSRI
MDD, SSNRI
C
impact of risk factors
Pp=0.84
Pp=0.99
Pp=0.99
HC, no risk
HC, genetic risk
HC, environmental risk
HC, both risks
posterior parameter estimate

Poster Topics

T21 Motor Systems

T22 Homeostatic and Neuriendocrine Systems, Stress Response

T23 Neural Networks and Rhythm Generators

T24 Attention, Motivation, Emotion and Cognition

T25 Learning and Memory

T26 Computational Neuroscience

T27 Techniques and Demonstrations

Poster Topic

T1: Stem cells, Neurogenesis and Gliogenesis

T1-1A Chromatin remodeling BAF (mSWI/SNF) complexes regulate oligodendrogenesis in the embryonic forebrain
Eman Abbas, Kamila A. Kiszka, Linh Pham, Jochen F. Staiger, Tran C. Tuoc

T1-2A Automated and manual patch clamp data of human induced pluripotent stem cell-derived dopaminergic neurons
Denise Franz, Hervør Lykke Olsen, Jan Gimsa, Rüdiger Köhling

T1-3A Nr2f1 transcriptional gradient in the developing mouse cerebral cortex depends on histone demethylase KDM1a activity
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T1-4A Appropriate markers to identify glioblastoma stem cells in vitro
Diana Freitag, Fritz Klippel, Rolf Kalff, Christian Ewald, Jan Walter

T1-1B Recovery of olfactory induced behavior indicates successful network restoration after olfactory nerve transection in larval *Xenopus laevis*
Sara Joy Hawkins, Yvonne Gärtner, Lukas Weiss, Thomas Hassenklöver, Ivan Manzini

T1-2B Loss of Brg1 in hGFAP-positive cells impairs cerebral and cerebellar development
Dörthe Holdhof, Melanie Schoof, Malte Hellwig, Ulrich Schüller

T1-3B Generation of functionally active and mature neurons from ADHD patients carrying copy number variants of *SLC2A3* to study its impact on neuronal metabolic as well as neurodevelopmental processes
Charline Jansch, Andrea Forero, Sina Kollert, Sina Wäldchen, Jonas Waider, Frank Edenhofer, Erhard Wischmeyer, Klaus-Peter Lesch

T1-4B Stress impedes neuronal differentiation via *ZBTB16* in human cerebral organoids
Anthodesmi Krontira, Cristiana Cruceanu, Simone Röh, Silvia Matrinelli, Elisabeth Binder

T1-1C Characterization of Electrophysiological Properties
Of Human iPSC-derived Neurons in Autaptic Culture
Hong Jun Rhee, Ali Shaib, ChoongKu Lee, Oliver Bruestle, Nils Brose, JeongSeop Rhee

T1-2C Characterization of Morphological Properties of Human iPSC-Derived Neurons in Autaptic Culture System
Ali Shaib, Hong Jun Rhee, ChoongKu Lee, Peter Seif, Oliver Bruestle, Nils Brose, JeongSeop Rhee

T1-3C Wharton's Jelly - source of MSC which are able to differentiate in NSC.
Adam Osowski, Ewa Kruminis-Kaszkiel, Ewa Bejer-Olenska, Joanna Wojtkiewicz

T1-4C Profilin1 mutant mice display features of a gyrencephalic neocortex
Marco Rust, Sophie Meyer, Jan Kullmann, Fabrizia Pipicelli, Felix Schneider, Nora Bartels, Silvia Cappello

T1-1D Neurogenic effect of Wnt signaling pathway on isolated murine and human progenitor cells of the enteric nervous system
Melanie Scharr, Peter Neckel, Katharina Nothelfer, Ying Zhang, Karin Seid, Florian Obermeyr, Lothar Just

T1-2D EGFL7: a novel modulator of neural homeostasis in the hippocampus
Mirko HH Schmidt, Verica Vasic, Frank Bicker

T1-3D Analysing schizophrenia risk variants in NRXN1 using functional and mature neuronal cultures from patient-derived iPS cells
Annika Liisa Majer, Matthias Jung, Jessica Reinsch, Jovita Schiller, Ina Giegling, Dan Rujescu

T1-4D Assessment of electrophysiological properties of human iPSC-derived serotonergic neuron model
Evgeniy Svirin, Sina Kollert, Charline Jansch, Erhard Wischmeyer , Tatyana Strekalova, Klaus-Peter Lesch

T1-5D Is the coat color reflecting neuronal layering in the olfactory bulb in the Female American Mink (*Neovison vison var. spec.*)?
Elke Weiler, Willi Bennegger

Chromatin remodeling BAF (mSWI/SNF) complexes regulate oligodendrogenesis in the embryonic forebrain

Eman Abbas[1], Kamila A. Kiszka[1,2], Linh Pham[1], Jochen F. Staiger[1,2], Tran C. Tuoc[1,2]

[1]Institute for Neuroanatomy, University Medical Center, Georg-August University Goettingen, Germany
[2]DFG, Center for Molecular Physiology of the Brain (CMPB), 37075 Goettingen, Germany

In the developing mammalian brain, the ventral telencephalic neural stem cells (NSCs) produce distinct cell types such as GABA-expressing interneurons, astrocytes, and oligodendrocytes (OLs). OLs myelinate axons in the CNS that lead to accelerating their signal transduction. However, little is known about the epigenetic regulatory mechanisms that control the oligodendrogenesis. In this study, we focused on the role of the chromatin remodeling BAF complex in the OL production. Our findings revealed that the BAF complex is highly expressed in the oligodendrocyte lineage including oligodendrocyte precursor cells (OPCs), immature oligodendrocytes (iOLs) as well as, mature oligodendrocytes (mOLs). Using next-generation RNA sequencing, we identified the transcriptomes of the mutant BAF155/170 double knockout mice via applying the hGFAP-Cre. Remarkably, we observed many oligodendrocyte-expressing genes are significantly downregulated. Therefore, we deleted the BAF 155/170 subunits specifically in the Olig2-Cre line that is expressed in the ventral NSCs and oligodendrocyte lineage in the forebrain to generate the conditional BAF155/170 double knockout mice. Noticeably, this conditional BAF complex loss resulted in a depleted pool of iOLs and mOLs in both ventral and dorsal telencephalon. Furthermore, our data exhibited that the BAF complex is required for the proper proliferation of OPCs. Thus, our findings substantiated that the BAF complex has a crucial function to regulate the OL development during the embryogenesis.

Automated and manual patch clamp data of human induced pluripotent stem cell-derived dopaminergic neurons

Denise Franz[1], Hervør Lykke Olsen[2], Jan Gimsa[3], Rüdiger Köhling[1]

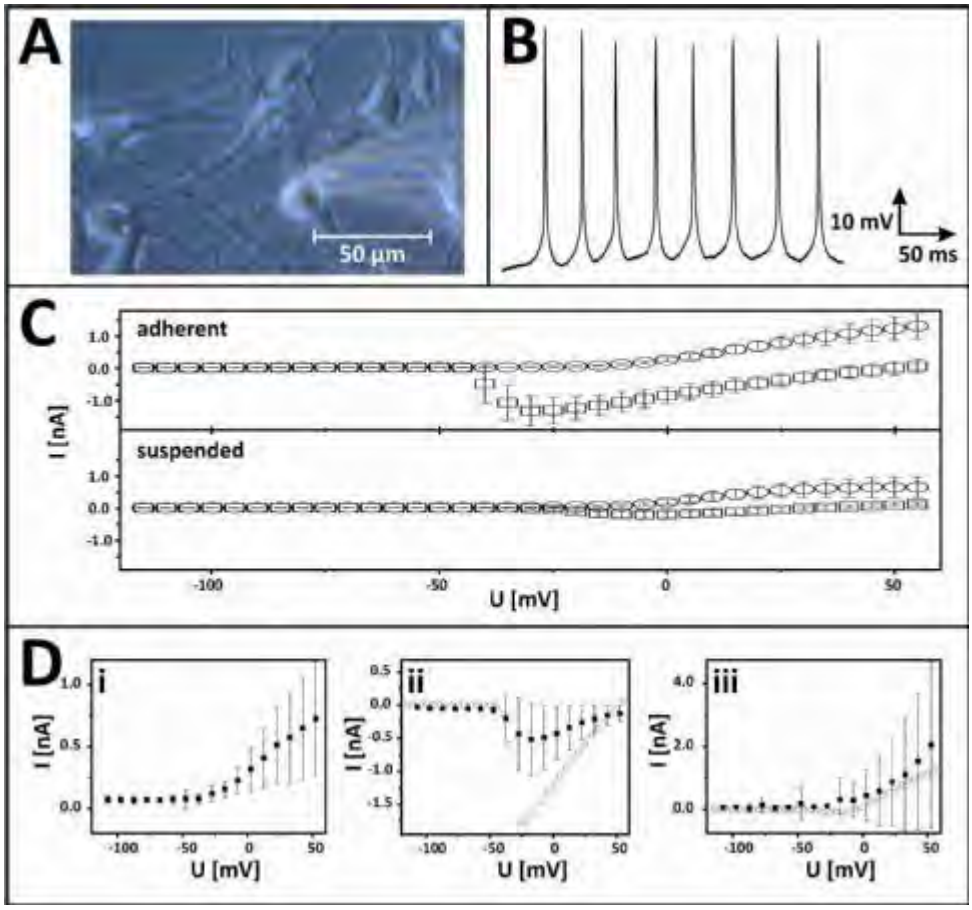
[1]University Medicine Rostock, Oscar Langendorff Institute of Physiology, Gertrudenstraße 9, 18057 Rostock, Germany

[2]Sophion – Biolin Scientific, Baltorpvej 154, 2750 Ballerup, Denmark

[3]Chair of Biophysics, University of Rostock, Gertrudenstraße 11, 18057 Rostock, Germany

Human induced pluripotent stem cells (hiPSCs) are a new option for modeling the cellular effects of human neurodegenerative diseases. hiPSCs are obtained by reprogramming the somatic cells of individual patients, and the hiPSCs then can be redifferentiated into neuronal cells. Automated patch clamp measurements were used for the electrophysiological characterization of hiPSC-derived dopaminergic neurons (Dopa.4U neurons, Axiogenesis, Cologne, Germany), which may be important for Parkinson research. Manual patch clamp measurements were used to verify the results. Dopa.4U neurons expressed voltage-gated sodium (Na_V) and potassium (K_V) channels and showed neuron-like spontaneous electrical activity. In suspended Dopa.4U neurons, delayed rectifier K^+ current (delayed K_V) and rapidly inactivating A-type K^+ current (fast K_V) were identified. Examination of the fast K_V current with inhibitors yielded IC_{50} values of 0.4 mM (4-aminopyridine, n=10) and 0.1 mM (tetraethylammonium, n=7). In adherent Dopa.4U neurons, fast K_V current could not be detected, while the delayed K_V current showed an IC_{50} of 2 mM (n=9) for 4-aminopyridine. The Na_V channels in adherent and suspended Dopa.4U neurons showed IC_{50} values for tetrodotoxin of 27 nM (n=4) and 2.9 nM (n=9), respectively. GABA-induced currents could be detected in adherent but not in suspended Dopa.4U neurons. Application of current pulses to the cells resulted in three diverse reactions (type I to type III) of induced action potentials. The automated patch clamp analysis provides qualitative and quantitative electrophysiological parameters of single neurons. Here, it enabled detection of the fast K_V channel. In contrast, the ligand-gated GABA receptors could only be investigated with the conventional approach because automation required the cells to be in suspension. Our results proved the feasibility of automated electrophysiological characterization of neuronal cells.

Figure text: Automated and manual patch clamp data of human induced pluripotent stem cell-derived dopaminergic neurons. **A** Patched Dopa.4U neuron in neuronal network after 10 DIV. **B** Whole-cell recording after 5 DIV showed spontaneous electrical activity. **C** Current-voltage relationship of adherent and suspended Dopa.4U neurons detected with the manual patch clamp indicated same ion channel composition, but with altered magnitude. **D** Current-voltage relationship of suspended Dopa.4U neuron detected with the automated QPatch system showed three voltage-gated ion channel types: **i)** Fast voltage-gated potassium channel; **ii)** Voltage-gated sodium channel (dotted line represents detection of suspended single cell measured with manual patch clamp); and **iii)** Delayed voltage-gated potassium channel (dotted line represents detection of suspended single cell measured with manual patch clamp).


A
50 µm
B
10 mV
50 ms
C
adherent
suspended
I [nA]
U [mV]
D
i
ii
iii
I [nA]
U [mV]

Nr2f1 transcriptional gradient in the developing mouse cerebral cortex depends on histone demethylase KDM1a activity

Henriette Franz[1], Tanja Vogel[1]

[1]Albert-Ludwigs University Freiburg - Institute for Anatomy and Cell biology, Department Molecular Embryology, Albertstrasse 17, 79104 Freiburg, Germany

Nr2f1 transcriptional gradient in the developing mouse cerebral cortex depends on histone demethylase KDM1a activity

Arealisation of the murine cerebral cortex is under control of morphogenetic gradients such as SHH (sonic hedgehog), FGFs (fibroblast growth factors) and BMPs (bone morphogenetic proteins). These important developmental signals induce subsequently gradients of transcription factors in the developing neocortex. Further regionalized gene activities result in functionally specialized areas of the neocortex that are recognized by variations in the neuronal composition. Here we present data showing that histone modifications of lysine 4 of histone H3 (H3K4) occur in a caudal-high to rostral-low gradient in the E14.5 developing mouse cerebral cortex. Both, H3K4 di-(me2) and tri-methylation (me3) activate gene transcription. We determined genome-wide distribution of H3K4me3 in the rostral and caudal E14.5 developing cortex and correlated this data to transcriptional alterations between both brain regions. We identified twelve developmental genes as differentially methylated and expressed between rostral and caudal regions, including the transcription factor Nrf2f1 (Nuclear Receptor Subfamily 2 Group F Member 1). Nrf2f1 was transcribed in a rostral-low to caudal-high gradient. Among the H3K4 modifying enzymes, activity of which could be responsible for the differential H3K4me2/me3 along the rostro-caudal axis, we identified Kdm1a, a H3K4me2 demethylase. Kdm1a was expressed in an opposing gradient, with rostral-high to caudal-low levels, both in mRNA and protein. Chromatin-immunoprecipitation followed by quantitative real-time PCR confirmed KDM1A location at the promotor of Nr2f1. KDM1A levels were lower in the rostral compared to the caudal telencephalon. Pharmacological inhibition of KDM1A in vivo with the specific inhibitor ORY-1001 during mouse brain development disrupted the Nr2f1 transcriptional gradient at E14.5. Together our data suggest an important role of KDM1A enzymatic function during corticogenesis by regulating Nr2f1 expression in a rostro-caudal gradient.

Appropriate markers to identify glioblastoma stem cells in vitro

Diana Freitag[1], Fritz Klippel[1], Rolf Kalff[1], Christian Ewald[2], Jan Walter[1]

[1]Universitätsklinikum Jena, Klinik für Neurochirugie, Am Klinikum 1, 07747 Jena, Germany
[2]Hochschulklinikum der MHB Theodor Fontane, Klinik für Neurochirurgie, Hochstrasse 29, 14770 Brandenburg an der Havel

Objective: Cancer stem cells are regarded as a reason for therapy failure and the malignant biology of glioblastoma multiforme. This aggressive tumor entity is often associated with the known cancer stem cell marker CD133 or with the ability to form spheroids. However, in recent years the relevance of the surface protein CD133 has been increasingly questioned and the need for one or even more suitable markers for glioblastoma stem cells has become more and more present.
This study aimed to analyze possible markers with respect to their gene expression level and to validate suitable candidate genes characterizing tumor stem cell properties in a glioblastoma stem cell model.
Methods: Isolated glioblastoma stem cell-like cells (GSCLCs; n=5), spheroidal cells of immortal glioblastoma cell lines (GCL_{sph}, n=4) and spheroidal cells of primary glioblastoma cultures (GPCs; n=6) were cultured. The cells were cultured under serum-free conditions to induce spheroidal growth. The different cell culture groups were characterized with respect to their physiological (MTT assay, migration assay, differentiation) and phenotypic (sphere formation assay, expression of specific marker proteins by immunofluorescence staining) properties. In the second step, stem cell and tumor specific markers (*CD133, MSI1, SOX2, OCT4, NANOG, NES, GFAP, NOTCH1*) were analyzed and statistically validated at their gene expression level (quantitative polymerase chain reaction) (Mann-Whitney-U-Test, Spearmann correlation).
Results: All three investigated cell groups formed spheroids, one of the main characteristics of tumor stem cell cultures. Nevertheless, the GSCLCs needed in average 12d longer (p=0.012) and formed in average larger spheres from which the cells emigrated more strongly (p=0.001). In addition, compared to the GCL_{sph} and GPC_{sph}, they showed a ten times less metabolic activity (p=0.004 to 0.016). This clearly corresponds to the stem cell-like character. However, the immunofluorescence staining showed that the previously used stem cell markers CD133 and SOX2 showed no differences in expression between the groups GSCLCs, GCL_{sph} and GPC_{sph}. Thus, a differentiation between glioma stem cells and spheroid glioma cells was not possible using these markers. However, the subsequent mRNA expression analysis clearly showed that there were no significant differences in the relative gene expression between the study groups for the stem cell and pluripotency-specific genes *NANOG, SOX2, OCT4* and *CD133*. In contrast, significantly higher relative expression for the genes *MSI1, NOTCH1* and *NES* in the GSCLCs compared to the GPC_{sph} and GCL_{sph} could be determined. All three genes showed a highly significant positive correlation to each other.
Conclusion: The collected data clearly show that the GSCLCs have the typical tumor stem cell characteristics, but the expression of the marker proteins does not clearly separate them from other similar cells. After evaluation of the additional mRNA expression data *MSI1* seems to be a valid marker for the identification of glioblastoma stem cells, especially in combination with increased expression of the genes *NES* and *NOTCH1*.

Recovery of olfactory induced behavior indicates successful network restoration after olfactory nerve transection in larval *Xenopus laevis*

Sara Joy Hawkins[1], Yvonne Gärtner[1], Lukas Weiss[1], Thomas Hassenklöver[1], Ivan Manzini[1,2]

[1]Institute of Animal Physiology, Department of Animal Physiology and Molecular Biomedicine, University of Giessen, 35392, Giessen

[2]Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), University of Göttingen, 37073, Göttingen

The olfactory system is ideal to study the process of neuronal recovery, as it is known for its lifelong capacity to replenish olfactory receptor neurons lost during natural turnover, as well as its remarkable ability to regenerate after severe lesion. Although the regenerative capacity of the olfactory system has been illustrated numerous times in various organisms, many questions still remain as to how this system is capable of recovering from damage. We previously investigated the timing of degeneration and subsequent regeneration of the olfactory system of larval *Xenopus laevis* after transection of the olfactory nerve. We showed, using fast two-photon Ca^{2+} imaging, that activity in the olfactory bulb in response to olfactory stimuli is lost after nerve transection due to the loss of synaptic input, and that it can again be seen between 3 and 7 weeks after transection. These results indicate that there is some degree of successful rewiring of the olfactory network after lesion, but leave open questions as to how accurately this system can recover.

We now describe how the different cells that make up the olfactory bulb are affected by the loss of synaptic input and how they recover from olfactory nerve transection. Using fast two-photon Ca^{2+} imaging we show that the bimodal processing stream of olfactory information into a lateral and a medial stream described in healthy larval *X. laevis* is again present after the complete re-innervation of the olfactory bulb post-nerve transection. We have developed behavior analysis methods and successfully identified odorant dependent behavior in healthy larval *X. laevis*. We find that the specific behavioral responses to olfactory stimuli that the larvae exhibit, are lost after nerve transection, and subsequently recover in 6-7 weeks. Together, our results show that not only is activity again visible in the olfactory bulb after recovery, but that re-innervation is accomplished with a remarkable accuracy, illustrated by the restoration of the lateral and medial olfactory processing pathways, and the recovery of the ability to successfully detect odorants in the environment.

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Loss of Brg1 in hGFAP-positive cells impairs cerebral and cerebellar development

Dörthe Holdhof[1,2], Melanie Schoof[1,2], Malte Hellwig[1,2], Ulrich Schüller[1,2,3]

[1]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Germany
[2] Research Institute Children's Cancer Center Hamburg, Germany
[3] Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Germany

The SWI/SNF (switch/ sucrose non-fermenting) complex is involved in regulation of gene expression, DNA repair, differentiation and development by functioning as an ATP-dependent chromatin remodeler. Brg1 (Brahma related protein, SMARCA4) is one of the two mutually-exclusive catalytic subunits and has been described as a tumor suppressor in e.g. rhabdoid tumors or a subtype of ovarian cancer. Furthermore, BRG1 mutations have been identified in patients with Autism Spectrum Disorders (ASD) or Coffin-Siris-Syndrome (CSS) highlighting the diverse and context-dependent roles of Brg1.
Here, we investigate the functional involvement of Brg1 in brain development. For this purpose, we used the Cre/ loxP system to delete Brg1 in hGFAP-positive multi-potential stem cells starting at embryonic day (E) 13.5. A heterozygous Brg1 loss in hGFAP-cre::Brg1 fl/wt mice did not result in any alterations of the brain or survival compared to wildtype animals. hGFAP-cre::Brg1 fl/fl mice with a homozygous knockout were also born with the expected Mendelian Ratio, but died approximately two weeks after birth. Macroscopic examination of hGFAP-cre::Brg1 fl/fl brains revealed a severe hydrocephalus and a significantly decreased brain weight from postnatal day 7 onwards. Histological analyses uncovered several anomalies in the brain. The cerebrum presented with a significantly decreased cortical thickness accompanied by an altered neuronal morphology. Furthermore, the hippocampus was severely underdeveloped in mutant mice and, finally, the cerebellum of hGFAP-cre::Brg1 fl/fl mice was hypoplastic recapitulating a phenotype already known from other mouse models investigating Brg1 deficiency. This study illustrates the importance of Brg1 in different aspects of brain development. As a next step, functional studies are necessary to identify molecular pathways in order to understand how BRG1 mutations cause disease features in ASD or CSS.

Generation of functionally active and mature neurons from ADHD patients carrying copy number variants of *SLC2A3* to study its impact on neuronal metabolic as well as neurodevelopmental processes

Charline Jansch[1], Andrea Forero[1], Sina Kollert[2], Sina Wäldchen[3], Jonas Waider[1], Frank Edenhofer[4], Erhard Wischmeyer[2], Klaus-Peter Lesch[1]

[1]Division of Molecular Psychiatry, Center of Mental Health, University of Würzburg, Würzburg, Germany
[2]Institute of Physiology, Molecular Electrophysiology, University of Würzburg, Würzburg, Germany
[3]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Würzburg, Germany
[4]Department of Genomics, Stem Cell Biology and Regenerative Medicine, Institute of Molecular Biology & CMBI, Leopold-Franzens-University Innsbruck, Innsbruck, Austria

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder that is characterized by inattention, hyperactivity and increased impulsivity. Several genes have been associated with an increased risk for ADHD. One of those so-called candidate or risk genes is *SLC2A3*, which encodes the glucose transporter-3 (GLUT3). Although, GLUT3 has been found to be expressed in a variety of cell types with very specific and high energy needs, it is most specifically expressed in neurons where it facilitates the transport of glucose across the plasma membrane. Given its essential role in neuronal activity, dysfunction of GLUT3 might impact neuronal metabolic processes, as well as maturation and synaptogenesis of neurons and might therefore contribute to the pathophysiology of neuropsychiatric disorders. To examine the effect of an altered *SLC2A3* expression on glucose metabolism as well as on neurodevelopmental processes, we have generated human induced pluripotent stem cells (iPSCs) from fibroblasts of ADHD patients carrying either a duplication or a deletion of *SLC2A3* and differentiated those pluripotent cells into neurons. All generated iPSC lines display key features of pluripotent cells, such as a typical embryonic stem cell (ESC)-like morphology and growth behaviour, homogeneously expressed pluripotency specific markers as well as the ability to differentiate towards mesodermal, endodermal and ectodermal layer specific cells. Since serotonin (5-HT) neurons and their vast innervation have been implicated in the etiology of several psychiatric disorders, we have specifically aimed to establish a robust protocol to efficiently generate 5-HT neurons from human iPSCs. Obtained 5-HT neurons express specific markers for serotonergic neurons, such as tryptophan hydroxylase 2 (TPH2), exhibit typical electrophysiological characteristics and show synaptic structures after only a few weeks of neuronal maturation. Besides the subset of 5-HT neurons, our protocol additionally yields in a similar high amount of catecholaminergic neurons - most likely dopaminergic neurons - and a small proportion of GABAergic neurons. Examined *SLC2A3* mRNA expression levels have been found to be either increased or decreased in those human iPSC-derived neurons carrying a duplication or a deletion of this gene, respectively. Additional exposure to different glucose concentrations further changes expression levels of *SLC2A3*. Thus, we have developed a useful human iPSC-derived neuronal culture system not only to examine the implication of neurotransmitters, but also of an altered glucose metabolism in the pathogenesis of neuropsychiatric disorders, such as ADHD.

Stress impedes neuronal differentiation via *ZBTB16* in human cerebral organoids

Anthodesmi Krontira[1,2], Cristiana Cruceanu[1], Simone Röh[1], Silvia Matrinelli[1], Elisabeth Binder[1,3]

[1]Max Planck Institute of Psychiatry, Department of Translational Psychiatry, RG Binder, Kraepelinstr. 2, 80804 Munich, Germany

[2]International Max Planck Research School of Translational Psychiatry (IMPRS-TP)

[3]Department of Psychiatry and Behavioral Sciences, Emory University, School of Medicine, Atlanta, GA.

Background/Hypothesis: Stress during early development has been linked to poor outcomes and vulnerability to mental illness, suggesting a dysregulation of a critical process during a critical period. The hypothalamic-pituitary-adrenal (HPA) axis is a major component of the stress response. The HPA axis is activated by stress hormones called glucocorticoids (GCs) and has been implicated extensively in the causality and the treatment of depression. *ZBTB16* (zinc finger and BTB domain containing 16) is a transcription factor activated by GCs that regulates the differentiation of Neural Progenitor Cells (NPCs) to neurons. We hypothesize that GCs impede the neuronal differentiation pathway and that this trajectory change is mediated via *ZBTB16*.

Methods/Results: The human Cerebral Organoids (COs) are 3D in vitro neuronal cultures from human induced pluripotent stem cells (hiPSCs). The COs are derived by differentiation of hiPSCs and self-organization into multi-dimensional tissue. The COs recapitulate hallmarks of human neurogenesis and brain anatomy, making them an informative model of the developing human brain. We sequenced the transcriptome of COs in different developmental time points and observed that *ZBTB16* is dynamically expressed. *ZBTB16* expression is high in the early developmental stages and it is co-expressed with NPCs' markers, such as *SOX2* and *PAX6*, whereas its expression is decreased when the neuronal markers, such as *MAP2* or *SATB2*, arise. Immunohistochemical analysis of the COs at different developmental stages shows *ZBTB16* is expressed by the NPCs in the basal side of the Sub Ventricular Zone, as it is co-expressed with *TBR2* and *HOPX*. We mimicked the activation of the HPA axis via dexamethasone treatment. Dexamethasone is a synthetic glucocorticoid receptor (GR) agonist that binds selectively to the GR and activates the HPA axis. Acute (4 and 12 hours) and chronic (7 days) dexamethasone treatment of the organoids at 2 different developmental time points increases *ZBTB16* RNA expression as seen by q-PCR. In addition, dexamethasone treatment increases ZBTB16 expression in the NPCs and results in decreased neuronal differentiation as shown both in 2D human neuronal cultures and in organoids.

Conclusions: In conclusion, glucocorticoid exposure during neurodevelopment alters the neuronal differentiation pathway via *ZBTB16*.This could pose a potential mechanism on how stress during development affects the progression of mental diseases in adulthood.

Characterization of Electrophysiological Properties Of Human iPSC-derived Neurons in Autaptic Culture

Hong Jun Rhee[1], Ali Shaib[1], ChoongKu Lee[1], Oliver Bruestle[2], Nils Brose[1], JeongSeop Rhee[1]

[1]Molecular Neurobiology, MPI Experimental Medicine, Goettingen, Germany
[2]Institute of Reconstructive Neurobiology, Univ. Bonn, Bonn, Germany

Studies of human CNS are fundamental demands to understand variety of neuropsychiatric and neurological disorders but there are limits to investigate development and function of human. Instead of acute human brain study, stem cell researches have been suggested and provided as an alternative approaches to solve these limits. Besides classical embryonic stems (ESs) studies, induced pluripotent stem cells (iPSCs) and induced neurons (iNs) technologies have been shown to generate several types of neurons from epithelial cells.
In this study, we performed the morphological and functional quantitative analyzes in induced human cortical neurons using both human iPSC-derived glutamatergic and GABAergic neurons and induced glutamatergic and GABAergic neurons. In addition, we established the single cell autaptic neuronal culture system, which is suitable for understanding the most important parameters underlying synaptic communication in a quantitative fashion.

Characterization of Morphological Properties of Human iPSC-Derived Neurons in Autaptic Culture System

Ali Shaib[1], Hong Jun Rhee[1], ChoongKu Lee[1], Peter Seif[1], Oliver Bruestle[2], Nils Brose[1], JeongSeop Rhee[1]

[1]Max Planck Institute of Experimental Medicine, Department of Molecular Neurobiology, Göttingen, Germany
[2]Institute of Reconstructive Neurobiology, University of Bonn Medical Faculty, Bonn, Germany

Bypassing the animal testing to study human brain diseases, the development of the human-induced pluripotent stem cells-derived or Neurogenin-2 forcefully-induced neurons methods have overcome the limitations of the direct use of human neurons. However, beyond the authenticity of the neuron formation, the specific properties of each neuron by itself are still illusive. So, we introduce an autaptic human neuronal culture system with single neurons grown on astrocyte feeder islands. We performed extensive morphological and functional characterization of cultured neurons. Whereby, we studied synaptic release parameters triggered by action potentials, morphological analysis, neuron maturation, dendrite complexity and synaptogenesis during developmental process. The current study provides for the first time systematic and reproducible tools that enable isolating delicate electrophysiological and morphological properties from derived human neurons, setting a solid model for investigating psychological disorders.

Wharton's Jelly - source of MSC which are able to differentiate in NSC.

Adam Osowski[1], Ewa Kruminis-Kaszkiel[1], Ewa Bejer-Olenska[1], Joanna Wojtkiewicz[1]

[1]Department of Pathophysiology, School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, 30 Warszawska Str., 10-082 Olsztyn, Poland

Neural stem cells (NSC) belong to multipotent stem cells and play a unique role in regenerative medicine, because they are able to differentiate towards neurons, astrocytes and oligodendrocytes. Transplantation of neural stem cells capable of regenerating CNS cells is a promising strategy of CNS diseases and injuries treatment as well as CNS diseases modelling. Among all possible methods of generating neural stem cells, differentiating them from mesenchymal stem cells (MSC) seems to be an effective and less problematic method.

The aim of this study was to develop feasible, efficient and repeatable method for inducing MSC derived from Wharton's jelly (hWJ-MSC) differentiation towards NSC-like cells. Induction of hWJ-MSC in a monolayer culture with adhesive conditions using growth factors: EGF, bFGF and supplements N2, B27 was performed. After 10 days, phenotype of cultured cells was examined using microscopy, flow cytometry, immunocytochemistry and qPCR.

The induction method enabled to obtain cells with some distinct morphological features, ability to proliferate and expression of certain neural markers on protein and mRNA level. Flow cytometry and immunocytochemistry confirmed the expression of neural markers such as nestin, SOX2, SOX1, MAP2, GFAP. Gene expression analysis using qPCR revealed significantly enhanced expression of nestin and MAP2 in differentiatied cells.

To sum up, it is possible to obtain cells with NSC-like phenotype through inducing hWJ-MSC in 2D culture using practical method. However, therapeutic effectiveness of such cells should be extended with in vitro research to confirm ability to terminal differntiation of NSC-like cells and electrophysiological properties of neurons derived from them. Safety and efficacy of transplanted generated NSC-like cells should be determined in animal models.

Profilin1 mutant mice display features of a gyrencephalic neocortex

Marco Rust[1,2,3], Sophie Meyer[1], Jan Kullmann[1,3], Fabrizia Pipicelli[4], Felix Schneider[1,2,3], Nora Bartels[1], Silvia Cappello[4]

[1]Philipps-University Marburg, Institute of Physiological Chemistry, Karl-von-Frisch-Str. 1 , 35043 Marburg, Germany; [2]DFG Research Training Group, Membrane Plasticity in Tissue Development and Remodeling, GRK 2213; [3]Center for Mind, Brain and Behavior (CMBB), Research Campus of Central Hessen (RCCH), Germany; [4]Max-Planck Institute for Psychiatry, 80804 Munich, Germany

The neocortex is unique to mammals and relevant for higher cognitive functions. Evolutionary expansion of the neocortex resulted in a highly convoluted, gyrencephalic structure as present in primates. The mechanisms underlying neocortex gyrification largely remained unknown. We report that genetic depletion of the cytoskeletal regulator profilin1 was sufficient to induce features of a primate neocortex in mice, including an enlargement of the basal radial glia (bRG) pool and an additional germinal zone within the subventricular zone. Transient bRG overproduction in profilin1 mutants increased neurogenesis and induced neocortex folds resembling rudimentary gyri. We found a critical role for profilin1 in actin filament (F-actin) assembly in neural stem cells and in division of apical radial glia (aRG), the progenitors of bRG. We propose a model, in which profilin1-dependent F-actin assembly controls aRG division, and thereby profilin1 restricts bRG-derived neurogenesis and suppresses gyrification in mice. Our data strongly support the 'radial cone hypothesis' claiming that elevated bRG activity is responsible for neocortex gyrification. Moreover, they identified F-actin assembly as a novel mechanism relevant for evolution of the gyrencephalic neocortex.

Neurogenic effect of Wnt signaling pathway on isolated murine and human progenitor cells of the enteric nervous system

Melanie Scharr[1], Peter Neckel[1], Katharina Nothelfer[1], Ying Zhang[1], Karin Seid[1], Florian Obermeyr[2,3], Lothar Just[1]

[1]Department of Anatomy, Institute for Clinical Anatomy and Cell Analysis, University of Tübingen, Germany
[2]Department of Pediatric Surgery, University Children's Hospital Tübingen, Germany
[3]Department of Pediatric Surgery, University Children's Hospital, Marburg, Germany

Stem and progenitor cells of the postnatal enteric nervous system (ENS) can be isolated from gastrointestinal tract and subsequently expanded and differentiated in cell culture.
However, little is known about their physiological role and the cellular and molecular mechanisms involved in their homeostasis. Increasing evidence indicates that the Wnt signaling pathway has a regulatory effect on the proliferation of enteric neuronal progenitor cells *in vitro*.

Therefore, we investigated the influence of Wnt signaling on the proliferation of isolated postnatal ENS progenitors from murine and human intestine using gene-chip analysis,
BrdU-incorporation assays, immunohistochemistry, Western blot, RT-PCR and FACS experiments.

Gene expression analysis of proliferating enterospheres verified mRNA expression of
Wnt-receptors and upregulation of known Wnt-target genes axin2, lef1, and lgr5 after activation of Wnt signaling pathway. These results provided molecular evidence for the activity of the canonical Wnt pathway in ENS derived progenitor cells. In addition, our cell culture experiments demonstrated that activation of canonical Wnt signaling increases the proliferation of enteric neural progenitors and leads to a higher yield of differentiated neurons *in vitro*, both in human and mouse model. Based on these results, we identified the Wnt-receptor frizzled-4 as a potential novel marker expressed on human postnatal ENS progenitor cells. To elucidate the role of frizzled-4, we used histological evaluation and FACS to show the Wnt-receptor frizzled-4 is expressed partly overlapping with the putative stem cell marker $P75^{NTR}$ in the human colon and in tunica muscularis derived enterospheres. To obtain a purified culture, we carried out FACS experiments using PE-conjugated frizzled-4 antibodies. Frizzled-$4^{positive}$ cells gave rise to neurosphere-like bodies and ultimately differentiated into neurons as revealed by BrdU-proliferation assays and immunocytochemistry, whereas in frizzled-$4^{negative}$ cultures we did not detect any neural cells.

Our results give first insights in the Wnt dependent regulation of neuronal progenitor cells derived from the neonate and adult ENS. Moreover, we show that frizzled-4 is expressed by this cell pool and can be utilized as a marker for their isolation.
In ongoing experiments, we are analyzing the frizzled-$4^{positive}$ cell pool as well as the intrinsic Wnt regulation of the enteric nervous system in more detail.

EGFL7: a novel modulator of neural homeostasis in the hippocampus

Mirko HH Schmidt[1], Verica Vasic[1], Frank Bicker[1]

[1]University Medical Center, Institute for Microscopic Anatomy and Neurobiology, Langenbeckstr. 1, 55131 Mainz, Germany

Neurogenesis in the adult brain originates from neural stem cells (NSCs) residing in specialized niches, i.e., the subventricular (SVZ) or subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus. Throughout life these cells give rise to adult-born interneurons in the olfactory bulb (OB) or granule cells in the DG in a Notch-dependent manner, thus contributing to neural plasticity and pattern discrimination.
The Notch ligand EGFL7 is secreted at high levels by neurons and blood vessels in the adult brain (Nat Cell Biol, 2009). EGFL7 edits NSC niches by modulating surface Notch receptors and ligands. Loss-of-EGFL7 (EGFL7-/-) caused an accumulation of activated NSCs (aNSCs) in the SVZ and promoted Dll4-induced Notch signaling at the blood vessel-stem cell interface. Less inhibitory neurons formed in the OB of adult EGFL7-/- mice, which increased the signal conducted from the mitral cell layer but decreased neuronal network synchronicity. Consequently, EGFL7-/- mice displayed physiological defects in olfactory behavior and perception (Nat Commun, 2017).
In comparison, the amount of aNSCs and adult-born neurons in the SGZ of the DG was found increased in constitutive and tissue-specific EGFL7-/- mice. RNASeq analyses revealed that promitotic cytokines enlarged the aNSCs pool. This was accompanied by an increase in adult-born neurons in the DG and enhanced neural spine density. Interestingly, EGFL7-/- mice displayed greater intelligence as measured in Morris water maze, touchscreen or Intellicage assays.
In conclusion, EGFL7 is a feedback protein secreted by mature granule cells in the DG to contain excessive, unproductive neurogenesis from adult NSCs.

Analysing schizophrenia risk variants in NRXN1 using functional and mature neuronal cultures from patient-derived iPS cells

Annika Liisa Majer[1], Matthias Jung[1], Jessica Reinsch[1], Jovita Schiller[1], Ina Giegling[1], Dan Rujescu[1]

[1]Martin Luther University Halle-Wittenberg

Schizophrenia is a complex psychiatric disorder that effects about 1% of the world's population. The highly disabling and heritable disease is clinical characterized by two symptom complexes. Positive symptoms include delusions and hallucinations. Negative symptoms show emotional, social and motivational deficits and often lead to strong impairment of patient's life. Although pharmacological treatment is available, therapies are often inadequate, which highly recommends the analysis of molecular and cellular disease mechanisms. There is strong evidence that heterozygous deletions in the Neurexin 1 (NRXN1) gene contribute to onset and progression of schizophrenia.
Human induced pluripotent stem cells (iPS cells) provide a tool for investigating healthy and altered function of NRXN1 for the analysis of known and unknown disease mechanisms.

We reprogrammed B-lymphoblasts obtained from schizophrenia patients carrying heterozygous deletions in NRXN1. We proved pluripotency and generated cortical neurons. Transcript and protein analysis were applied for the characterization of neurons from healthy subjects and NRXN1 carriers. Cells were differentiated into mature neurons by using a four-step culture system via iPS cells and neural stem cells.

Human iPS cells were succesfully differentiated into derivatives of the three germ layers and showed specific pluripotency markers (OCT4, NANOG, and SOX2) on RNA and protein level. Human iPS cells were differentiated into neural stem cells, neural progenitor cells and mature neurons. Transcript and immunofluorescence analysis of specific markers confirmed glial and neuronal cells (TUBB3, STX, GFAP, CD68). The presence of different neuronal subtypes such as GABAergic and glutamatergic neurons was shown (SLC17A7, GAD1, GABBR1, GRIA2 and GRIN1).

In summary, schizophrenia-specific iPS cells and their differentiation into neurons has potential to elucidate the impact of specific genetic variations and to enable the identification of potential therapeutic targets in schizophrenia.

Assessment of electrophysiological properties of human iPSC-derived serotonergic neuron model

Evgeniy Svirin[1,2], Sina Kollert[1,2], Charline Jansch[1], Erhard Wischmeyer [1], Tatyana Strekalova[1,2,3], Klaus-Peter Lesch [1,2,3]

[1]University of Würzburg, Center of Mental Health, Division of Molecular Psychiatry, Würzburg, Germany
[2]I.M. Sechenov Moscow State Medical University, Laboratory of Psychiatric Neurobiology, Moscow, Russia
[3]Maastricht University, School for Mental Health and Neuroscience (MHeNS), Department of Translational Psychiatry, Maastricht, The Netherlands

The serotonergic system of the brain plays a crucial role in regulating brain functions and has been implicated in various psychiatric disorders, including ADHD and other neurodevelopmental disorders. Thus, studying the role of risk genes in functional properties of human serotonergic neurons is of fundamental importance for understanding their pathogenesis. However, human serotonergic neurons are not readily available for in vitro studies. Here, we established an in vitro serotonergic neuron model derived from human induced pluripotent stem cells (hiPSC) for electrophysiological assessment. This model can be useful for evaluation of the impact of risk genes in a patient-specific manner.
Serotonergic neurons from three different differentiations from a hiPS cell line were used. Electrophysiological patch-clamp recordings were performed to investigate functional maturation of induced serotonergic neurons. Whole-cell patch clamp recordings were performed weekly within six weeks and properties of serotonergic neurons were analyzed according to electrophysiological criteria discussed in literature, of which action potentials half-high width (HHW) > 1.2 ms and firing rate < 12 Hz show the lowest error rate in identifying serotonergic neurons.
We recorded repetitive firing elicited by current injections, as well as spontaneous firing in neurons over the whole measurement period. Frequency of measured action potentials varied over time but always matched the criteria for serotonergic neurons and was less than 12 Hz. In course of six weeks the amplitude of action potentials increased and HHW decreased, however action potentials with amplitude and HHW typical for serotonergic neurons were recordable during all six weeks.
The hiPSC-derived serotonergic neurons exhibited an electrophysiological signature characteristic for raphe serotonergic neurons as previously reported from in vivo studies. However, cell types cannot be distinguished based on their electrophysiological properties alone, so further validation e.g. by using specific serotonergic agonists/antagonists is ongoing.

Is the coat color reflecting neuronal layering in the olfactory bulb in the Female American Mink (*Neovison vison var. spec.*)?

Elke Weiler[1], Willi Bennegger[2,3]

[1]Max-Planck-Institut für Biologische Kybernetik, Physiologie kognitiver Prozesse, Max-Planck-Ring 8, 72076 Tübingen, Germany; [2]Faculty of Natural Sciences, Institute of Neurobiology, University of Ulm, Ulm, Germany; [3]Maria-von-Linden-Schule, Heckentalstr. 86, 89518 Heidenheim, Germany

A key organizational feature of the brain is the layering pattern of each area. Changes in layering indicate changes in function and developmental or pathological phenomena. The layering reflects neuronal composition and depends on several factors, including specific genes, growth factors and enzymes. In a few areas the neuronal system is a „colored“ system, such as the substantia nigra which received its name by the dark color based on the neuromelanin of dopaminergic neurons. Melanin is a pigment of skin and hair, so the question arose, if there might be a correlation of the coat color and brain areas containing dopaminergic neurons such as the olfactory bulb. Therefore we investigated the olfactory bulbs of female American minks bred specifically for their coat color and measured the absolute layer volumes of the four color varieties: dark black “standard” (*Neovison vison var. atratus*), light black “silverblue” (*Neovison vison var. glaucus*), light brown “pastel” (*Neovison vison var. suffuscus*), dark brown “wild” (*Neovison vison var. carinum*) using a morphometric system. The volume of the glomerular layer, including the periglomerular dopaminergic neurons, revealed a significant difference between the pale brown variety (*suffuscus*: 20.68 ± 4.73 mm^3) versus the black varieties (*glaucus*: 14.79 ± 0.91 mm^3 and *atratus*: 15.35 ± 1.21 mm^3). Significant differences were also observed in the mitral cell layer (including passing periglomerular cells) of *suffuscus* (5.30 ± 1.55 mm^3) versus the black varieties *glaucus* (3.54 ± 0.65 mm^3) and *atratus* (3.78 ± 0.37 mm^3) and in the internal plexiform layer (*suffuscus*: 5.36 ± 0.86 mm^3; significant different versus *glaucus*: 3.54 ± 0.65 mm^3 and *atratus*: 2.90 ± 0.33 mm^3). No differences were found among any of the color varieties in the volumes of the fila, external plexiform, granule cell and subependymal layer, which are all composed of much fewer or no dopaminergic neurons. Our results indicate that, based on gene expression, the coat color might reflect neuronal structures and, potentially, different information processing.

Poster Topic

T2: Axon and Dendrite Development, Synaptogenesis

T2-1A SHANK3 transient silencing is accompanied by alterations in adhesion molecules partially restored by oxytocin
Jan Bakos, Martina Zatkova, Alexandra Reichova, Annamaria Srancikova, Veronika Meliskova, Zuzana Bacova

T2-2A Developmental neurotoxicity testing for axonal navigation defects in an intact locust embryo
Gerd Bicker, Karsten Bode, Michael Stern

T2-3A Remodeling of M1 layer Vb pyramidal cell axon initial segments and their axo-axonic innervation pattern after spinal cord lesion
Dominik Dannehl, Bruno Benedetti, Christian Thome, Jan Maximilian Janssen, Lara Sophie Bieler, Corinna Corcelli, Sébastien Couillard-Déspres, Maren Engelhardt

T2-1B Role of Ndr2 kinase in substrate-specific neurite growth and spine development
Yunus Demiray, Atsuhiro Tsutiya, Deniz Madencioglu, Dain Lee, Oliver Stork

T2-2B The morphology of pyramidal cells with axon-carrying dendrites in rat visual cortex
Eugenia Dutova, Ina Gasterstedt, Lisa Rennau, Steffen Gonda, Maren Engelhardt, Alexander Jack, Petra Wahle

T2-3B Neuroplastin Promotes Spinogenesis and Regulates E/I Synapse Balance through TRAF6
Rodrigo Herrera-Molina, Sampath Kumar Vemula, Ayse Malci, Lennart Junge, Anne-Christine Lehmann, Johannes Hradsky, Ricardo A. Matute, Ramya Rama, Michael Naumann, Constanze I. Seidenbecher, Eckart D. Gundelfinger

T2-4B Visual map formation without postsynaptic lamina neurons in *Drosophila*
Monika Kauer, Egemen Agi, Charlotte Wit, P. Robin Hiesinger

T2-1C GluK2-NETO2 signalling regulates dendritic spine morphology in developing hippocampus
Sebnem Kesaf

T2-2C retracted: The altered expression of cell adhesion molecule contactin-3 in tuberous sclerosis complex
Anatoly Korotkov, James D. Mills, Armand Blondiaux, Fanny Jaudon, Jasper J. Anink, Jackelien van Scheppingen, Constanze Seidenbecher, Lorenzo Cingolani, Erwin A. van Vliet, Eleonora Aronica

T2-3C Posttranslational modification of hyaluronan receptor CD44 modifies its functions in regulation of neuronal morphology.
Josephine Labus, Alexander Wirth, Saskia Borsdorf, Evgeni Ponimaskin

T2-1D *In vivo* time-lapse imaging of olfactory sensory neuron birth, differentiation and axogenesis
Thomas Offner, Sara Joy Hawkins, Lukas Weiss, Thomas Hassenklöver, Ivan Manzini

T2-2D Genetically encoded calcium indicators (GECIs) can impair developmental dendrite growth in rat cortical neurons
Petra Wahle, Tobias Stahlhut, Alexander Jack

T2-3D Molecular mechanisms underlying Ankyrin2-dependent control of synaptic plasticity
Tobias Weber, Johanna Buchheit, Raiko Stephan, Jan Pielage

T2-4D Roles of Dscams in the Development of *Drosophila* Central Neuron Dendrites
Nicole Wilhelm, Shikha Kumari, Carsten Duch

SHANK3 transient silencing is accompanied by alterations in adhesion molecules partially restored by oxytocin

Jan Bakos[1,2], Martina Zatkova[1,2], Alexandra Reichova[1], Annamaria Srancikova[1], Veronika Meliskova[2], Zuzana Bacova[1]

[1]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Dubravska cesta 9, 84505 Bratislava, Slovakia

[2]2Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Oxytocin is a small neuropeptide known for its role in control of social behavior and neuronal cell morphology. Alterations of oxytocin receptor signaling are supposed to contribute to the pathology of neurodevelopmental disorders. We have previously demonstrated that oxytocin increases neurite outgrowth and affects expression of actin-binding proteins. Given the known presence of oxytocin receptors in the presynaptic and postsynaptic membranes it could be hypothesized that oxytocin regulates scaffolding proteins connecting several membrane domains, ion channels and neuronal cytoskeleton. The aim of the present study was to investigate the oxytocin effects on cell morphology and expression of selected scaffolding proteins, adhesion molecules and GTPases in the model of induced downregulation of expression of SHANK3 scaffolding protein in SH-SY5Y cells. Transient silencing of SHANK3 with specific siRNA resulted in decrease of expression of SHANK3 without effect on SHANK1 and SHANK2 proteins. Silencing of SHANK3 has been accompanied by lower levels of postsynaptic protein Neuroligin3. Both SHANK3 and Neuroligin 3 decreases have been partially restored by oxytocin treatment. Incubation of cells in the presence of oxytocin stimulated expression of Neurexin 1α, 1β, 2α, 2β regardless of SHANK3 silencing. Oxytocin treatment significantly increased gene expression of RhoB. No effect has been observed for Rac1 and RhoA mRNA levels. GTPase RhoB is responsible for polymerization of actin, while RhoA is more important for depolymerization processes, therefore it can be concluded that oxytocin can be related to the actin remodeling and consequently to morphology changes of neuronal cells. Overall, it appears that oxytocin contributes to the regulation of expression of scaffolding proteins known to be associated with clusters of calcium channels at the cell membrane. The present data also suggest that SHANK deficit may be modulated by activation of oxytocin receptors. Supported by VEGA 2/0116/16, APVV-15-205 and APVV-15-0045.

Developmental neurotoxicity testing for axonal navigation defects in an intact locust embryo

Gerd Bicker[1], Karsten Bode[1], Michael Stern[1]

[1]University of Veterinary Medicine Hannover, Cell Biology, Bischofsholer Damm, 30173 Hannover, Germany

Developmental neurotoxicity (DNT) poses a serious threat to the health of children. However, far too few industrial chemicals have yet been tested, mainly because current assays require the use of high numbers of laboratory animals. The formation of a functional brain requires the precisely timed navigation of axons. We address this complexity by monitoring defects in axonal navigation of pioneer neurons of intact locust embryos after exposure to chemicals. Mechanisms of axonal guidance, such as growth cone navigation along molecular semaphorin gradients are evolutionary conserved. Thus, assays monitoring axonal navigation in insects will be indicative for the DNT potential of industrial chemicals in humans.
Locust embryos are kept in culture overnight in the presence of test chemicals, followed by biochemical viability measurement and immunolabeling of leg bud pioneer neurons. Defects in axonal outgrowth and navigation of pioneer axons are detected via fluorescence microscopy. Currently, the assay is being calibrated against a range of positive compounds with known DNT potential, and negative compounds, which are toxic, but have no specific DNT potential. For example, the mitochondrial respiratory chain inhibitor rotenone inhibited both pioneer neuron growth and correct pathfinding in the same concentration range as found in human neurons.
This insect assay will serve as complementary test system to other alternative DNT testing methods.

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Remodeling of M1 layer Vb pyramidal cell axon initial segments and their axo-axonic innervation pattern after spinal cord lesion

Dominik Dannehl[1,2,3,5], Bruno Benedetti[2,3,5], Christian Thome[4], Jan Maximilian Janssen[1], Lara Sophie Bieler[2,3], Corinna Corcelli[1], Sébastien Couillard-Déspres[2,3,5], Maren Engelhardt[1,5]

[1]Institute of Neuroanatomy, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
[2]Institute of Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria
[3]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria
[4]Institute of Physiology and Pathophysiology, Heidelberg University, Germany
[5]Equal contribution

The axon initial segment (AIS) is an electrogenic microdomain usually located at the proximal axon in most cortical neurons. Its molecular architecture, length, and position as well as synaptic innervation pattern make it a key player in modulation of neuronal excitability. Previous studies have shown that after changes in synaptic drive in vivo, AIS can undergo significant remodeling in terms of length and location, which corresponds to changes in cellular excitability of the affected neuron. To which extent the modification of axonal output parameters, e.g. the ability to propagate action potentials to postsynaptic targets can affect AIS structure and function remains unclear. Thus, our aim is to understand if M1 pyramidal neurons retain the ability to regulate their excitability when axonal output is compromised. To this end, 12 week old rats underwent laminectomy of C4 vertebra and wire-knife lesion of the dorsal corticospinal tract (CST). Lesioned CST neurons were visualized via retrograde tracer injection using hydroxystilbamidine (FluoroGold). Utilizing immunofluorescence, confocal microscopy, whole-cell patch-clamp recordings, and surface reconstruction of inhibitory synaptic complexes and the axonal cisternal organelle, we addressed several structural and functional AIS parameters. AIS length analysis showed that already 3 days after surgery, axotomized layer Vb pyramidal neurons have significantly longer AIS than non-lesioned controls; this condition persists throughout 5 and 7 days after surgery (lesioned AIS 3 days post-surgery 34.25 µm ± 0.94 µm vs. control 31.77 µm ± 0.92 µm STDEV, $p < 0.001$; lesioned AIS 5 days post-surgery 33.81 µm ± 0.31 µm vs. control 31.46 µm ± 1.3 µm STDEV, $p < 0.001$; lesioned AIS 7 days post-surgery 33.88 µm ± 0.85 µm vs. control 31.27 µm ± 0.70 µm STDEV, $p < 0.001$; One Way ANOVA;). Intriguingly, also pyramidal neurons in layer II/III dynamically adapt their AIS length in response to axotomy of infragranular pyramidal neurons, but in the opposite direction (lesioned animals 3 days post-surgery 31.90 µm ± 0.61 µm vs. control 30.50 µm ± 0.85 µm STDEV, $p = 0.004$; lesioned animals 5 days post-surgery 28.51 µm ± 0.51 µm vs. control 30.75 µm ± 0.3 µm STDEV, $p < 0.001$, One Way ANOVA). Strikingly, 7 days post-surgery, AIS return to control length in layer II/III (lesioned animals 31.05 µm ± 0.37 µm vs. control 30.75 µm ± 0.78 µm STDEV, $p = 0.920$, One Way ANOVA). Furthermore, the number of axo-axonic inhibitory GABAergic synapses at the AIS of axotomized neurons is significantly reduced 3, 5 and 7 days after surgery (lesioned AIS 7 days post-surgery 4.13 ± 1.62 synapses STDEV vs. control 7.14 ± 1.62 synapses STDEV, $p < 0.001$, Mann-Whitney Rank Sum Test). Whether these morphological changes are accompanied by electrophysiological adaptations is currently under investigation. Taken together, our results suggest that M1 pyramidal neurons of both supragranular and infragranular layers dynamically adapt their AIS length and innervation pattern in response to distal axotomy.

Role of Ndr2 kinase in substrate-specific neurite growth and spine development

Yunus Demiray[1], Atsuhiro Tsutiya[1], Deniz Madencioglu[1], Dain Lee[1], Oliver Stork[1,2]

[1]Departmen of Genetics and Molecular Neurobiology , Institute of Biology, Magdeburg, Germany

[2] Center for Behavioral Brain Sciences, Magdeburg, Germany

Neurons express a variety of α/β integrin heterodimers, which allow them to control neurite growth on different extracellular substrates. The nuclear Dbf2-related protein kinases 2 (Ndr2) has been suggested to play an important role in such integrin-mediated neuronal development and function. However, how Ndr2 determines the substrate specificity of the neurite growth has not been resolved yet. Here we show that stable overexpression of Ndr2 in PC12 cells increases the phosphorylated β1 integrin in the growth tips, reflecting the previously observed increase in neurite growth of primary neurons upon Ndr2 overexpression. By contrast, the expression of α1 integrin was markedly reduced in the growth tips of Ndr2 overexpressing PC12 cells, resulting in a reduced growth response to soluble and deposited α1β1 integrin substrates. Laminin-111 is a neural extracellular matrix substrate, which can specifically bind and induce growth through α1β1 integrin dimers. By culturing primary hippocampal primary neurons on PDL vs. Laminin-111 substrate, we could demonstrate that Ndr2 kinase also determines the substrate specificity of the dendritic growth of neurons, likely involving the above mentioned α1 and β1 integrin subunit modulation. Whether these mechanisms also translate to changes in synapse formation and function is currently under investigation. Overall, our findings confirm the previously observed increase in dendritic growth of neurons by Ndr2 kinase and suggest that Ndr2 is also involved in determining the substrate specificity of neurite growth.

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The morphology of pyramidal cells with axon-carrying dendrites in rat visual cortex

Eugenia Dutova[1], Ina Gasterstedt[1], Lisa Rennau[1], Steffen Gonda[1], Maren Engelhardt[2], Alexander Jack[1], Petra Wahle[1]

[1]Ruhr University, Developmental Neurobiology, Universitatsstraße 150, 44801 Bochum, Germany
[2]Institute of Neuroanatomy, Medical Faculty Mannheim, CBTM, Heidelberg University, Germany

The classical “text-book” neuron is built of a cell body which gives rise to a number of dendrites and usually one axon emerging from the soma. In recent years it became increasingly recognized that neurons in many brain regions deviate from this morphology in that they carry usually one axon (or more) on a dendrite. Interneurons frequently have axons from up to tertiary dendrites (Höfflin et al., Front Cell Neurosci 11, 2017). In mouse hippocampus, up to 50% pyramidal neurons in CA1 display axon-carrying dendrites, AcD, which have been shown to receive privileged synaptic input (Thome et al., Neuron 83, 2014)., Here, we asked if apical and basal AcD of visual cortical pyramidal cells in organotypic slice cultures from rat visual cortex have a growth patttern distinct from dendrites not carrying an axon. Morphology was revealed by gene-gun transfection, immunohistochemistry for EGFP and Neurolucida reconstruction. The analysis of nearly 800 pyramidal cells from supra- and infragranular layers at DIV10 and DIV15 revealed about 12% clear-cut AcD neurons (“shared root” cases excluded). The apical as well as a basal dendrite can give rise to an axon. In neurons of layers II/III and V/VI at DIV10 and at DIV15, basal dendrites carrying the axon are longer than 1) the basal dendrites not carrying an axon and 2) the basal dendrites of neurons with a somatic axon or 3) with an apical AcD: moreover, these basal AcD tend to have more segments. Of the infragranular neurons at DIV15, apical dendrites carrying an axon are longer than 1) apical dendrites of neurons with a somatic axon or 2) with an axon from a basal dendrite. The results suggest that dendrites which carry axons have a growth pattern distinct from “regular” dendrites, presumably driven by their higher excitability and action potential backpropagation.

Neuroplastin Promotes Spinogenesis and Regulates E/I Synapse Balance through TRAF6

Rodrigo Herrera-Molina[1], Sampath Kumar Vemula[1], Ayse Malci[1], Lennart Junge[1], Anne-Christine Lehmann[1], Johannes Hradsky[1], Ricardo A. Matute[2], Ramya Rama[1], Michael Naumann[3], Constanze I. Seidenbecher[1], Eckart D. Gundelfinger[1]

[1]Leibniz Institute for Neurobiology, Magdeburg, Germany
[2]California Institute of Technology, California, United States
[3]Institute of Experimental Internal Medicine, Otto von Guericke University, Magdeburg, Germany

Neuroplastins, type-1 transmembrane glycoproteins with extracellular Ig-like domains, have been associated to cortical thickness and intelligence in human as well as with retrograde amnesia, deficits in cortical processing, and synapse plasticity in mice. We have identified Neuroplastin (Np) isoforms as candidates to mediate formation of glutamatergic hippocampal synapses and as an essential partner of plasma Ca2+ ATPases (PMCAs). Here, we show that Np promotes formation of excitatory postsynapses efficiently via a hitherto unanticipated pathway, i.e. via tumor necrosis factor receptor-associated factor 6 (TRAF6)-dependent regulation of key synaptogenic signalling intermediates. By combining in silico modelling and surface plasmon resonance with biochemical, molecular and cell biological analyses and confocal microscopy of hippocampal primary neurons and HEK293 cells; we characterized the binding of Np to TRAF6 as structurally different from the binding to PMCAs. Indeed, Np capacity to promote PMCA protein levels and to co-localize with the pump was not affected by elimination of the TRAF6 interaction. Gain-of-function, knock-down and small molecule inhibition (SMI) experimentation argue that Np restores impaired synaptogenesis in Np-deficient (Nptn-/-) hippocampal neurons and that it is required for normal genesis of wild-type postsynapses by interacting with TRAF6. Documenting Np capacity for fostering spinogenesis, we show that the formation of dendritic protrusions by binding of Np65 specific-extracellular Ig-like domain is not depending critically of PMCA activity but strongly associated to the TRAF6-dependent activation of PI3K-AKT, ERK1/2 and NF-kappaB. Furthermore, examination of pre-to-postsynapse matching revealed that the synaptogenic capacity of Np-TRAF6 is essentially involved in the formation of future mature glutamatergic, but not inhibitory, synapses. These findings provide new molecular insights into glutamatergic synapse development and show that Np is critical for the establishment of an appropriate excitation-inhibition balance in hippocampal circuits.

Visual map formation without postsynaptic lamina neurons in *Drosophila*

Monika Kauer[1,3], Egemen Agi[1,3], Charlotte Wit[1,3], P. Robin Hiesinger[2,3]

[1]equal contribution
[2]correspondence: robin.hiesinger@fu-berlin.de
[3]Freie Universitaet Berlin, Division of Neurobiology, Institute for Biology, Berlin, Germany

'Target field regulation', i.e. molecular guidance of incoming axons through target area cues, is a core idea of the chemoaffinity theory by Roger Sperry and colleagues based on the example of vertebrate retino-tectal projections. However, Sperry envisioned such chemoaffine cues to explain *'not a precise cell-to-cell connectivity, but rather cell-to-focal tectal area with extensive terminal overlap among neighboring fibers'* (Meyer and Sperry 1976). In *Drosophila*, each of the simultaneously growing ~4800 photoreceptor (PR) axons overlap with >10 other PR growth cones and target lamina neurons (L-cells), including both correct and incorrect target cells (Langen et al., 2015). These observations raise the question to what extent the postsynaptic L-cells participate in the guidance of PR growth cones to the correct target areas.

Using intravital imaging in intact, normally developing pupae, we now show that PR axons target correctly after complete ablation of all potential target L-cells. Lack of L-cells in the correct target area leads some PRs to probabilistically fail to adhere and consequently either retract or attach at incorrect target areas (lamina cartridges). The results are consistent with predictions from our previously proposed computational model that L-cells are not absolutely required but may increase accuracy (Langen et al., 2015).

In the absence of all L-cells PR growth cones initially extend correctly in a symmetric, albeit shrunken, 'sorting field'. Sparse ablation of lamina neurons leads to higher variability in the sorting field. PR growth cones of a single subtype self-correct for these inaccuracies and all extend simultaneously with the same precise angle. At the same time as PRs target correctly, remaining L-cells after sparse ablation already fuse and form a distorted interaction network independent of the PR growth cones. We conclude that PR growth cones interact to self-correct angles, while target L-cells interact amongst themselves; in contrast, PR growth cones do not interact with target L-cells until targeting is complete. Finally, loss of N-Cadherin in all L-cells (Schwabe et al., 2013), phenocopies loss of all L-cells with respect to PR targeting. We conclude that the presynaptic photoreceptors and postsynaptic lamina neurons undergo independent, synchronous pattern formation that ensures their presence in the correct target areas prior to synapse formation. As a consequence, this spatiotemporal pre-sorting of synaptic partners allows for largely promiscuous synaptogenesis thereafter.

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GluK2-NETO2 signalling regulates dendritic spine morphology in developing hippocampus

Sebnem Kesaf[1]

[1]University of Helsinki, Molecular and Integrative Biosciences, Biocenter 3, Viikinkaari 1, 00014 HELSINKI, Finland

Kainate receptors (KARs) are a subtype of ionotropic glutamate receptors, composed of five different subunits (GluK1-5) in tetrameric assemblies. They are highly expressed during early brain development to modulate synaptic transmission, network excitability, neuronal maturation and synaptogenesis. Recent evidence highlights a robust increase in axonal filopodia by overexpression GluK1-GluK5 while shRNA-mediated knockdown of GluK2/5 reduces the density of filopodia, suggesting a role for KARs in structural plasticity of synaptic contacts. NETO2 is an auxiliary subunit of KARs which modulates their functional properties. However, the role of NETO2 on the KAR-dependent neuronal maturation remains to be unknown.

In this study, we showed that absence of NETO2 significantly reduced the proportion of dendritic spines in cultured hippocampal neurons, and it was rescued by the overexpression of GluK2. We also studied the effect of NETO2 on the actin dynamics using live-cell imaging. Indeed, the absence of NETO2 had a significant effect on actin dynamics by increasing the stability of F-actin filaments in dendritic spines of hippocampal cultures. In conclusion, our results demonstrate that GluK2-NETO2 signalling developmentally regulates dendritic spine formation and its stability.

retracted

The altered expression of cell adhesion molecule contactin-3 in tuberous sclerosis complex

Anatoly Korotkov[1], James D. Mills[1], Armand Blondiaux[4], Fanny Jaudon[6], Jasper J. Anink[1], Jackelien van Scheppingen[1], Constanze Seidenbecher[4,5], Lorenzo Cingolani[6], Erwin A. van Vliet[1,2], Eleonora Aronica[1,3]

[1]Department of (Neuro) Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam Neuroscience, Amsterdam, The Netherlands

[2]Center for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

[3]Stichting Epilepsie Instellingen Nederland (SEIN), The Netherlands

[4]Leibniz Institute for Neurobiology, Magdeburg, Germany

[5]Center for Behavioral Brain Sciences, Magdeburg, Germany

[6]Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia

Background:
Tuberous sclerosis complex is a multisystem genetic disorder that results from mutations in one of two tumor suppressor genes, TSC1 and TSC2, resulting in dysregulation of mechanistic target of rapamycin (mTOR) signaling pathway. TSC is frequently associated with autism spectrum disorders (ASD), cognitive disabilities and epilepsy, which is present in 70-80% of the patients. These neurological features of TSC are highly associated with cortical tubers - developmental cortical malformations in the brain. A large-scale transcriptomic analysis revealed a great variety of dysregulated protein coding genes and small non-coding RNAs in resected brain tissue of patients with TSC, which was associated with brain inflammation and alteration of cell adhesion molecules (CAMs). The preliminary analysis revealed a strong down-regulation of the contactin-3 (CNTN3) gene. CNTNs are a family of CAMs, which belong to immunoglobulin superfamily, and are thought to be involved in neural cell migration, axon guidance, organization of myelin subdomains and neurite outgrowth. We aim to elucidate the role, expression and regulation of CNTNs in TSC, particularly the lesser known CNTN3.

Methods:
The expression of CNTN3 was studied in resected and autoptic cortical brain tissue from 35 patients with TSC (7 months-47 years) and compared to autoptic brain tissue from 28 postnatal (0-44 years) and 15 fetal (GW 15-41) controls. Gene expression was analyzed by RT-qPCR and protein expression was analyzed by Western blot and immunohistochemistry. Functional studies were carried out in human neuronal cell line SH-SY5Y and involved overexpression of CNTN3 by CRISPR-dCas9 activation system and inhibition by siRNAs and miRNAs, differentiation of the neuronal cell line with retinoic acid and stimulation with brain derived neurotrophic factor (BDNF).

Results:
CNTN3 was expressed in neurons. It was detected in human brain as early as gestational week (GW) 15. CNTN3 expression was lower in resected cortical brain tissue from patients with TSC as compared to controls. This was most obvious in young (0-10 years) TSC patients. The expression of CNTN3 was higher in differentiated cells of the human neuronal cell line SH-SY5Y as compared to non-differentiated cells.

Conclusions:
CNTN3 is dysregulated in TSC and further investigation of CNTN3 in this pathology may provide a better insight into its function, regulation and therapeutic potential.

Posttranslational modification of hyaluronan receptor CD44 modifies its functions in regulation of neuronal morphology.

Josephine Labus[1], Alexander Wirth[1], Saskia Borsdorf[1], Evgeni Ponimaskin[1]

[1]Hannover Medical School, Cellular Neurophysiology, Carl-Neuberg-Str.1, 30625 Hannover, Germany

The extracellular matrix (ECM) and its modifiers function as important regulators of neuronal morphology and synaptic plasticity contributing to physiological processes such as learning and memory. One important player in ECM signalling is the hyaluronan receptor CD44, which has been proposed to regulate myelination, axonal growth, dendritic arborisation, synaptogenesis as well as neuronal excitability. CD44 undergoes multiple posttranslational modifications, which modulates its cellular localisation and signalling properties. Palmitoylation is the most common posttranslational lipid modification of proteins, which represents the reversible attachment of the C16 saturated fatty acid palmitate to cysteine residue(s). Even though CD44 is known to be palmitoylated, the functional consequences of CD44 palmitoylation in the brain have not been studied yet.

Here, we investigated the molecular mechanism of CD44 palmitoylation and its role in CD44-mediated regulation of neuronal morphology. We demonstrated that in rat hippocampal neurons, CD44 exists either in a non-palmitoylated or mono-palmitoylated state. Using site-directed mutagenesis, we found the cytoplasmic cysteine residue 298 to be the only palmitoylation site in rat CD44. Furthermore, we identified the palmitoyltransferases (so called DHHC proteins) responsible for palmitoylation of CD44 in neurons. By silencing endogenously expressed CD44 accompanied with the over-expression of a palmitoylation-deficient CD44 mutant, we studied the effects of this lipid modification on CD44 function in hippocampal neurons.

In vivo time-lapse imaging of olfactory sensory neuron birth, differentiation and axogenesis

Thomas Offner[1,2], Sara Joy Hawkins[2], Lukas Weiss[2], Thomas Hassenklöver[2], Ivan Manzini[1,2]

[1]University of Göttingen, Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany

[2]Justus-Liebig-University Giessen, Department of Animal Physiology and Molecular Biomedicine, Göttingen, Germany

The vertebrate olfactory system has the lifelong capacity to compensate for the loss of olfactory sensory neurons via adult neurogenesis. Additionally to the regular turnover of olfactory sensory neurons for tissue maintenance, the neuronal circuitry is capable of regenerating and rewiring after injury. The bipolar sensory neurons are generated from stem cells in the basal layer of the olfactory mucosa and project their axon via the olfactory nerve to second order neurons in the olfactory bulb. Larval *Xenopus laevis* has proven to be a powerful model to observe olfactory system regeneration after olfactory nerve axotomy on multiple levels of the olfactory system.

In this work we present *in vivo* time-lapse imaging of olfactory sensory neuron regeneration after injury, from early progenitor level to the point of axonal pathfinding. Two days after olfactory nerve transection we electroporated progenitor cells of the olfactory epithelium with a genetic probe (LifeAct_P2A_tdTomato) which allowed us to observe cellular morphology and actin cytosceleton dynamics for several days. By expressing LifeAct_P2A_tdTomato under different promotors of genes known to play major roles in vertebrate neuronal development (Pax6, Sox3, NCAM, NbT), we were able to correlate the observed cellular morphologies with the stage in neuronal differentiation. Finally, a combination of sparse cell labelling and immunohistochemistry against those developmental markers complemented our *in vivo* data, providing unprecedented insights into injury-induced neuroregeneration from the single cell to the population level.

Genetically encoded calcium indicators (GECIs) can impair developmental dendrite growth in rat cortical neurons

Petra Wahle[1], Tobias Stahlhut[1], Alexander Jack[1]

[1]Ruhr University, Developmental Neurobiology, Universitatsstraße 150, 44801 Bochum, Germany

Genetically encoded calcium indicators (GECIs) are one of the most commonly used methods of detecting cellular calcium signals. Two major types of GECIs exist: the single fluorophore sensors containing calmodulin as calcium-binding moiety fused with green fluorescent protein (GFP; GCaMP family), and the FRET-based sensors containing calmodulin or troponin C as calcium-binding moiety fused with GFP/YPF fluorophores. A battery of GECIs with different binding kinetics, calcium affinities and fluorescence signals has evolved over the recent years. They allow for long-term calcium imaging in vitro and in vivo after virus-mediated or transgenic expression in the cells of interest. However, despite the advantages, perturbations have been reported. These include nuclear accumulation of the sensor and aberrant neurite growth in dissociated cortical neurons. It seems evident that overexpression of such calcium catchers interferes with cellular calcium homeostasis and signaling pathways which are particularly important during neural differentiation. Our objective is to investigate whether the overexpression of the commonly used GECIs GCaMP3, GCaMP5, GCaMP6m and the FRET-based sensor TNXXL in early developmental time windows could have an effect on the morphological maturation of pyramidal cells and interneurons. For this, biolistic transfections of CMV promoter containing plasmids encoding GECIs and EGFP as control had been done in organotypic slice cultures of rat visual cortex. After 10 days of overexpression, transfected neurons were immunostained for GFP and Neurolucida-reconstructed. The data show that none of the GECIs seemed to elicit neuronal degeneration. However, GCaMP3 overexpression severely impaired dendrite growth of pyramidal cells of supra- and infragranular layers, and TNXXL overexpression yielded a statistical trend towards stunted growth. GCaMP5 and GCaMP6m transfectants were not different from EGFP control neurons. GCaMPX-C reported to not affect maturation is currently under investigation. Growth-impaired or too-small-for-age neurons might not display the physiological responses typical for their wildtype sisters, and thus, results obtained with certain GECIs have to be interpreted with caution.

Molecular mechanisms underlying Ankyrin2-dependent control of synaptic plasticity

Tobias Weber[1], Johanna Buchheit[1], Raiko Stephan[2], Jan Pielage[1]

[1]Department of Zoology and Neurobiology, Technical University of Kaiserslautern, Erwin-Schrödinger-Str. 13, 67663 Kaiserslautern, Germany

[2]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

One essential hallmark of neuronal circuit plasticity is the precise regulation of synaptic connectivity and morphology. Synapse formation, stability, function and plasticity critically depend on the highly organized assembly of cell adhesion molecules (CAMs) through interactions with the actin and microtubule cytoskeleton. However, the precise molecular mechanisms controlling the interaction of the cytoskeleton with synaptic cell adhesion molecules remain largely unknown.
We previously identified two giant isoforms of the adaptor molecule Ankyrin 2 as essential regulators of synapse stability and organization. Within the presynaptic terminal, the Ank2-L isoform controls synaptic stability upstream of the microtubule-organizing isoform Ank2-XL. Both isoforms share conserved, N-terminal Ankyrin-repeat domains and a spectrin-binding domain that are thought to mediate interactions with transmembrane proteins and the subcellular spectrin skeleton to link the cytoskeleton to ion channels and cell adhesion molecules. To investigate the specific contributions of these domains for Ankyrin2 localization and for the organization of synaptic cell adhesion molecules and microtubules we generated a series of domain-specific deletions using a Pacman-based rescue approach. First analysis of the deletion mutants demonstrated a hierarchical interaction of the Ank2 isoforms with Ank2-L controlling the synaptic localization of Ank2-XL. In addition, our analysis revealed unique and specific requirements of different Ankyrin domains for the organization of synaptic scaffolds. Together these data provide novel insights into the sequential organization of the presynaptic nerve terminal to control synaptic maintenance and plasticity.

Roles of Dscams in the Development of *Drosophila* Central Neuron Dendrites

Nicole Wilhelm[1], Shikha Kumari[1], Carsten Duch[1]

[1]Johannes Gutenberg-University, Institute of Developmental Biology and Neurobiology, Hanns-Dieter-Hüsch-Weg 15, 55128 Mainz, Germany

CNS development and function critically rely on correct neural circuitry wiring. Dendrites are the predominant neuronal compartments for receiving synaptic input, and thus form the blueprint for correct brain wiring. To cover their respective synaptic input spaces completely and non-redundantly, dendrites of one neuron should ideally be even spaced but intermingled with dendrites of other neurons which need access to the same synaptic partners. Largely based on seminal studies on mechanosensory neurons, it has been proposed that the large isoform diversity of the Down syndrome cell adhesion molecule 1 (Dscam1, 38.000 isoforms) provides a nervous system-wide code for self-recognition and homophilic repulsion in the *Drosophila* nervous system (Kise & Schmucker, 2013, Curr Opini Neurobiol). Given that Dscam1 is widely expressed in all central neuropils during dendrite differentiation, we tested whether this code applies to different types of central *Drosophila* neurons.

Surprisingly, we found that Dscam1 is not required for even spacing of adult motoneuron dendrites, but instead for dendrite growth (Hutchinson et al., 2014, J Neurosci). This challenges the view of a nervous system-wide code for self-recognition and avoidance. To further test for this, we extended our analysis to multiple types of central neurons. We found that Dscam1 is not required for spacing but for dendritic growth of larval and adult glutamatergic motoneurons (RP2 and MN5), and efferent aminergic neurons (TDC2). Therefore, Dscam1 provides not a spacing but a growth signal for all efferent neurons tested. By contrast, neither dendrite development of cholinergic interneurons (Giant Fiber) nor of local inhibitory interneurons (period neurons) is affected by Dscam.

We now test the other Dscam family members (Dscam2-4) for their roles in the dendritic structure development of motoneurons and interneurons by localization and genetic knockdown strategies. Endogenously tagged proteins (protein traps) reveal broad expression patterns in central neuropils during critical stages of circuit differentiation. However, RNAi knockdown of any of the Dscam2-4 reveals no dendrite spacing defects but growth defects to different degrees. In the adult flight MN5 for example Dscam2 and 4 knockdown causes a reduction of dendritic field dimension by favoring longitudinal branch growth at the cost of new branch formation.

Our data support neuron-type specific functions of different Dscams for different aspects of dendrite and neural circuit development, but not for self-recognition and spacing. Therefore, three dimensional dendrites with massive synaptic input likely use alternative principles for proper dendrite spacing.

Poster Topic

T3: Developmental Cell Death, Regeneration and Transplantation

T3-1B Identifying transcriptional response of developing corticospinal neurons to spinal axotomy
Philipp Abe, Karthikeyan Devaraju, Natalia Baumann, Denis Jabaudon

T3-2B TGF-β2 Regulates Development of Serotonergic Neuron Subgroups: Evidence from mutant mice
Belal Mahmoud Rahhal, Eleni Roussa

T3-1C Hypoxic reprogramming of HeLa Kyoto tumor cells.
Anastasia Alekseevna Elizarova, Elena Ivanovna Erlykina, Vladimir Georgievich Pimenov, Mikhail Mikhailovich Palkin, Maria Maksimovna Lukina, Maria Vadimovna Shirmanova

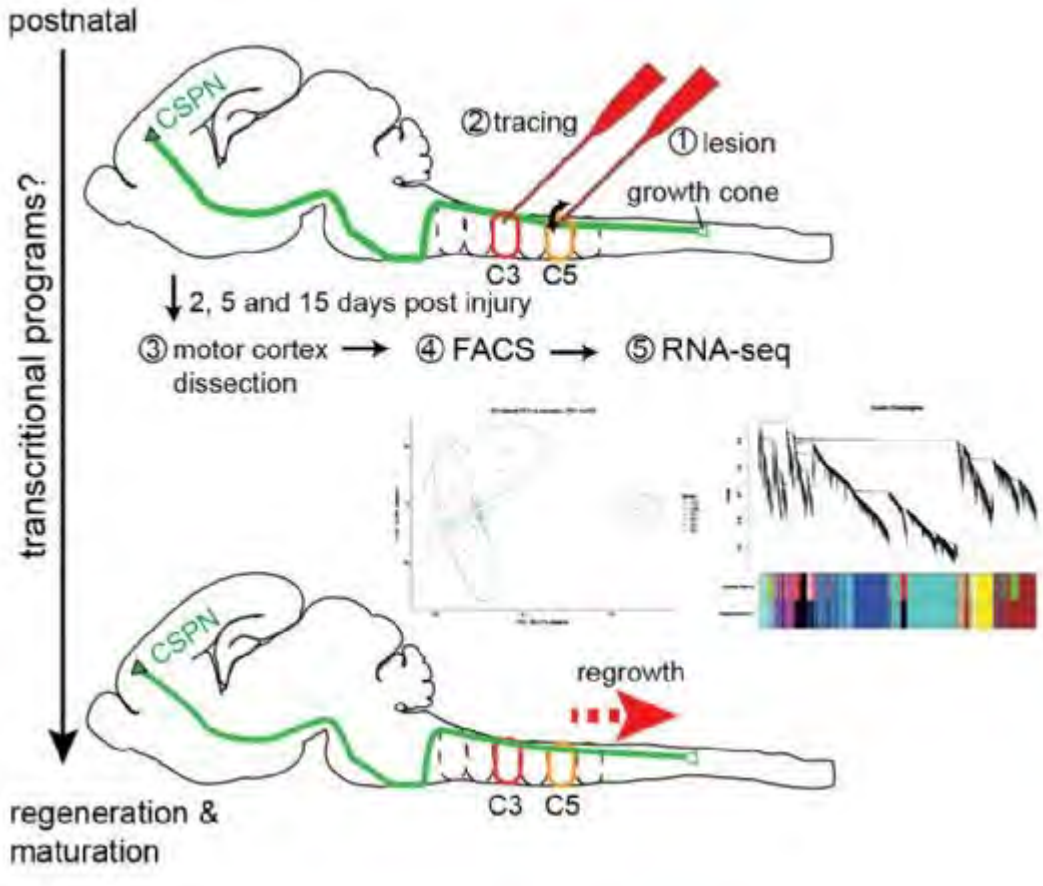
T3-2C Bioinformatics analysis of oxidative and elemental status as a factor in the early diagnosis of brain tumors
Anna Vladimirovna Shcherina, Larisa Mikhailovna Obukhova, Oksana Vladimirovna Barinova, Kirill Vladimirovich Kuzmichev, Igor Alexandrovich Medyanik, Ilya Igorevich Evdokimov

Identifying transcriptional response of developing corticospinal neurons to spinal axotomy

Philipp Abe[1], Karthikeyan Devaraju[1], Natalia Baumann[1], Denis Jabaudon[1]

[1]University of Geneva, Dept. of Basic Neurosciences, 1 rue Michel Servet, CH-1211 Geneva, Switzerland

In the adult mammalian central nervous system, neurons have a limited capacity to regenerate their axons after injury. During development, by contrast, neurons are thought to be more plastic and extend their axons effectively. To better characterize this plasticity and to identify transcriptional signatures of axon regeneration in young animals, we developed a minimally-invasive spinal cord injury (SCI) model in mice and performed RNA-sequencing of pure populations of corticospinal neurons. At postnatal P3, the dorsal corticospinal tract (CST) was lesioned at C5 segment by using ultrasound guidance, followed by retrograde labeling at C3 level and collection of corticospinal projection neurons (CSPN) either two-, five- or 15-days post injury for RNA-sequencing. Principle component analysis, differentially expressed gene and weighted correlation network analysis identified the dynamics of the transcriptional responses of CSPN, including lesioned-induced changes in differentiation, axon growth and synaptogenesis. Our data suggest that injury adjusts normal developmental genetic programs to allow axon regeneration in young animals.



TGF-β2 Regulates Development of Serotonergic Neuron Subgroups: Evidence from mutant mice

Belal Mahmoud Rahhal, Eleni Roussa[2]

[11]Institute of Anatomy and Cell Biology, Department of Molecular Embryology, Faculty of Medicine, University of Freiburg, Freiburg, GermanySchool of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine, Institute of Anatomy and Cell Biology, Department of Molecular Embryology, Faculty of Medicine, University of Freiburg, Freiburg, GermanySchool of Medicine and Health Sciences, An-Najah National University, Albertstrasse 17, 79104 Freiburg, 7 Nablus, Germany

Abstract:
Molecular and functional diversity within hindbrain serotonergic (5-HT) neurons has emerged as a relevant feature that could underlie selective vulnerability of neurons in clinical disorders. We have investigated the role of transforming growth factor beta 2 (TGF-β2) during development of mouse hindbrain 5-HT subgroups. Therefore, we performed a phenotypic analysis during development of the hindbrain serotonergic system in TGF-2β mutant mice. The results show a significant decrease in the number of 5-HT neuronsin TGF-2β-deficient mice at embryonic day (E) 12, whereas at E14 and E16 the number of 5-HT neurons was comparable between wildtype and mutant mice. At E18 a selective significant decrease in the hindbrain paramedianraphe 5-HT neurons in the mutant was observed, compared to wildtype.
These results highlight a selective growth factor dependency of individual rostral hindbrain serotonergic subpopulations, emphasize the impact of TGF-2β during development of 5-HT subgroups, and suggest TGF-2β as potent candidate to establish diversity within the hindbrain serotonergic system.

Hypoxic reprogramming of HeLa Kyoto tumor cells.

Anastasia Alekseevna Elizarova, Elena Ivanovna Erlykina[1], Vladimir Georgievich Pimenov [2], Mikhail Mikhailovich Palkin[1], Maria Maksimovna Lukina[1], Maria Vadimovna Shirmanova[1]

[1]Federal State Budgetary Educational Institution of Higher Education «Privolzhsky Research Medical University» of the Ministry of Health of the Russian Federation, medical Faculty, square Minin and Pozharsky, 10/1, 603950 Nizhny Novgorod, Russia

[2]Federal State Budgetary Institution of Science "Institute of Chemistry of High-Purity Substances to them. G.G. Ninth Russian Academy of Sciences", st. Tropinina, 49, 603137, Nizhny Novgorod, Russia

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The aim of the study was to analyze changes in the characteristics of cancer cells under conditions of intracellular hypoxia.

Materials and methods. We studied HeLa Kyoto cells (human cervical cancer) cultured according to standard methods. Control - human skin fibroblasts. The concentration of glucose, lactate, acetylcholinesterase (AChE), catalase activity was determined spectrophotometrically. Myoglobin - immunoturbidimetric test and in the reaction of passive hemagglutination. The level of trace elements by atomic emission spectrometry iCAP6300Duo. Free radical activity by the method of induced biochemiluminescence. The products of lipid peroxidation (LPO) - diene, triene conjugates (DC, TC), Schiff bases (SB) were determined in heptane-isopropanol fractions.

Results. The glucose content in tumor cells is lower (2.19±0.08 mM/l/g) than in fibroblasts (3.24±0.1 mM/g protein cells). The consumption of glucose by cells from the medium is higher (0.14±0.001 mM/g versus 0.069±3 × 10-3 mM/g protein in fibroblasts) which is explained by the anaerobic oxidation of glucose and, as a result, the accumulation of lactate (0.66±0.007 mM/g of protein in the absence of fibroblasts). The formation of lactate contributes to metastasis (Koblyakov, 2014). The predominance of anaerobic glycolysis suggests hypoxic reprogramming of the cell with the participation of PI3K/AKT/mTOR. Perhaps this is due to an increase in the content of Cu (1.8±0.06 mg/g) and Zn (8.48±0.73 mg/g) in comparison with fibroblasts (0.33±0.04 mg/g; 2.54±0.19 mg/g) as they are involved in the activation of this signaling pathway. The increase in myoglobin (6.87 × 10-3±0.0001 g/g) compared with fibroblasts (3.49 × 10-3±0.0001 g/g protein of cells) also indicates the involvement of PI3K/AKT/mTOR (Semenza, 2013). Cellular hypoxia induces production of hypoxia-inducible factor-1, that stimulates the production of myoglobin. An increase in the iron content was observed in the cells (12.71±0.89 mg/g of cell protein versus 2.22±0.16 mg/g of protein). Its release from macrophages and transport to tumor cells during hypoxia (Tarasova, 2012). Free radical activity in tumor cells increased (576±67 imp./sec versus 296±24 imp./sec). Integral indicators of damage to biological structures - SB (4.96±0.02 rel. units/mg), DC (4.96±0.02 rel. units/mg), TC (0.093±0.001 rel. units/mg) were higher in comparison with those in fibroblasts (4.519±0.01 rel. units/mg; 0.024±0.001 rel. units/mg of protein). The state of oxidative stress is characterized by the inability of the body's antioxidant system to adequately respond to the production of reactive oxygen species (ROS). Catalase activity in tumor cells increased (0.23±0.08 mM/min/mg protein versus 0.192±0.03 mmM/min/ mg protein in the control). The tumor cell is protected from apoptosis by maintaining the ROS level optimal for cell activity under hypoxic conditions (Vakhidova, 2010). The adaptive mechanism is also a decrease in the activity of AChE (0.03±0.0065/g versus 0.7±0.01/g of protein) - this leads to inhibition of apoptosis and uncontrolled cell division (Greenberg, 2012).

Conclusions. The hypoxic reprogramming of the metabolism of cancer cells is a factor in the adaptation

of tumor cells to the altered conditions of their vital activity.

Bioinformatics analysis of oxidative and elemental status as a factor in the early diagnosis of brain tumors

Anna Vladimirovna Shcherina, Larisa Mikhailovna Obukhova[1], Oksana Vladimirovna Barinova [1], Kirill Vladimirovich Kuzmichev[1], Igor Alexandrovich Medyanik[1], Ilya Igorevich Evdokimov[2]

[1]Federal State Budgetary Educational Institution of Higher Education «Privolzhsky Research Medical University» of the Ministry of Health of the Russian Federation, medical Faculty, square Minin and Pozharsky, 10/1, 603950 Nizhny Novgorod, Russia

[2]Federal State Budgetary Institution of Science "Institute of Chemistry of High-Purity Substances to them. G.G. Ninth Russian Academy of Sciences", st. Tropinina, 49, 603137, Nizhny Novgorod, Russia

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The aim of the work is the proteomic analysis, the analysis of mineral status and some biochemical parameters as a factor of early differential diagnosis of brain tumors.

Blood and tissue of tumor neoplasms of the brain of patients in the age group of 39-59 years were studied: 12 patients with malignant brain tumors (glioma, glioblastoma, astrocytoma), 7 patients with benign brain tumors (meningioma, subependymoma) before treatment. The brain tissue of persons who died as a result of injury (death time: up to 10 hours) - 7 people, and blood from 10 practically healthy people was used as a control. Proteomic bioinformatics analysis was performed on databases using Cytoscape software. Catalase (Cat), acetylcholinesterase (AChE) activities were determined spectrophotometrically, myoglobin was determined using erythrocyte diagnosticum, elemental analysis was determined by atomic emission spectrometry, and free radical activity (FRA) by induced biochemiluminescence.

The study showed that with malignant neoplasms of the brain compared with practically healthy people and people with benign tumors, significant changes in the studied parameters occur. Changes in the oxidative status were found: FRA of malignant tumor tissues (6.42 mV vs. 1.5 mV; 3.85 mV) and plasma (18.96 mV against 1.25 mV; 6.96 mV) increases. Cat activity in the tumor tissues increases (18.42 mmol/l versus 14.37 mmol/l; 17.45 mmol/l), and decreases in erythrocytes (4.47 mmol/l versus 15.24 mmol/l; 10.96 mmol/l). The content of macro-micro elements changes. In the tissue of malignant tumor, there is an increase in the concentration of Ca (by 3.9 times), Fe, Zn, Cu by 3.5; 2.9; 4.3 times, respectively. In the blood plasma, an increase in the Ca content is observed in comparison with the results of the control group by 1.3 times, Fe, Zn, respectively, by 2; 2.1 times and a decrease in Cu by 1.7 times. AChE red blood cells lower by 25%. Myoglobin is detected in gliomas (74.86 mg/ml versus 0 mg/ml; 24.36 mg/ml).

Intermittent hypoxia activates NADPH oxidase, increasing FRA and activating Ca release. Ca activates protein kinase C which promotes the progression of gliomas through regulation of p21 (Waf1/Cip1) and suppression of p53-mediated activation of IGFBP3 (Besson, 2000).

For glioma, activation of epidermal growth factor receptor synthesis is shown which increases Ca release and activates the small G-protein Ras, triggering uncontrolled cell proliferation.

In glioblastoma cells, AChE in combination with the framework protein RACK1 and protein kinase C affects the proliferation of tumor tissue.

Under hypoxic conditions, the cell produces myoglobin, which is an inactivator of tumor growth and a factor in the adaptation of cells to hypoxia.

Tumor cells proliferate due to the redox-mediated enhancement of signaling in the MAP kinase cascade through the activation of ERK1/2 and Ras. The expression of Cat, heme-(Fe)-containing enzyme, increases compensatory protecting the tumor cells from oxidative stress and induction of apoptosis (Salazar-Ramiro A et al., 2016). Zn can act as a ligand for proteins of the Hedgehog signaling pathway, in particular, the Hedgehog interacting protein (Bosanac I et al., 2009), actively synthesized in glioma

cells.
Thus, the study of the oxidative and mineral status of the body can be a method of early differential diagnosis of malignant and benign brain tumors.

Poster Topic

T4: Neurotransmitters, Retrograde messengers and Cytokines

T4-1A Impaired anandamide/palmitoylethanolamide signaling in hippocampal glutamatergic neurons alters synaptic plasticity, learning and emotional responses
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Impaired anandamide/palmitoylethanolamide signaling in hippocampal glutamatergic neurons alters synaptic plasticity, learning and emotional responses

Annika Beer[1], Tina Zimmermann[1], Julia C. Bartsch[2], Ermelinda Lomazzo[1], Stephan Guggenhuber[1], Maren Lange[2], Laura Bindila[1], Hans-Christian Pape[2], Beat Lutz[1]

[1]Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

[2]Institute of Physiology I, Westfälische Wilhelms-University Münster, Münster, Germany

Fatty acid amide hydrolase (FAAH) is a serine hydrolase highly expressed in the brain and is the major degrading enzyme of the endocannabinoid anandamide (AEA), which is produced and released on demand, and acts as a partial agonist at the presynaptic cannabinoid CB1 receptor (CB1), and as a full agonist at the transient receptor potential vanilloid 1 (TRPV1). Additionally, FAAH hydrolyses a number of other N-acyl ethanolamides (NAEs), including palmitoylethanolamide (PEA), which activates the peroxisome proliferator-activated receptor alpha (PPARá) and the G protein-coupled receptor 55 (GPR55). While the consequences of genetic deletion or pharmacological inhibition of FAAH have been investigated, finding that increased AEA signaling leads to anxiolytic, antidepressant and analgesic effects in rodents, reduced the impact of decreased AEA signaling have not yet been explored. To this end, we generated a mouse model in which FAAH is selectively overexpressed in hippocampal glutamatergic neurons. We used the Cre/loxP system combined with an adeno-associated virus (AAV)-mediated delivery system to overexpress FAAH specifically in the glutamatergic neurons of the CA1-CA3 region. This led to increased FAAH expression and activity, consequently, decreased levels of AEA and PEA. Electrophysiological recordings revealed an increase in long-term potentiation (LTP), without any alterations in inhibitory long-term depression (LTD), and in depolarization induce suppression of excitation (DSE) and inhibition (DSI). Furthermore, mice overexpressing FAAH in hippocampal glutamatergic neurons showed an increase in anxiety-like behavior and impairment in hippocampal-dependent memory formation. This study established a valid genetic system to analyze the role of AEA/PEA signaling in in brain functions.

Electrophysiological properties of CA1 pyramidal neurons and their dopaminergic modulation along the longitudinal hippocampal axis

Swantje Beythien[1], Volkmar Leßmann[1,2], Elke Edelmann[1,2]

[1]Otto von Guericke Universität Magdeburg, Institut für Physiologie, Leipziger Straße 44, 39120 Magdeburg, Germany
[2]Center for Behavioral Brain Sciences (CBBS), Magdeburg
39120 Magdeburg, Germany

The hippocampus as a critical brain area involved in learning and processing of spatial information has long been described as a homogeneous structure. In contrast, recent research suggests functional and structural diversities along the longitudinal as well as radial and transversal axis. The restriction of specific functions to sub regions of the hippocampus hints at underlying regional differences in information processing and storage at the single cell level as well as its regulation by neuromodulators such as dopamine. This is supported by recent findings of graded expression of different dopaminergic receptors and varying densities of dopaminergic fibers along the longitudinal hippocampal axis (e.g. Dubovyk and Manahan-Vaughan, 2018 Hippocampus; Edelmann and Lessmann, 2018 Cell Tissue Res.).
While the processing of information at the single cell level is not fully understood yet, valuable insights can be derived from electrophysiological characterizations of single neurons. Therefore we investigated electrophysiological properties of CA1 pyramidal neurons as well as basal synaptic transmission at Schaffer collateral synapses in the mouse hippocampus along its longitudinal axis. Furthermore, we examined region specific neuromodulatory effects of dopamine on the above-mentioned parameters.
We performed whole cell patch clamp recordings of CA1 pyramidal neurons in acute hippocampal slices of juvenile C57BL/6J mice (P25—P35) to assess intrinsic excitability, passive and active membrane properties as well as basal synaptic transmission at CA3-CA1 synapses in dorsal, intermediate and ventral sections of the hippocampus. Additional experiments were carried out in the presence of bath applied dopamine and dopamine subtype specific receptor agonists and antagonists to investigate neuromodulatory effects of dopamine and their regional differences. All experiments were performed in the presence of 100 µM picrotoxin. Our experiments revealed a graded efficiency in synaptic transmission along the longitudinal axis that with highest magnitudes in the ventral hippocampus. In accordance with these findings transmitter release probability decreases from the ventral to the dorsal pole. We found no regional differences in intrinsic excitability or active and passive membrane properties. Neither dopamine nor the dopaminergic agonists and antagonists used showed effects on any of the parameters analyzed.
While these results demonstrate region specific differences especially in the efficiency of glutamatergic synaptic transmission that could play a role in the functional heterogeneity of the hippocampus, dopamine showed no neuromodulatory effects on basal electrophysiological properties of CA1 pyramidal neurons and synaptic transmission at Schaffer collateral-CA1 synapses under our experimental conditions. Further investigations need to clarify whether different experimental approaches are required to inquire into a possible role of this neuromodulator in electrophysiological diversity along the longitudinal hippocampal axis.

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Morphological and Behavioural Characteristics of the Tryptophan Hydroxylase Knockout Rat

Sabrina Ilonka Hanswijk[1], Jan K Buitelaar[1], Judith R Homberg[1]

[1]Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition, and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands

Serotonin is involved in a wide range of physiological systems including gastrointestinal motility and secretion, cardiovascular regulation, the sleep–wake cycle, appetite, nociception, and stress. Alterations in the serotonin homeostasis are related to a variety of behavioural traits and personality disorders, including alcoholism, aggression, suicidal behaviour, depression, and anxiety.

One of the major factors regulating serotonin neurotransmitter levels is tryptophan hydroxylase (TpH). TpH is the rate-limiting enzyme of serotonin synthesis, TpH1 is expressed in the periphery, and TpH2 in the brain. Changes in Tph1 function affect serotonin levels in blood, heart function, vessel contraction, and gastrointestinal tract function. TpH1 activity alterations may affect autonomic responses to fear and stress and thus impair our fight-or-flight response resulting in the variety of behaviour traits described above. Indeed, earlier studies reported an increased risk for depressive and impulsive/aggressive disorders due to TpH1 gene variation as well as changes in cardiovascular and gastrointestinal functions. This implies that Tph1 – by influencing peripheral serotonin levels – links the periphery with the central nervous system. In support, Tph1 allelic variants influence monoamine levels in the cerebrospinal fluid.

To this end we assessed a rat line deficient in peripheral serotonin biosynthesis. This TpH1 knock-out Wistar WKY rat model is generated by use of the CRISPR/SpCas9 technology. We measured olfactory function at infancy, social play at adolescent age, and anxiety- and depressive-like behaviour during young adulthood. We also measured developmental milestones such as eye opening and reflex development, and morphological development such as body weight, brain weight and heart size. The data of these tests will be presented at the 13th Göttingen Meeting of the German Neuroscience Society. Potentially we are able to present data concerning peripheral serotonin metabolism and brain morphology as well.

Characterization of this novel rat model will provide insides in the usefulness of this model to study behavioural and neurobiological consequences of disturbed peripheral serotonin biosynthesis.

Postsynaptic exocytosis of endogenous BDNF vesicles in BDNF-GFP knock-in mice

Volkmar Lessmann[1], Robert Eckenstaler[1], Julia Leschik[2], Thomas Endres[1], Thomas Munsch[1], Karin Richter[3], Oliver Kobler[4], Klaus Fischer[3], Werner Zuschratter[4], Tanja Brigadski[1], Beat Lutz[2]

[1]Otto-von-Guericke-University, Institute of Physiology, Leipziger Strasse 44, 39120 Magdeburg, Germany
[2]Johannes Gutenberg University,Institute of Physiological Chemistry, 55128 Mainz, Germany
[3]Otto-von-Guericke-University, Institute of Biochemistry and Cell Biology, 39120 Magdeburg, Germany
[4]Leibniz-Institut for Neurobiology, Lab for Electron and Laserscan microscopy, 39118 Magdeburg, Germany

Brain-derived neurotrophic factor (BDNF) is a crucial secreted messenger for induction of synaptic plasticity. Accordingly, altered or reduced expression, targeting, or secretion of BDNF is suspected to cause neurological deficits and neurodegenerative disorders. Due to its extremely low abundance the dynamics of physiological BDNF secretion in vivo are unexplored.
Here, we describe an innovative BDNF-GFP knock-in (KiBE) mouse model, in which GFP-labeled BDNF is expressed under the normal transcriptional control of the unaltered endogenous mouse BDNF gene regulatory elements. BDNF-GFP release and biological activity are apparently not affected by the C-terminal GFP tag, since homozygous KiBE mice, which lack wildtype BDNF, exhibit no obvious dysfunctions and have a normal life expectancy. The fluorescently labelled endogenous BDNF in KiBE mice allowed us to detect single BDNF-containing vesicles with live cell imaging. Our results disclose synaptic targeting of endogenous BDNF-GFP vesicles to dendrites (harboring ~70% of BDNF-GFP vesicles in hippocampal neurites) and axons (remaining 30%). Endogenous BDNF-GFP vesicles are found mostly several 100 nm away from pre- and postsynaptic marker proteins. Thus, they are localized rather close to synaptic structures but are not accumulated underneath pre- or postsynaptic membranes like e.g. transmitter vesicles or proteins of the postsynaptic density. Endogenous BDNF-GFP vesicles have an acidic intra-vesicular pH of approximately 5.8, an apparent diameter of 50-100 nm (determined with STED imaging), and their load with BDNF cargo depends on the amount of expressed BDNF in a neuron, whereas – unexpectedly – the number and the density of BDNF vesicles remain unchanged by low BDNF levels. Exocytosis of dendritic BDNF vesicles commences typically within the first 30 s after a strong depolarizing stimulus and continues for ~100 s thereafter, revealing an astonishingly delayed and prolonged release of endogenous BDNF from postsynaptic structures.
This novel KiBE mouse model will be a valuable tool to discover BDNF dynamics in vivo that so far escaped real time analysis.

Novel molecular tools for single cell imaging of C-to-U RNA editing

Jochen Meier[1], Andrea Knoll[2], Oliver Seitz[2], Svenja Kankowski[1]

[1]Technische Universität Braunschweig, Spielmannstr.7, 38106 Braunschweig, Germany
[2]Humboldt-Universität zu Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany

RNA editing as a native nucleic acid modification influences splicing, stability of RNA, translation and protein function. One protein affected by this modification is the glycine receptor (GlyR) where C-to-U RNA editing confers a gain-of-function of the receptor protein and may contribute to neuropsychiatric symptoms of patients with temporal lobe epilepsy (TLE).
State-of-the-art methods use bulk material and enable the detection of mRNA editing sites in ensemble of cells only. As recent findings suggest that GlyR RNA editing is a neuron type specific pathological mechanism of the disease, it is necessary to study GlyR C-to-U RNA editing at the single neuron level.
For this purpose, we developed Forced Intercalation (FIT) probes able to detect the single nucleotide exchange by enhancement of fluorescence upon hybridization only with fully complementary RNA target. To further improve the technology we also present a binary probe system based on Förster resonance energy transfer (FRET) that allows to distinguish between edited and unedited GlyR mRNA.
These probes should be useful for the identification of neuron types with increased RNA editing of GlyR-coding mRNA in TLE and elucidation of neuron type specific pathophysiological mechanisms of the disease.

GABAergic synaptic transmission and plasticity is unaltered in the lateral amygdala of heterozygous BDNF knockout mice

Susanne Meis[1,2], Thomas Endres[1], Thomas Munsch[1,2], Volkmar Lessmann[1,2]

[1]Otto-von-Guericke University, Institute of Physiology, Leipzigerstrasse 44, 39120 Magdeburg, Germany
[2]Center for Behavioral Brain Sciences, Magdeburg, Germany

Brain-derived neurotrophic factor (BDNF) has previously been shown to play an important role in glutamatergic synaptic plasticity in the amygdala, correlating with cued fear learning. While glutamatergic neurotransmission is enhanced by BDNF signalling, its mechanism of action at inhibitory synapses in the amygdala is far less understood. We therefore analysed the impact of chronic BDNF depletion on $GABA_A$ mediated synaptic transmission in BDNF heterozygous knockout mice ($BDNF^{+/-}$). Projection neurones of the lateral amygdala (LA) were examined using the whole cell patch clamp technique in an *in vitro* slice preparation. GABA responses were elicited by focal stimulation in the presence of inhibitors of glutamatergic synaptic transmission to focus on GABAergic synapses.

Inhibitory synaptic efficacy in LA was unaltered in $BDNF^{+/-}$ mice. Neither input-output relationships nor IPSC kinetics showed any differences between genotypes. Analysis of miniature IPSC revealed no pre- or postsynaptic changes. In addition, paired-pulse facilitation as well as synaptic fatigue of evoked IPSCs showed no difference between the two experimental groups. Long-term potentiation (LTP) of IPSCs could be reliably induced in LA neurons by pairing postsynaptic depolarisation with tetanic presynaptic stimulation. LTP was not significantly different between $BDNF^{+/-}$ mice and wildtype littermates. These results argue against impaired efficacy and plasticity at GABAergic synapses due to a chronic BDNF reduction in the LA, neither at the postsynaptic nor at the presynaptic site. Interestingly, facilitation of sIPSCs by norepinephrine was reduced in $BDNF^{+/-}$ mice. This diminished GABAergic tone due to BDNF deficiency may therefore lead to amygdala hyper-excitability in response to states of high arousal.

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Desensitization of partially occupied kainate receptor heteromers

Stefan Pollok[1], Andreas Reiner[1]

[1]Ruhr-University Bochum, Cellular Neurobiology, Universitätsstraße 150, 44801 Bochum, Germany

Ionotropic glutamate receptors (iGluRs) mediate fast excitatory synaptic neurotransmission as well as neuromodulation in the central nervous system of vertebrates. iGluRs are ligand gated ion channels assembled from four subunits. Although AMPA and kainate receptor subunits can form homomers, they are typically expressed as heteromers *in vivo*.[1] In these two iGluR subfamilies, activation, which occurs in the sub-millisecond range, is followed by a pronounced, ligand-induced desensitization that develops within milliseconds.
Subunit-specific agonists and antagonists should act on certain receptor subtypes while not affecting other iGluR subtypes. They should thus reduce the side effects on overall neuronal circuit function that are associated with the use of rather nonspecific iGluR drugs. However, little is known about how subtype-specific agonists and antagonists affect the gating of heteromeric receptors, where they can lead to partial receptor occupancy. To study gating in partially occupied heteromers, we heterologously expressed two kainate receptor heteromers: GluK1/GluK2 heteromers, which only encompass 'low-affinity' subunits, and GluK1/GluK5 heteromers, which are composed of 'low and high-affinity' subunits. Activation and desensitization of these channels was assessed electrophysiologically using fast, piezo-driven ligand application to outside-out patches. We find, for instance, that application of saturating glutamate concentrations in combination with a subunit-specific antagonist resulted in reduced activation but varying amounts of desensitization. In extreme cases, the use of subunit-specific antagonists can cause a complete block of desensitization. Moreover, we are able to directly control receptor occupancy of GluK1 and GluK2 receptors with a family of photoswitchable tethered ligands (PTLs), the maleimide-azobenzene-glutamate (MAG) ligands.[2] These photoswitches are covalently coupled to a cysteine substitution in the LBD and ligand binding and unbinding of the glutamate head group can be controlled with short pulses of light triggering the cis/trans isomerization of the azobenzene group. Combining these PTLs with electrophysiological recordings and pharmacological manipulations might provide more detailed insight into the gating processes of heteromeric kainate receptors and, ultimately, their role in the nervous system.

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[2]Reiner and Isacoff: Tethered ligands reveal glutamate receptor desensitization depends on subunit occupancy, Nat. Chem. Biol. (2014) 10: 273-280.

Towards highly specific genetic manipulation of the mouse cannabinoid CB1 receptor using CRISPR/Cas9: cell-type selective and region-specific CB1 knockout in the adult brain and generation of a CB1 point-mutation mouse line

Floortje Remmers[1], Leonid Eshkind[2], Maren D. Lange[3], Hans-Christian Pape[3], Beat Lutz[1]

[1]University Medical Center Johannes Gutenberg University Mainz, Institute of Physiological Chemistry, Duesbergweg 6, 55128 Mainz, Germany

[2]University Medical Center Johannes Gutenberg University Mainz, Transgenic Facility Mainz, Translational Animal Research Center, Hanns-Dieter-Hüsch-Weg 19, 55128 Mainz, Germany

[3]Westfaelische Wilhelms-University, Institute of Physiology I, Robert-Koch-Straße 27b, 48149 Münster, Germany

The endocannabinoid system is involved in the regulation of many physiological processes via retrograde suppression of neurotransmitter release. Previously, cell-type specific involvement of the cannabinoid type 1 (CB1) receptor in these processes was demonstrated using conditional knockout mouse lines by crossing CB1-floxed mice with cell-type specific Cre-driver lines, whereas local viral administration of Cre-recombinase has been used to identify brain-region specific roles. However, combining cell-type specificity with brain-region selectivity has been challenging with these tools. Further, more subtle mutations than a complete gene-knockout, such as base pair substitutions, have been difficult to achieve with conventional genetic manipulations. New tools making use of the CRISPR/Cas9-system can address both these issues of combining cell-type specificity with brain-region selectivity and fast and cost-effective generation of mutant mouse lines.

In the first project, we use a Cre-dependent Cas9 mouse in combination with cell-type specific Cre-driver lines and brain region-specific stereotactic injections of adeno-associated viral vectors containing guide RNA (AAV-gRNA) targeting the CB1 gene to induce locally restricted and cell-type specific knockdown of the CB1 receptor. CB1 receptor expression and functionality is analyzed 3-4 weeks post-injection. In the second project, to generate a CB1 point-mutation mouse line with enhanced CB1 receptor activity and signaling, mouse zygotes are electroporated with a mix of Cas9 protein, guideRNA and a single-stranded donor DNA template to induce the targeted mutation. The mutation of interest is verified by a diagnostic restriction digest in DNA harvested from 4-day-old blastocysts or from tail biopsies from 3-week-old mice. These novel mouse models will allow us to unravel the underlying mechanisms of different functions of the endocannabinoid system with ever more specificity and precision.

Neuromuscular transmitters in arthropods

Michael Stern[1], Gerd Bicker[1]

[1]University of Veterinary Medicine Hannover, Division of Cell Biology, Bischofsholer Damm 15/102, 30173 Hannover, Germany

In contrast to the vertebrates and many other taxa which use acetylcholine as chemical messenger at the neuromuscular junction, in some well-studied crustacean and insect preparations glutamate has been shown as the excitatory transmitter. However, it is currently unknown whether glutamate acts as a neuromuscular transmitter in other arthropod lineages. In this cytochemical/immunofluorescence investigation, we studied potential neurotransmitters in ventral ganglia and neuromuscular junctions of selected species of Myriapoda, Chelicerata, basal Hexapoda. and pterygote insects. We could co-localize glutamate with synapsin labelling in synaptic boutons on skeletal muscles of both body wall and walking legs in all studied arthropod specimens. Acetylcholine esterase (AChE) activity as a marker for cholinergic synapses was found abundantly in the central nervous systems, but not at neuromuscular junctions. Our data indicate that glutamate, and to a lesser extent, GABA are most likely neurotransmitters at arthropod neuromuscular junctions, whereas acetylcholine is very unlikely to play a role in neuromuscular transmission. As outgroup we localized AChE at the neuromuscular junctions of the Annelida Nereis, Platynereis, Lumbricus and Eisenia. Together with our previous findings of mixed cholinergic/glutamatergic neuromuscular innervations in the onychophoran sister group, the new data support our hypothesis of the exclusive excitatory skeletal neuromuscular transmitter glutamate as a phylogenetic trait of Arthropoda. Furthermore, we could show that in the chilopod Lithobius forficatus, a large number of leg sensory neurons displayed GABA-immunofluorescence and was also labeled with an antiserum against the GABA-synthesizing enzyme, glutamate decarboxylase.

Reference: Langeloh H, Wasser H, Richter N, Bicker G, Stern M (2018) Neuromuscular transmitter candidates of a centipede (Lithobius forficatus, Chilopoda). Front. Zool. 15:28
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C-terminal truncation at serine 505 increases EAAT2 activity and is not involved in EAAT2 downregulation associated with staurosporine-induced caspase 3 activation

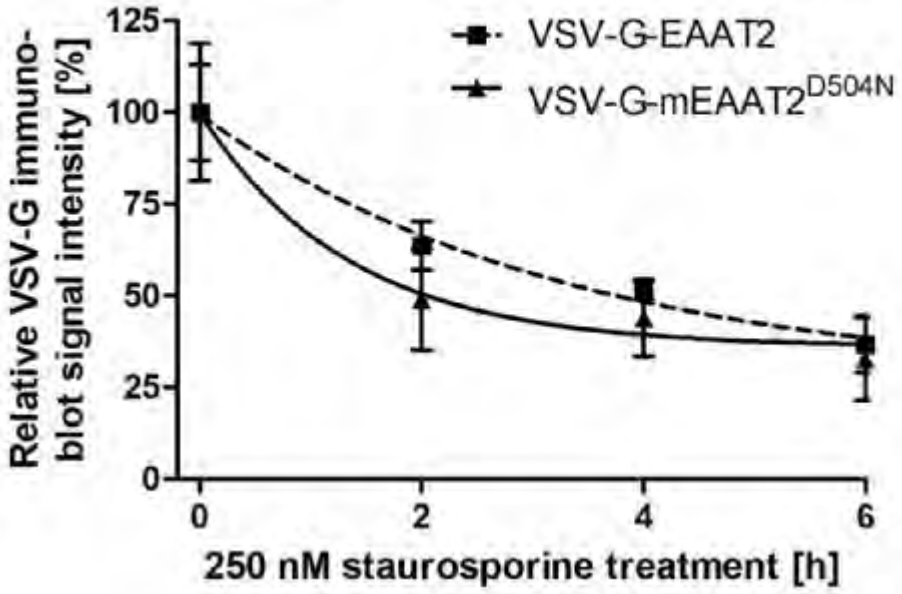
Timo-Daniel Voss[1], Jan Lewerenz[1]

[1]Neurology, Experimental Neurology, Helmholzstr. 8/1, 89081 Ulm, Germany

Introduction: Downregulation of the excitatory amino acid transporter 2 (EAAT2) observed in diseases like amyotrophic lateral sclerosis, Alzheimer's and Huntington's disease is thought to contribute to glutamate (Glu) excitotoxicity. Post-transcriptional mechanisms have been proposed to underlie this downregulation, including caspase 3 (C3)-mediated cleavage of the EAAT2 protein at the C-terminus.
Aim: To study the mechanisms that regulate activity and protein stability of the EAAT2 protein.
Methods: A172 and HT22 cells were transiently co-transfected with VSV-G-tagged wild-type ($EAAT2^{wt}$), C3-cleavage site mutant ($EAAT2^{D504N}$) or C-terminal truncated (Δ505-572) EAAT2 (tEAAT2) cloned into pBI-5 and tetracycline transactivator to allow doxycycline(Dox)-inhibitable EAAT2 expression. EAAT2 activity was measured as DL-threo-β-benzyloxyaspartate-inhibitable ^{3}H-Glu uptake in A172 cells. C3 was activated in HT22 cells by staurosporine (STR). EAAT2 protein levels and C3 activation in HT22 cells were semiquantitatively assessed by immunoblotting.

Results: When expressed in A172 cells, $EAAT2^{D504N}$ showed 19% higher activity than $EAAT2^{wt}$. In addition, in HT22 cells $EAAT2^{wt}$ protein levels were downregulated to 37±8% upon STR-induced C3 activation (250 nM, 6h). This downregulation was partially reversed by the pan-caspase inhibitor Q-VD-OPh (5 µM). However, STR-induced C3 activation was also associated with decreased EAAT2 protein levels upon $EAAT2^{D504N}$ overexpression (33±11%). Moreover, the size shift of EAAT2wt protein expected upon C3 cleavage could not be observed. In addition, the ubiquitin-activating enzyme E1 inhibitor PYR-41 (250 µM) rescued STR-mediated $EAAT2^{wt}$ protein downregulation more effectively than Q-VD-OPh. Finally, tEAAT2 overexpression compared to $EAAT2^{wt}$ revealed that the truncated protein leads to 21% higher EAAT2 activity. As ubiquitinated lysines are reportedly located at the EAAT2 C-terminus, we evaluated the half-life of EAAT2 activity after blocking transcription by Dox. While EAAT2 activity was downregulated to 61±3 and 32±2% in $EAAT2^{wt}$-overexpressing A172 cells after 24h and 48h, respectively, it was only reduced to 72±5% and 43±1% upon tEAAT2 expression.
Conclusion: We found no evidence that EAAT2 downregulation associated with STR-induced C3 activation is mediated by cleavage at the EAAT2 C-terminus in our cell models. In contrast, mimicking C3 cleavage of the EAAT2 protein increased its activity, most possibly by stabilizing the protein as C-terminal deletion removes lysines reportedly involved in EAAT2 ubiquitination. Thus, how the D504N mutation increases EAAT2 activity remains to be explored.

Wildtype and mutant D504N EAAT2 protein levels



Poster Topic

T5: G Protein-linked and other Receptors

The adhesion-GPCR CIRL promotes mechanosensory signal discrimination

Sven Dannhäuser[1,2], Thomas Lux[3], Jeremy Chen[3], Nadine Ehmann[1,2], Chun Hu[4], Peter Soba[4], Heike Rittner[3], Robert J. Kittel[1,2]

[1]Department of Animal Physiology, Institute of Biology, Leipzig University, 04103 Leipzig, Germany
[2]Carl-Ludwig-Institute for Physiology, Leipzig University, 04103 Leipzig, Germany
[3]Department of Anaesthesiology, University Hospital Würzburg, 97080 Würzburg, Germany
[4]Neuronal Patterning and Connectivity, Center for Molecular Neurobiology, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany

Research on the sense of touch has long been a center stage for receptors that directly convert mechanical force into electrical signals, and the function of such mechanosensing ion channels remains a topical research focus. In contrast, evidence for mechano-metabotropic signal transduction and compelling models of force conversion into an intracellular second messenger response are limited, despite the vital role of metabotropic modulation in all corners of physiology.

Adhesion-type G protein-coupled receptors (aGPCRs), a large molecule family with over 30 members in humans, operate in a vast range of physiological processes. Correspondingly, these receptors are associated with diverse human diseases, such as developmental disorders, defects of the nervous system, allergies and cancer. Several aGPCRs have recently been linked to mechanosensitive functions suggesting that processing of mechanical stimuli may be a common feature of this receptor family, not only in classical mechanosensory structures.

Drosophila Latrophilin/CIRL (ADGRL), one of the oldest members of the aGPCR family, modulates mechanosensory signal transduction. In addition to shaping sensory responses to gentle touch and sound, we show here that CIRL also influences mechanonociception *in vivo*. *Cirl* is expressed in peripheral larval nociceptors where it adjusts nocifensive behaviour under physiological conditions and in a chemical neuropathy model. By combining behavioural analyses with optogenetic manipulation of cyclic AMP levels *in vivo*, we find that CIRL exerts opposing modulatory effects in low-threshold mechanosensory neurons and high-threshold nociceptors. This bipolar action likely facilitates the differentiation of mechanosensory signals carrying different physiological information. Currently, we are testing for putative interaction partners and for an evolutionary conservation of CIRL function in rodent models of nociception.

CaMello-XR: Visualization and optogenetic control of $G_{q/11}$ signals and receptor trafficking in GPCR-specific domains

Dennis Eickelbeck[1], Raziye Karapinar[1], Alexander Jack[2], Petra Wahle[2], Stefan Herlitze[1]

[1]Ruhr University Bochum, Department of Zoology and Neurobiology, Universitätsstr. 150, 44780 Bochum, Germany
[2]Ruhr University Bochum, AG Entwicklungsneurobiologie, Universitätsstr. 150, 44780 Bochum, Germany

G protein-coupled receptors (GPCRs) modulate intracellular signaling cascades, thereby linking external cues to internal responses via activation of different GPCR pathways. The 5-HT_2 serotonin receptor family (5-HTR) for example activates the $G_{q/11}$ pathway, leading to an increase in intracellular Ca^{2+} levels. The characteristics of these signals highly depend on the subcellular localization and trafficking of the GPCR. To illuminate these subcellular effects, we engineered CaMello (Ca^{2+}-melanopsin-local-sensor) and mLocal for -simultaneous or separate- active modulation and passive visualization of traffic-dependent Ca^{2+} signals in different receptor domains. We show that the specific localization of the GPCR to its receptor domain, e.g. to 5-HT_{2A} receptor domains, drastically alters the dynamics and localization of these Ca^{2+} events in different neuronal populations in the cerebral and cerebellar cortex *in vitro* and *in vivo*.

The brain oxytocin system and its complex impact on stress and anxiety

Benjamin Jurek[1]

[1] University of Regensburg, Department of Behavioral and Molecular Neurobiology, Universitaetsstr. 31, 93051 Regensburg, Germany

In recent years, the neuropeptide oxytocin (OXT) attracted considerable attention in science and public due to its ability to modulate aspects of socio-emotional behavior and stress. Studies on intranasal OXT in human patients or healthy probands are accumulating, reporting a barrage of effects depending on the context, sex, dosage, and duration of the treatment. This is in part due to the plethora of signaling cascades that are coupled to the OXT receptor (OXTR), such as the MEK1/2, p38, ERK5, CamK, PI3K, Calcineurin, eEF2, or PKA/PKC. The activity of these cascades depend on the sex, species and duration of treatment (Jurek and Neumann, 2018), while signal/target specificity is brought about by the exclusive or combined activation of a yet unknown subset of signaling cascades. OT's concerted effects on specific cascades lead to altered activity of a defined set of nuclear transcription/translation factors, (e.g. CREB, CRTC3, MEF-2, or eEF2, see graphic below) to alter gene expression, but also cytoplasmic targets, like channel proteins, to alter neuronal excitability (van den Burg et al., 2015) or morphology (Meyer et al., 2018). Our research aims to determine how the combination of nuclear and cytoplasmic effects interact to lead to the observed behavioural effects of OXT.
We could unravel the role of the MAPK pathways in OT's anxiolytic effect (Jurek et al., 2012), as well as the involvement of the CREB/CRTC (Jurek et al., 2015) and MEF-2A/C (Meyer et al., 2018) pathways in OT's transcriptional regulation of stress-related factors, such as corticotropin releasing factor (CRF), CRF receptor 2, or NPY5R. The transcriptional regulation of those factors manifests then in altered anxiety-like behavior. With this work we hope to contribute to a better understanding of the underlying mechanisms of altered socio-emotional behavior, which is essential for the development of effective and safe treatment of anxiety- or stress-related disorders.

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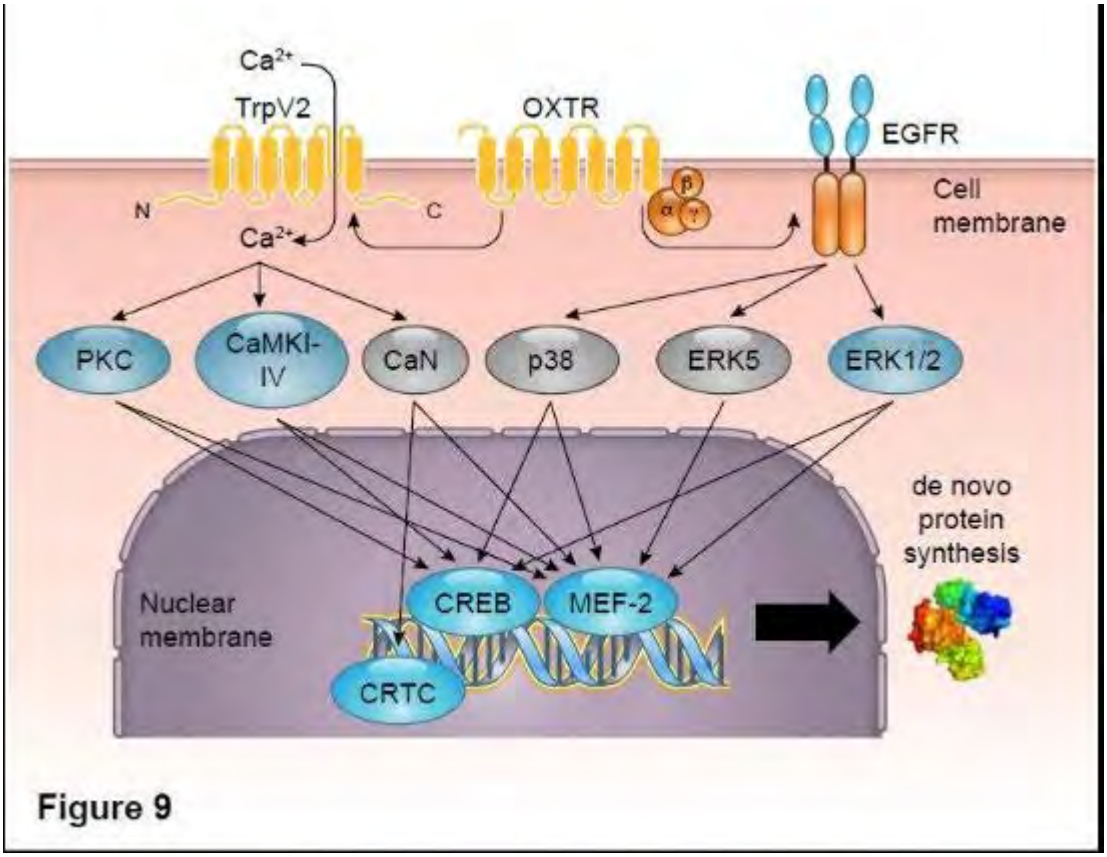
Ca2+
TrpV2
OXTR
EGFR
N
C
Ca2+
Cell membrane
PKC
CaMKI-IV
CaN
p38
ERK5
ERK1/2
de novo protein synthesis
Nuclear membrane
CREB
MEF-2
CRTC

Figure 9

ADAMTS 4/5-mediated proteolysis of neural extracellular matrix upon D1-like dopamine receptor stimulation

Jessica Mitlöhner[1], Rahul Kaushik[2], Christine Gee[3], Alexander Dityatev[1,2,5], Renato Frischknecht[1,4,5], Constanze Seidenbecher[1,5]

[1]Leibniz Institute for Neurobiology, Neurochemistry and Molecular Biology, Magdeburg, Germany
[2]DZNE, Molecular Neuroplasticity, Magdeburg, Germany
[3]Center for Molecular Neurobiology Hamburg, Institute of Synaptic Physiology, Hamburg, Germany
[4]University of Erlangen-Nuremberg, Dept. of Biology, Animal Physiology, Erlangen, Germany
[5]Center for behavioral Brain Sciences, CBBS, Magdeburg, Germany

The extracellular matrix (ECM) of the central nervous system largely consists of chondroitin sulfate proteoglycans such as aggrecan and brevican bound to hyaluronic acid, link proteins and tenascins. The brain's ECM has been shown to enwrap pre- as well as postsynapses and to be formed and remodeled in an activity-dependent manner. Evidence accumulates that the ECM plays an important role in synaptic plasticity, learning and memory formation and stability.
Dopamine (DA), one crucial neuromodulator for motivated learning, acts through either D1- or D2-like DA receptors in the brain. These receptors can either stimulate or inhibit protein kinase A (PKA) activity. D1/D5 DA receptor-dependent PKA activation was shown to lead to enhanced extracellular tissue-type plasminogen activator (tPA) activity probably associated with an increased release of this protease. Hence, we hypothesized that a similar mechanism may underlie DA-dependent ECM remodeling by proteases, such as ADAMTS 4 and 5.
Here, we studied the impact of DA in ECM modification by proteolytic cleavage and its relevance for synaptic plasticity. Therefore, D1-like or D2-like receptors were activated by receptor agonists SKF81297 and Quinpirole, respectively. Immunocytochemical analysis revealed that perisynaptic brevican cleavage is increased only after D1-like receptor activation at excitatory synapses. We could block this effect by the D1-like DA receptor antagonist SCH23390 as well as by using an inhibitor of PKA, ADAMTS 4/5 and by shRNA-mediated knock down of ADAMTS 4 and ADAMTS 5. In addition, we could show that DA-dependent Brevican cleavage depends on intracellular cAMP levels using light modulation of cellular cAMP by bPAC (Stierl et al. 2011). Taken together, these findings point to an interplay between the dopaminergic system and activity-induced ECM remodeling which also involves NMDA receptor and CaMKII activity.

Characteristics of 5-HT_7 receptor-expressing neurons in the mouse ventral dentate gyrus

Marcin Siwiec[1], Krzysztof Tokarski[1]

[1]Institute of Pharmacology of the Polish Academy of Sciences, Department of Physiology, Smetna 12 Street, 31-343 Kraków, Poland

The 5-HT_7 receptor is a potential therapeutic target for affective and neurodevelopmental disorders. Its anatomy and physiological function as well as the cellular mechanisms associated with its signaling pathways have not yet been fully understood. Depending on the brain region, 5-HT_7 receptors can be found on primary cells or local interneurons, or both. A particularly high density of 5-HT_7 receptors are present in the rodent hippocampus, in CA subfields as well as the dentate gyrus.

Our experiments aimed to investigate the impact of 5-HT_7 receptor activation on the mouse ventral dentate gyrus network, and to characterize 5-HT_7-expressing neurons in this structure. To this end, we used a transgenic mouse line expressing eGFP under the Htr7 promoter to visually identify 5-HT_7-expressing cells. This allowed us to perform selective patch clamp recordings from eGFP-positive neurons under fluorescence microscopy and to examine their electrophysiological characteristics. This was followed by morphological and neurochemical analysis of recorded cells, which were filled with biocytin during the recordings and subjected to immunohistochemical staining protocols.

We found that the eGFP signal was abundant in interneurons of the hilar region and present up to the border with the granular layer on putative basket cells. However, there were no eGFP-positive cells among dentate granule cells. The immunohistochemical data was supported by electrophysiological recordings, where 5-HT_7 receptor agonists elicited direct responses in eGFP-positive cells. Recording of synaptic activity showed that application of 5-HT_7 receptor agonists increased the frequency of spontaneous inhibitory postsynaptic currents recorded from dentate granule cells.

To conclude, we found that 5-HT_7 receptor signalling influences single cell and network activity in the mouse ventral dentate gyrus and were able to characterize the neurons expressing this receptor within the dentate hilar region. These results shed more light on serotonin signaling in the hippocampus which may prove relevant to the pathophysiology of affective disorders.

Dual action of D2 dopamine receptor activation in nucleus incertus – potential source of sex differences in food intake

Agata Szlaga[1], Anna Gugula[1], Anna Blasiak[1]

[1] Jagiellonian University in Krakow, Department of Biology, Institute of Zoology and Biomedical Research, Krakow, Poland

Aims: Nucleus incertus (NI) is a main source of relaxin-3 in the brain and is involved in control of stress, food intake and arousal related processes. Relaxin-3 is an orexigenic (promoting food intake) peptide with stronger actions in female rats. The goal of the current study was to asses the involvement of dopaminergic receptors in NI neurons activity modulation.
Methods: The influence of dopaminergic D2 receptor agonists on NI neurons activity was recorded using whole-cell patch clamp technique. Combined tract-tracing with anti-tyrosine hydroxylase (TH) immunohistochemical staining was used to indicate the source of TH in NI.
Results: Electrophysiological recordings revealed expression of functional D2 receptors on NI neurons. Bath application of D2R agonist quinpirole (20 μM) exerted both inhibitory (increase in outward current by 27.53 ± 11.07 pA (mean change ± SEM) and decrease in action potentials firing frequency by 2.3 ± 0.9 Hz) and excitatory influence (increase in inward current: 24.79 ± 8.16 pA and increase in frequency of action potentials by 0.72 ± 0.09 Hz) on neuronal activity within the NI. Both excitatory and inhibitory actions of quinpirole persisted in the presence of tetrodotoxin and GABA/glutamate receptors antagonists, what indicates postsynaptic site of its action. Using tract-tracing technique, we indicated hypothalamic dopaminergic groups A11 and A13 as a source of TH-immunoreactive fibers in the NI.
Conclusions: TH-immunoreactive fibers arising from A11 and A13 are possible source of dopamine in the NI. Taking into account that both A11 and A13 are sensitive to sex hormones, their presumably dopaminergic projections may control the sex differences in food intake governed by nucleus incertus relaxin-3-positive neurons.

MrgD is expressed by neurons in the forebrain

Oliver von Bohlen und Halbach[1], Nora Bödecker[1], Thomas Walther[2], Anja Tetzner[2], Viola von Bohlen und Halbach[1]

[1]Universitätsmedizin Greifswald, Institut für Anatomie und Zellbiologie, Friedrich Loeffler Str. 23c, 17489 Greifswald, Germany

[2]Dept. Pharmacology and Therapeutics, School of Medicine and School of Pharmacy, University College Cork (UCC), Cork, Ireland

Aside from the periphery, a renin-angiotesin-system (RAS) exists within the brain. Not only the classic members of the RAS (angiotensin II, angiotensin II (1-7), angiotensin IV and their specific cognate receptors AT1, AT2, AT4, Mas) have been identified in the brain. In addition, further members, as e.g. pro-renin and its receptor have been identified and it has been shown that the pro-renin receptor, among others, seems to play a role in adult hippocampal neurogenesis (Schäfer et al., 2015).
About 15 years ago a large family of GPCRs, now called Mas-related G protein-coupled receptors (Mrgprs or Mrg) has been identified. The most prominent members of this family are MrgA, MrgB, MrgC, and MrgD. While it is known that MrgD is specifically expressed in a subpopulation of sensory neurons in the skin, the existence of MrgD-positive neurons in the brain is unknown. Therefore, we used adult mice in which MrgD-expressing neurons were marked by a genetically encoded axonal tracer (Zylka et al., 2005) and examined the expression pattern within the forebrain. Concerning the cortex, we could detect positive labelled cells especially in the prefrontal cortex and in the piriform cortex. Labelled cells were also found, at a lower frequency, in the motor cortex, whereas in the somatosensory cortex, only some labelled cells were detected. Labelled cells in the hippocampus were mainly mapped to area CA3 and in the amygdala mainly in the anterior amygdaloid area, the posteriomedial cortical nucleus of the amygdala and the basomedial nucleus of the amygdala. However, the strongest signal was detected in the basal ganglia, namely the caudate putamen and the substantia nigra pars compacta.
MrgD-expressing neurons in the periphery and in the spinal chord have been associated with nociception of epidermal areas (Zylka et al., 2005). The expression of MrgD in neurons of the forebrain, however, is not restricted to areas involved in nociception, but is prominent in areas involved in motor-functions, as e.g. the motocortex, the caudate-putamen and the substantia nigra.

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Poster Topic

T6: Ligand-gated, Voltage-dependent Ion Channels and Transporters

T6-1A Low-voltage-activated calcium and TTX-sensitive sodium currents are present at young and adult mouse retinal horizontal cells
Norbert Babai, Johann Helmut Brandstätter, Andreas Feigenspan

T6-2A Control of circadian ATP release in organotypic cultures of the rat suprachiasmatic nucleus by purinergic P2X and P2Y receptors
Anirban Bhattacharyya, Irena Svobodová , Milorad Ivetic, Zdenka Bendová , Hana Zemková

T6-3A Expression of the BK channel γ subunit LRRC52 (γ2) in mouse inner hair cells and the „BK channel activation paradox“
Jutta Engel, Isabelle Lang, Barbara A. Niemeyer, Martin Jung, Peter Ruth

T6-4A Mechanism of GLUT1 and GLUT3 palmitoylation
Noemi Gmahl, Nataliya Gorinski, Britta Stapel, Kai G. Kahl, Evgeni Ponimaskin

T6-5A Mechano-gating properties of *Drosophila* NOMPC
Philip Hehlert, Thomas Effertz, Martin Göpfert

T6-6A Probing the function of $\alpha_2\delta$ voltage gated calcium channel subunits in the genetic model system *Drosophila melanogaster*
Laurin Heinrich, Christopher Bell, Stefanie Ryglewski

T6-1B Quantification of alternative splicing within ionotropic glutamate receptors (iGluRs) using human RNA-Seq data
Robin Herbrechter, Andreas Reiner

T6-2B A novel RNA editing sensor tool and specific agonist determine neuronal protein expression of RNA-edited glycine receptors
Florian Hetsch, Benjamin Förstera, Aline Winkelmann, Pina Knauff, Erich E. Wanker, Xintian A. You, Marcus Semtner, Svenja Kankowski, Jochen C. Meier

T6-3B Effect of fasting/refeeding on purinergic modulation of GABA-ergic synaptic transmission in the rat supraoptic nucleus
Milorad Ivetic, Anirban Bhattacharya, Hana Janouskova, Hana Zemkova

T6-4B Alternative splicing as a mechanism to increase ion channel diversity
Lukas Kilo, Stefanie Ryglewski

T6-5B RNA-edited glycine receptors are potential targets for pharmacotherapy in temporal lobe epilepsy
Larissa Kraus, Svenja Kankowski, Florian Hetsch, Nicolai Dorka, Marcus Semtner, Martin Holtkamp, Jochen C. Meier, Pawel Fidzinski

T6-1C The role of L-type Dmca1D calcium channels at the Drosophila neuromuscular synapse
Niklas Krick, Carsten Duch

T6-2C Maintaining excitation and inhibition at single cell level
Marie-Luise Kümmel, Uli Müller

T6-3C Thyroid Hormone Effects on Metabolic Rate: Correlation of Na^+ Influx and Expression of Na^+/K^+-ATPase
Heiko Michael Leßlich, Lisa Bachmann, Sascha Döring, Sivaraj Mohana Sundaram, Irmgard D. Dietzel

T6-4C Interactions of calcium channel ($Ca_v1.2$) circuitries with early life stress and their involvement in the pathogenesis of psychiatric disorders
Srivaishnavi Loganathan, Jan M Deussing

T6-5C TRPM3 channels in non-neuronal cells of somatosensory dorsal root ganglia
Johannes Oberwinkler, Sandeep Dembla, Raissa Enzeroth, Behrendt Marc

T6-6C Nano-scale dynamics of voltage gated Ca^{2+} channels: an in vivo single molecule analysis
Tina Ghelani, Hylkje Geertseema, Ulrich Thomas, Martin Lehmann, Felix Ewers, Martin Heine, Stephan J. Sigrist

T6-1D Effect of solute carriers (SLC) on CA1 pyramidal cells, synaptic transmission and hippocampal network activity
Marco Rohde, Vanessa Ziesak, Andreas Birkenfeld, Rüdiger Köhling

T6-2D Alanine scanning mutagenesis of the rat P2X7 receptor highlights the requirement for lysine and aspartate in the first transmembrane domain
Marian Rupert, Anirban Bhattacharya, Hana Janouskova, Stanko Stojilkovic, Hana Zemkova

T6-3D Role of the Na^+-activated K^+ channel Slack (Slo2.2) for hearing function and noise vulnerability in mice
Pauline Schepsky, Anne Bausch, Robert Lukowski, Katharina Sorg, Dietmar Hecker, Bernhard Schick, Peter Ruth, Simone Kurt, Jutta Engel

T6-4D The auxiliary Ca^{2+} channel subunits $\alpha_2\delta2$ and $\alpha_2\delta3$ are required for proper $Ca_v2.1$ currents in cultured spiral ganglion neurons and for the development of endbulb of Held synapses
Friederike Stephani, Kerstin Blum, Jutta Engel

T6-5D *In silico* current prediction and noise analysis elucidates gating properties of heterodimeric rClC-

K1 chloride channels
Stefan Thiemann, Birgit Begemann, Toni Becher, Martin Fischer

T6-6D Ionic channels involved in spontaneous and CRH-induced excitability and calcium signaling of mice corticotrophs
Hana Zemkova, Melania Tomic, Marek Kucka, Greti Aguilera, Stanko S Stojilkovic

Low-voltage-activated calcium and TTX-sensitive sodium currents are present at young and adult mouse retinal horizontal cells

Norbert Babai[1], Johann Helmut Brandstätter[1], Andreas Feigenspan[1]

[1]University of Erlangen-Nürnberg, Department of Biology, Animal Physiology, Staudtstraße 5, 91058 Erlangen, Germany

Purpose: For the normal maturation of synaptic wiring among neurons, a highly specialized configuration of voltage- and ligand-gated ion channels is necessary. These ion channels expressed early in development regulate the activity of the neuron. The properties of ion channels expressed in developing neurons usually differ from those of adult cells.
Retinal horizontal cells (HCs) are interneurons conveying lateral information flow across the first synaptic layer of the retina, playing an important role in the center-surround organization. Ion channels of developing horizontal cells have not been described yet. The aim of this study was to examine the physiological properties of the voltage-gated currents and to identify the different current components present in mouse retinal horizontal cells.

Methods: The biophysical properties of inward currents in HCs of four groups of mice of different postnatal age (P8 – 11, P12 – 16, P21 – 30, P>60) were studied using a Cs^+- and TEA^+-based intracellular solution. High- (HVAC) and low- (LVAC) voltage-activated currents were discriminated using different holding potentials. Current activation, voltage- and time-dependent inactivation, and time-dependent recovery from inactivation at -30 mV holding potential were analyzed. To dissect currents, compounds such as the sodium channel blocker TTX and the T-type calcium channel blockers $NiCl_2$ and ML218 were used.

Results: Both HVAC and LVAC progressively attenuated with increasing postnatal age (from P8 to P60). At all examined ages, V50 activation of HVAC and LVAC was around -4 mV and -17 mV, respectively. HVAC stayed relatively constant during test pulse while LVAC showed a more transient response. Voltage-dependent inactivation of the steady current was shifted from -13 mV to -6.3 mV. Time-dependent recovery of the peak current from inactivation at -30 mV was reduced from 11.9 ms (P8) to 5.7 ms (P>60). All other current parameters did not significantly change during postnatal retinal development. TTX and ML218 (also $NiCl_2$) revealed the presence of sodium and T-type calcium channels in both young and adult mouse retinal HCs. The off response of the light-induced membrane potential changes in adult HCs was slightly decreased in the presence of 2 µM ML218; however, 1 µM TTX did not change the kinetics of the light response.

Conclusion: The larger HVAC and LVAC amplitude in mouse HCs at younger ages probably reflects a higher number of ion channels that are necessary for the functional organization of the OPL during postnatal retinal development. The evidence that HCs express T-type calcium channels and sodium channels even at adulthood opens up several questions for future studies about their role in lateral information transfer between photoreceptors and bipolar cells.

Control of circadian ATP release in organotypic cultures of the rat suprachiasmatic nucleus by purinergic P2X and P2Y receptors

Anirban Bhattacharyya[1], Irena Svobodová [1], Milorad Ivetic[1], Zdenka Bendová [2], Hana Zemková [1]

[1]Institute of Physiology Academy of Sciences of the Czech Republic, Department of Cellular and Molecular Neuroendocrinology, Prague,Czech Republic

[2]Department of Physiology, Faculty of Science, Charles University in Prague, Czech Republic

The circadian rhythms in physiological and behavioral functions are driven by a pacemaker located in the suprachiasmatic nucleus (SCN). The rhythms continue in constant darkness and depend on cell-cell communication between neurons and glia. The SCN neurons generate a circadian rhythm in arginine vasopressin secretion that parallels the rhythm in electrical activity. The SCN also generates a circadian rhythm in the extracellular adenosine 5'-triphosphate (ATP) accumulation, which negatively correlates with the electrical activity and AVP secretory rhythm, indicating that ATP is released from astrocytes, but molecular mechanisms that regulate ATP release are poorly understood. Here, we tested the hypothesis that ATP is released via the plasma membrane purinergic P2X7 receptors (P2X7Rs) and P2Y receptors (P2YRs) which have been previously shown to be expressed in the SCN tissue at transcriptional level. We have investigated this hypothesis using SCN organotypic cultures, primary cultures of SCN astrocytes, ATP bioluminescent assays, immunohistochemistry, patch-clamping, and calcium imaging. We found that extracellular ATP accumulation in organotypic cultures followed a circadian rhythm, with a peak between 24:00 – 04:00 h, and the trough at approximately 12:00 h. ATP rhythm was inhibited by application of AZ10606120, A438079 and BBG, specific blockers of P2X7R, and potentiated by GW791343, a positive allosteric modulator of this receptor. Double-immunohistochemical staining revealed high expression of the P2X7R protein in astrocytes of SCN slices. PPADS, a non-specific P2 antagonist, and MRS2179, specific P2Y1R antagonist, also abolished ATP rhythm, whereas the specific P2X4R blocker 5-BDBD was not effective. The pannexin-1 hemichannel blocker carbenoxolone displayed a partial inhibitory effect. The P2Y1R agonist MRS2365 and the P2Y2R agonist MRS2768 potentiated ATP release in organotypic cultures and increase intracellular Ca2+ level in cultured astrocytes. Our data provide evidence for the involvement of P2X7, P2Y1 and P2Y2 receptors in circadian ATP release from astrocytes in SCN organotypic cultures. Purinergic signaling via P2X7Rs is well known to play important roles in neurodegeneration, neuroprotection and neuro-regeneration, and our results might improve our understanding of the roles of these receptors in the healthy central nervous system.

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Expression of the BK channel γ subunit LRRC52 (γ2) in mouse inner hair cells and the „BK channel activation paradox“

Jutta Engel[1], Isabelle Lang[1], Barbara A. Niemeyer[2], Martin Jung[3], Peter Ruth[4]

[1]Saarland University, Department of Biophysics and CIPMM, Hearing Research, Homburg, Germany
[2]Saarland University, Department of Biophysics and CIPMM, Molecular Biophysics, Homburg, Germany
[3] Saarland University, Dept. of Biochemistry, Homburg, Germany
[4]University of Tübingen, Dept. of Pharmacology and Toxicology, Tübingen, Germany

The mammalian inner hair cell (IHC) transduces sound into depolarization, followed by transmitter release resulting in neuronal activation. The big conductance, voltage and Ca^{2+}-activated K^+ (BK) channels of the mature IHC are responsible for fast repolarization of the receptor potential and for the small time constant of the IHC, enabling it to respond very quickly to changes in membrane potential. BK channels of the mammalian IHC, which are localized at the IHC neck, are spaced at a large distance from $Ca_V1.3$ Ca^{2+} channels at ribbon synapses. Although they activate at negative voltages (V_{half} ~ -50 mV; Marcotti et al., J Physiol 2004) they are - unlike non-mammalian hair cells - largely insensitive to Ca^{2+} influx, a paradox that has not been resolved so far.

Ca^{2+}-independent activation of BK channels at negative potentials can be caused by the newly detected γ subunits, the LRRC proteins 26, 52, 55 or 78, which upon heterologous expression shift the voltage of half-maximum activation of the BK current by -140 mV, -100 mV, -50 mV, -20 mV, respectively (Yang and Aldrich, PNAS, 2012).

We consistently detected mRNA for LRRC52 but not for LRRC26 in cDNA synthesized from mRNA harvested specifically from IHCs of organs of Corti of 3-week-old wildtype (WT) mice using a nested PCR approach. Fluorescence immunolabeling for LRRC52 using confocal microscopy revealed perfect co-localization with spot-like immunoreactivity for BKα, the pore-forming subunit, at the IHC neck. This co-localization was confirmed using a proximity ligation assay, which indicates that both proteins are localized within a distance of less than 40 nm.

LRRC52 protein being absent from IHC until P10 was co-expressed and co-localized with BK protein from the onset of hearing at P12 onwards. LRRC52 was not expressed in three-week-old IHCs of BKα knockout mice. In IHCs of $Ca_V1.3$-deficient mice, which lack BKα protein, LRRC52 was missing, too.

In sum, LRRC52 is an intrinsic γ subunit of the mouse IHC BK channel complex from the developmental up-regulation of BK channel expression at the onset of hearing onwards and is a strong candidate for causing the Ca^{2+}-independent activation of BK currents at negative voltages in mouse IHCs.

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Mechanism of GLUT1 and GLUT3 palmitoylation

Noemi Gmahl[1], Nataliya Gorinski[1], Britta Stapel[2], Kai G. Kahl[2], Evgeni Ponimaskin[1]

[1]Cellular Neurophysiology, Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover, Germany
[2]Department of Psychiatry, Social Psychiatry and Psychotherapy, Hannover Medical School, Carl-Neuberg-Straße 1, 30625 Hannover, Germany

Protein palmitoylation, the posttranslational thioesterification of target proteins with C16 palmitic acid, is the most common lipid modification. It is widely accepted that the reversible attachment of palmitate to a protein affects, inter alia, its localization in specific membrane domains, the protein's stability and/or its functions. In the present study we analysed the palmitoylation levels of two glucose transporters, GLUT1 and GLUT3. GLUT1 is expressed in astrocytes and endothelial cells of the nervous system, whereas GLUT3 can be found in neurons. We have demonstrated that both GLUT1 and GLUT3 undergo basal palmitoylation in humane mononuclear cells and that treatment of cells with the antidepressant fluoxetine, a selective serotonin reuptake inhibitor (SSRI), rises palmitoylation of both glucose transporters. In the case of GLUT1, this subsequently increases the glucose uptake. We also investigated which of the 23 known DHHC-palmitoyltransferases can mediate palmitoylation of glucose transporters *in vitro* and which cysteine residues are being used as a target for the covalent attachment of palmitate. As palmitoylation often occurs in regions with close proximity to the plasma membrane, we focused on cysteines resided near transmembrane areas of GLUT1 and GLUT3. These cysteine residues were replaced with serine, followed by palmitoylation analysis by two different approaches: the Acyl-Biotinyl-Exchange (ABE) assay and labelling with [^{3}H] palmitate. Furthermore, using [^{18}F]-FDG assay we examined whether the palmitoylation-deficient mutants of GLUT1 and GLUT3 affect glucose uptake.

Mechano-gating properties of *Drosophila* NOMPC

Philip Hehlert[1], Thomas Effertz[2], Martin Göpfert[1]

[1]Georg-August-Universität Göttingen,Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Zelluläre Neurobiologie, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

[2]Universitätsmedizin Göttingen, Middle Ear Laboratory, Robert-Koch-Straße 40, 37075 Göttingen, Germany

The antennal ear of *Drosophila* comprises distinct clusters of ciliated mechanosensory cells for sound, wind and gravity transduction. Sound transduction involves NOMPC and Nan-Iav TRP channels, yet the respective roles of these mechanoelectrical-transduction (MET) channels are controversial.

The fly's sound receiver behaves like a critical harmonic oscillator that unifies the characteristics of the cochlear amplifier: self-sustained oscillations, free fluctuation, frequency specific amplification, power gain, and nonlinear compression.

These characteristics can be described by an adapted gating spring model. The rapid nature of sound transduction posits a direct gating of MET channels by mechanical stimuli. Additionally, it is assumed that these MET-channels operate in parallel and utilize an elastic element, the so-called gating spring, to relay stimulus forces to their respective gate. However, the molecular identity of this gating spring has not been found yet.

Previous work identified NOMPC as a candidate for the gating spring. Though, the long ankyrin repeats (ARs) of NOMPC (29 ARs) that may harbour some spring-like characteristics are very likely not the gating spring. Assuming that the gating spring behaves like a Hookean spring we searched for other parts of NOMPC with high compliance. We identified the PreS1-Linker domain as the region that may holds the potential to behave like a spring.

I will present new data on *Drosophila* NOMPC mechano-gating that hints at the molecular identity of the gating spring.

Probing the function of $\alpha_2\delta$ voltage gated calcium channel subunits in the genetic model system *Drosophila melanogaster*

Laurin Heinrich[1], Christopher Bell[1], Stefanie Ryglewski[1]

[1]Institute of Developmental Biology and Neurobiology, AG Duch, Hanns-Dieter-Hüsch Weg 15, 55128 Mainz, Germany

Voltage gated calcium channels (VGCC) are essential for the normal function of excitable cells. Calcium influx through VGCCs functions as charge carrier and plays an important role as a ubiquitary second messenger. In vertebrates there is a high diversity of the pore-forming α_1 subunit, which comprises three different subclasses (Ca_V1 – Ca_V3) with several members each amounting to 10 genes total encoding VGCCs. This diversity is further increased by interaction of the high voltage activated (HVA) calcium channel families Ca_V1 and Ca_V2 with regulatory subunits ($\alpha_2\delta$, β, γ). In this study we are analyzing the function of the $\alpha_2\delta$ subunits. In vertebrates as well as in *Drosophila* there are four different genes for $\alpha_2\delta$ ($\alpha_2\delta_1$ - $\alpha_2\delta_4$). Together with only two genes encoding HVA VGCCs in *Drosophila* this adds up to 8 $\alpha_2\delta$ – α_1 combinations as compared to 112 in vertebrates. $\alpha_2\delta$s are thought to modulate the biophysical properties, localization and stabilization of HVA channels. Additionally they seem to play a role in synaptogenesis. Therefore functional defects in $\alpha_2\delta$ subunits can cause a variety of neurological disorders. Still at present it is mostly unknown what the different functions of the numerous $\alpha_2\delta$ - α_1 combinations are. In this study we try to unravel the combinatorial code for functional α_1 and $\alpha_2\delta$ interactions, whether each combination serves a different function and if different $\alpha_2\delta$ subunits can compensate for each other.

A combination of molecular biological, neuroanatomical, electro- and optophysiological methods in the genetic model system *Drosophila melanogaster* is used to address this question.

Our data hints at a division of labor between the different $\alpha_2\delta$ - α_1 combinations. Endogenously tagged $\alpha_2\delta_1$ and $\alpha_2\delta_3$ subunits show distinctly different localizations in the *Drosophila* nervous system, suggesting different functions. Thereby $\alpha_2\delta_3$ seems important to maintain the amplitude of somatodendritic Ca_V2 calcium currents in identified adult flight motoneurons. By contrast, RNAi knock down of $\alpha_2\delta_1$ does not alter the biophysical properties of these channels. Instead $\alpha_2\delta_1$ seems to be involved in the dendritic and axonal localization of HVA channels in the same neuron. We also confirm that $\alpha_2\delta_3$ is crucial for synaptogenesis but not for maintenance of the neuromuscular synapse. However preliminary data indicate $\alpha_2\delta_1$ to play a role in the synaptogenesis of inhibitory synapses. To directly assess the interactions of different $\alpha_2\delta$ proteins with the different HVA channels we are now doing co-immunoprecipitation in fly strains in which these proteins are endogenously tagged with the “MiMIC protein trap” technique. Thereby we expect to unravel a combinatorial code for the different $\alpha_2\delta$ -α_1 combinations, that can be generalized for different types of *Drosophila* neurons and also hold in vertebrates.

Quantification of alternative splicing within ionotropic glutamate receptors (iGluRs) using human RNA-Seq data

Robin Herbrechter[1], Andreas Reiner[1]

[1]Ruhr-University Bochum, NG Cellular Neurobiology, Universitätsstraße 150, 44869 Bochum, Germany

Ionotropic glutamate receptors (iGluRs) are ligand gated ion channels, which mediate glutamatergic neurotransmission within the central nervous system. The 18 iGluR members are divided into AMPA, NMDA, KA and δ receptors. Their functional diversity is further increased by RNA editing and alternative splicing, which affect e.g. receptor trafficking, channel conductance, or receptor kinetics. Detailed knowledge about spatio-temporal iGluR isoform expression levels is vital for understanding the physiology of the glutamatergic system in development, health and disease.
We set out to quantify alternative splicing events within the iGluR family using the Tuxedo pipeline and human RNA-Seq data from different brain regions (730 Gbases in 36 data sets)[1,2]. Next to analyzing previously annotated splicing and editing events across different brain regions, we aimed to identify new potential iGluR isoforms. For that purpose we extracted all iGluR-related splice junctions detected by Tophat and evaluated them using a custom written Matlab pipeline. At this point, we have detected 337 of the 379 iGluR junctions that were annotated so far (88.9 %), and we found ~169 novel, potential junctions, which undergo subsequent manual evaluation. Findings will be validated/quantified using RT-PCR/qPCR. Using patch clamp recordings we will proof functionality and determine electrophysiological characteristics of selected, novel isoforms.

[1]Wu et al., Integrative analyses of RNA editing, alternative splicing, and expression of young genes in human brain transcriptome by deep RNA sequencing, J Mol Cell Biol. (2015) 7: 314-325.

[2]Labonté et al., Sex-specific transcriptional signatures in human depression, Nat Med. (2017) 23: 1102-1111.

A novel RNA editing sensor tool and specific agonist determine neuronal protein expression of RNA-edited glycine receptors

Florian Hetsch[1], Benjamin Förstera[2], Aline Winkelmann[3], Pina Knauff[4], Erich E. Wanker[3], Xintian A. You[5], Marcus Semtner[6], Svenja Kankowski[1], Jochen C. Meier[1]

[1]TU Braunschweig, Zellphysiologie, Spielmannstr. 8, 38106 Braunschweig, Germany

[2]Klinikum der Universität München, Feodor-Lynen-Straße 17, 81377 München, Germany

[3]Max-Delbrück-Centrum für Molekulare Medizin (MDC), Proteomics and Molecular Mechanisms of Neurodegenerative Diseases, Robert-Rössle-Str. 10, 13125 Berlin, Germany

[4]Charité Universitätsmedizin Berlin, Institute of Cell Biology and Neurobiology, Charitéplatz 1, 10117 Berlin, Germany

[5]Zuse Institute Berlin, Bioinformatics in Medicine, Takustraße 7, 14195 Berlin, Germany

[6]Max-Delbrück-Centrum für Molekulare Medizin (MDC), Cellular Neurosciences, Robert-Rössle-Str. 10, 13125 Berlin, Germany

C-to-U RNA editing of glycine receptors (GlyR) can play an important role in disease progression of temporal lobe epilepsy (TLE) as it may contribute in a neuron type-specific way to neuropsychiatric symptoms of the disease. It is therefore necessary to develop tools that allow identification of neuron types that express RNA-edited GlyR protein.

Here we identify NH4 as agonist of C-to-U RNA edited GlyRs. Furthermore, we generated a new molecular C-to-U RNA editing sensor tool that detects Apobec-1- dependent RNA editing. Using this sensor combined with NH4 application, we were able to identify C-to-U RNA editing-competent neurons and expression of C-to-U RNA-edited GlyR protein in neurons.

Bioinformatic analysis of 1,000 Genome Project Phase 3 allele frequencies coding for human Apobec-1 80M and 80I variants showed differences between populations, and the results revealed a preference of the 80I variant to generate RNA-edited GlyR protein.

We established a new PCR-based RFLP approach to profile mRNA expression with regard to the genetic APOBEC1 dimorphism of patients with intractable temporal lobe epilepsy (iTLE). Patients with expression of the Apobec-1 80I variant mostly suffered from simple or complex partial seizures, whereas patients with 80M expression exhibited secondarily generalized seizure activity.

Our method allows the characterization of Apobec-1 80M and 80I variants in the brain and provides a new way to classify iTLE according to the two different APOBEC1 alleles. Together, these results demonstrate Apobec-1-dependent expression of RNA-edited GlyR protein in neurons and identifies the Apobec1 gene dimorphism as a genetic risk factor for iTLE patients.

Effect of fasting/refeeding on purinergic modulation of GABA-ergic synaptic transmission in the rat supraoptic nucleus

Milorad Ivetic[1], Anirban Bhattacharya[1], Hana Janouskova[1], Hana Zemkova[1]

[1]Institute of Physiology ASCR, Molecular and Cellular Neuroendocrinology, Videnska, 14220 Prague, Czech Republic

The supraoptic nuclei (SON) of the hypothalamus produce hormones vasopressin and oxytocin that play important roles in the water balance, blood pressure, parturition and lactation. The secretion of these hormones is dependent on the rate and pattern of neuronal electrical activity that is regulated by glutamatergic and gamma-aminobutyric acid (GABA)ergic synaptic inputs, and modulated by gliotransmitter ATP stimulating purinergic P2X receptors (P2XRs). Although pre-synaptic and somatic P2XR subtypes have been already identified to modulate neuronal activity in SON neurons, the detailed mechanisms that underlie ATP-induced modulation and physiological meaning of this modulation are poorly understood. In the present study, we tested a hypothesis that the P2XR-mediated somatic depolarization or facilitation of GABAergic synaptic transmission in the SON might change under physiological conditions, for example during food state-related changes in hormone secretion. We have investigated this hypothesis using electrophysiological recording from SON neurons in hypothalamic slices prepared from 30-day-old rats under provision of food ad libitum and rats after 48h of fasting and subsequent refeeding with standard chow for 2 h. Coronal sections of the rat hypothalamus (~250 µm thick) were cut from approximately 1x1 mm tissue blocks containing the SON. Slices were incubated in oxygenated extracellular at 34°C for 1h before starting electrophysiological measurements. In normally fed rats, application of ATP evoked somatic current and increased the frequency of spontaneous GABAergic postsynaptic currents (sIPSCs) in about 50 % of SON neurons and similar effect was observed in fasted/refeed rats. However, the amplitude of ATP-induced inward current was significantly higher in fasted/refeed animals (118 +/- 22 pA) as compared with normally fed rats (70 +/- 15 pA). The averaged basal frequency of sIPSC was similar in both groups, 2.71 +/- 0.23 Hz and 2.11 +/- 0.22 Hz, respectively. Application of ATP induced increase in the frequency of sIPSCs in 63 % of neurons from control rats (to 1264 +/- 202%) and in 83% of neurons from fasted/refeed rats (to 1833 +/- 641 %), without changing their amplitude. In neurons without somatic ATP-induced current, ATP increased the frequency of sIPSC to 207 +/- 22% and 231 +/- 96 % in control and fasted/refeed rats, respectively. In conclusion, we observed significant changes in the effect of ATP in fasted/refeed vs normal rats, suggesting that alterations of purinergic signaling may occur in the SON in association with increased activity of magnocellular neurons.

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Alternative splicing as a mechanism to increase ion channel diversity

Lukas Kilo[1], Stefanie Ryglewski[1]

[1]Johannes Gutenberg University Mainz, Institute Of Developmental Biology And Neurobiology , Hanns-Dieter-Hüsch-Weg 15, 55128 Mainz, Germany

Throughout the animal kingdom, voltage gated calcium channels (VGCC's) play an essential role in excitability and overall function of neurons. Facilitating vesicle release, neuronal development, signal transduction, excitability and gene expression are among the most important functions of VGCC's. In humans and other mammals these channels are divided into 3 families, Cav1, 2, and 3, which encompass a total of 10 different genes. DmCa1A, alternatively called cacophony, the homolog for the Cav2 family in vertebrates, underlies three distinctly different calcium currents in the same wing depressor motoneuron (MN5) of Drosophila (Ryglewski et al. 2012; Ryglewski, Kilo, Duch 2014). These currents can be isolated by electrophysiological and pharmacological means into high voltage activated (HVA) sustained and low voltage activated (LVA) transient ones. Likely explanations for this high diversity, stemming from a single gene, are the involvement of alternative splicing and the possibility of different combinations with accessory subunits.
Here we address the impact of alternative splicing in cacophony channel diversity in more detail. Previous studies have shown that alternative splicing occurs in only a few locations in the cacophony gene, two of which are of special interest due to the parts of the protein they code for. These sites are part of the voltage sensor as well as an intracellular binding site for β-subunits and G-proteins. These two splice sites lead to several possible exon combinations resulting in different isoforms of cacophony. To elucidate the specific functions of these isoforms we took different approaches, including genomic manipulations of the cac locus by means of CRISPR/Cas9 to decrease isoform availability in vivo and probe the functional consequences. Taking the opposite approach, we express these single splice variants as transgenes in Drosophila and assess their ability to rescue cac null lethality as well as their impact on calcium current properties. Furthermore, we express the same specific isoforms in combination with various newly constructed Drosophila accessory subunits in HEK293T cells and record the resulting currents with whole cell patch clamp electrophysiology. In combination, these data allow us to discreetly differentiate the role individual cac isoforms play for the current properties.

RNA-edited glycine receptors are potential targets for pharmacotherapy in temporal lobe epilepsy

Larissa Kraus[1], Svenja Kankowski[2], Florian Hetsch[2], Nicolai Dorka[2], Marcus Semtner[3], Martin Holtkamp[1], Jochen C. Meier[2], Pawel Fidzinski[1]

[1]Charite, Experimental Epileptology, Chariteplatz 1, 10117 Berlin, Germany
[2]Technische Universität Braunschweig, Cell Physiology, Braunschweig, Germany
[3]Max Delbrueck Center for Molecular Medicine, Cellular Neuroscience, Berlin, Germany

Temporal lobe epilepsy (TLE) is one of the most common forms of focal epilepsy. Unfortunately, 40% of TLE patients develop pharmacoresistance and continue to suffer from seizures despite polytherapy with anti-epileptic drugs. We previously identified elevated expression of C to-U RNA-edited glycine receptors (edGlyR) in resected hippocampi of pharmacoresistant TLE patients. C-to-U RNA editing in GlyR mRNA leads to a gain of function, resulting in an increased affinity for glycine. In cell type-specific mouse models, edGlyR expression in glutamatergic neurons leads to hyperexcitability of the network and cognitive dysfunction, whereas expression in GABAergic interneurons decreases network excitability and facilitates persistence of contextual fear memory. Thus, edGlyR antagonism could present novel treatment strategies for TLE symptoms.
In this study, we identified Dimethylethanolamin (DMEA) as a specific edGlyR antagonist and investigated the effect on seizure-like activity in human epileptic tissue ex vivo. We were able to show concentration depended block of edGlyR currents in HEK cell experiments. In resected human tissue, 10 mM DMEA significantly decreases epileptiform activity in 8 out of 9 patients. In addition, we developed a RNA-editing sensor to identify C-to-U RNA editing competed neurons to better understand the functional mechanisms of edited GlyR in the disease background.
We propose that our research on TLE-associated modification of GlyR-coding gene transcripts can be useful for the identification of affected neuron types in intractable TLE and in the near future provide new personalized treatment options against maladaptive neuronal plasticity in TLE.

The role of L-type Dmca1D calcium channels at the Drosophila neuromuscular synapse

Niklas Krick[1], Carsten Duch[1]

[1]Institute of Developmental Biology and Neurobiology, AG Duch, Hans-Dieter-Hüsch Weg 15, 55128 Mainz, Germany

In neurons, Ca2+influx through voltage gated calcium channels (VGCCs) serves a dual function, as charge carriers upon membrane depolarization and as intracellular messenger. Therefore, VGCCs provide a means for the regulation of intracellular signaling coupled to neuronal activity. Arguably, activity induced synaptic vesicle (SV) exocytosis at chemical synapses is the best described coupling of neuronal action potential (AP) firing to intracellular signaling. At the Drosophila neuromuscular synapse, the Cav2 channel, Dmca1A (also named cacophony) localizes to active zones and has long been known to cause synaptic vesicle fusion with the presynaptic membrane upon AP invasion into a synaptic terminal.

We have identified a second VGCC, the L-type channel Dmca1D, in synaptic terminals. However, in contrast to cacophony, Dmca1D localizes not to the active zone, but to the peri-active region. Although, calcium imaging reveals a significant contribution of Dmca1D channels to the total calcium influx into the synaptic terminal upon presynaptic action potentials, Calcium influx through Dmca1D channel alone is not sufficient to trigger SV release, because temperature-sensitive inactivation of Dmca1A completely eliminates action potential triggered SV release. In an effort to isolate the acute presynaptic function of calcium influx through Dmca1D channels we currently establish a pharmacological profile of Dmca1D channels with different dihydropyridines. So far our data indicate that Dmca1D channels do not contribute significantly to synaptic vesicle endocytosis, but by contrast augment synaptic vesicle endocytosis. Pharmacological reduction of Dmca1D current with lanthanum or with Isradipine reduces the rate of synaptic vesicle uptake but leaves release normal as revealed by FM1-43 imaging. Moreover, genetic knock-out of Dmca1D in few motor terminals with the MARCM technique causes an increased rate of synaptic depression during sustained firing at 5 Hz. Therefore, our data indicate that L-type currents through Dmca1D facilitate SV endocytosis. Based on these findings we hypothesize a strict division of labor between L-type and PQ-type like calcium channels at the Drosophila neuromuscular terminal, Dmca1A (PQ-type) for Ca2+ triggered exocytosis and Dmca1D (L-type) for Ca2+ mediated endocytosis.

Maintaining excitation and inhibition at single cell level

Marie-Luise Kümmel[1], Uli Müller[1]

[1]Saarland University, Dept. NT - Zoology/Physiology, Neurobiology, 66123 Saarbrücken, Germany

Brain function is based on a well-balanced contribution of excitatory and inhibitory components within functional neuronal networks. Within these networks every single neuron has an important impact on the maintenance of the accurate balance and thus function of neuronal networks. However, the specific contribution of single cells is not completely understood. To pursue the question of how the balance between excitation and inhibition is maintained at the single cell level we focus on the cholinergic system, which plays an important role in human brain networks. We used Topiramate (TPM), a drug commonly used for treatment of epilepsy. Its unique bimodal action in networks causes a decrease in excitatory and increase in inhibitory signal transmission at the same time. By using the calcium imaging technique, the modulatory effects of TPM on the calcium response after stimulation with acetylcholine were examined on single primary kenyon cells of the honey bee (*Apis mellifera*) and single cells of the human line SHSY-5Y. The results reveal a concentration- and time-dependent modulation of basal cholinergic mechanisms by TPM in single bee neurons. The ongoing characterization of the action of TPM on the cholinergic system in isolated SHSY-5Y cells will complement the bee data and further the understanding of how single cells contribute to the excitation-inhibition balance within the neuronal networks in respect to the cholinergic signal transmission.

Thyroid Hormone Effects on Metabolic Rate: Correlation of Na^+ Influx and Expression of Na^+/K^+-ATPase

Heiko Michael Leßlich[1], Lisa Bachmann[1], Sascha Döring[1], Sivaraj Mohana Sundaram[2], Irmgard D. Dietzel[1]

[1]Ruhr-Universität Bochum, Department of Biochemistry II, Universitätsstr. 150, 44780 Bochum, Germany
[2]University of Lübeck, Institute of Experimental and Clinical Pharmacology and Toxicology, Ratzeburger Allee 160, 23562 Lübeck, Germany

Thyroid hormones – mainly the active form triiodothyronine (T3) – are known to accelerate many brain functions, such as EEG rhythms, reflexes and evoked potentials. Concomitant with these electroencephalographic changes, hyperthyroidism causes nervousness, restlessness, irritability and can even evoke epileptic seizures, while absence of thyroid hormones leads to retardation and slowed reflexes. In previous investigations, we observed an increase in Na^+ current density in neurons as a dominant underlying mechanism of the action of T3 (Hoffmann and Dietzel, 2004). Furthermore, we have obtained evidence, that in brain this regulation might be an indirect effect in contrast to muscle. Thus, the modulation of neuronal excitability is induced by factors, most prominently fibroblast growth factor 2 (FGF-2, formerly known as bFGF), secreted from surrounding glial cells especially astrocytes (Niederkinkhaus et al., 2009, Igelhorst et al, 2015). On the other hand, thyroid hormone T3 is mainly known for its regulation of metabolism. Several investigations have shown that T3 regulates the membrane expression of Na^+/K^+-ATPases which account for about 40% of resting metabolic rate. A selective increase in Na^+/K^+-ATPase activity alone, would, however, stabilize resting membrane potential and hyperpolarize cells, rendering them less excitable. To resolve this apparent contradiction, we assume that the regulation of the Na^+/K^+-ATPase is induced by the increased Na^+-load of the excited cells resulting from an upregulation of voltage-activated Na^+ channels and potentially also Na^+-coupled transporters. Here we obtained evidences, that in brain cortical cultures from postnatal rats, α1 and α2 subunits of the Na^+/K^+-ATPase, as well as ^{3}H-ouabain binding are upregulated by a treatment of the cultures for 4 days with T3 or FGF-2. In addition, we observed that pump currents in hippocampal neurons correlate with the Na^+ current density induced by these two factors. We also found that these effects are partially reversible by blocking voltage-activated Na^+ currents with tetrodotoxin, suggesting that at least to some extent enhancement of the basal metabolic rate induced by T3 is a consequence of increased cellular excitability.

Interactions of calcium channel ($Ca_V1.2$) circuitries with early life stress and their involvement in the pathogenesis of psychiatric disorders

Srivaishnavi Loganathan[1,2], Jan M Deussing[1]

[1]Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, 80804 Munich, Germany
[2]International Max Planck Research School for Translational Psychiatry, Max Planck Institute of Psychiatry, Kraepelinstr. 2-10, 80804 Munich, Germany

Psychiatric disorders display a considerable number of overlapping symptoms which may be explained by their shared genetic etiology. Numerous genome-wide association studies and clinical findings provide extensive evidence of genetic alterations within the *CACNA1C* gene, coding for the α1 subunit of the L-type calcium channel $Ca_V1.2$, associated with the pathology of psychiatric disorders. Utilizing conditional knockout mice with a specific deletion of *Cacna1c* in excitatory neurons of the forebrain revealed increased anxiety-related behavior, decreased sociability, hyperactivity and impaired cognitive performance, all resembling core symptoms of *CACNA1C*-associated psychiatric disorders. However, the underlying mechanisms which manifest the disease symptoms are not clearly understood.

The aim of the present study is to dissect CACNA1C-specific circuits and downstream signaling mechanisms. To reach this goal, we are currently performing a series of behavioral, cellular and molecular experiments using conditional *Cacna1c* mouse models to investigate: 1) changes in disease-related endophenotypes caused by cell type-specific *Cacna1c* deletion and its interaction with early life stress, 2) alterations in $Ca_V1.2$ downstream signaling pathways, structural plasticity and their functional implications and 3) consequences of $Ca_V1.2$ channel deletion on cell type-specific transcriptomic signatures. For investigating the interactions of early life stress with *Cacna1c* deletion, a conditional knockout mouse line with specific deletion of *Cacna1c* in excitatory neurons will be used. Simultaneously, the ribosomal protein RPL22 is tagged in excitatory forebrain neurons of these animals allowing cell type-specific isolation of translated mRNA and expression profiling. The animals will be subjected to an early life stress paradigm and tested in a series of behavior experiments broadly categorized into anxiety tests, cognitive functioning and depressive behaviors.

The brain tissues will be collected for RNA sequencing to identify changes in cell type-specific transcriptomic signatures as a consequence of *Cacna1c* deletion and exposure to stress. Furthermore, some tissues will also be subjected to other molecular investigations to unravel alterations in downstream signaling proteins, structural plasticity and their functional implications as a result of this interaction. Our study aims to fundamentally increase our understanding of the role L-type calcium channels play in the pathogenesis of psychiatric disorders for the development of better targeted therapeutic strategies.

TRPM3 channels in non-neuronal cells of somatosensory dorsal root ganglia

Johannes Oberwinkler[1], Sandeep Dembla[1,2], Raissa Enzeroth[1], Behrendt Marc[1]

[1]Philipps-Universität Marburg, Institut für Physiologie und Pathophysiologie, Marburg, Germany
[2]Charité Universitätsmedizin Berlin, Department of Anesthesiology and Operative Intensive Care Medicine, Berlin, Germany

TRPM3 channels are divalent-permeable cation channels activated by heat and endogenous small molecules, such as the steroid pregnenolone sulfate. It is well established that TRPM3 channels are expressed in somatosensory nociceptor neurons where they play a key role in noxious heat sensation and in the generation of inflammatory heat hyperalgesia. In many of these neurons, TRPM3 channels are under strong negative control from G_i-coupled receptors, such as μ-opioid receptors. The current mechanistic model indicates that activation of μ-opioid receptors liberates Gβ/γ proteins that directly bind to TRPM3 proteins, thereby inhibiting the channel activity.

However, in dorsal root ganglia where the cell bodies of somatosensory nociceptor neurons reside also other, non-neuronal cell types are present. Using Ca^{2+}-imaging, we investigated whether non-neuronal cells also express functional TRPM3 channels. We found that cells approximately 70% of non-neuronal cells reacted to TRPM3 channel agonists, while this percentage dropped to 2% in cells isolated from $TRPM3^{-/-}$ mice. These data indicated that non-neuronal cells in dorsal root ganglia express bona fide functional TRPM3 channels. However, when we checked in similar experiments for the presence of functional TRPV1 or TRPA1 channels in non-neuronal cells, we were, as expected, unable to detect them in these cells. In order to further identify and stratify these cells, we stained the cells after the Ca^{2+}-imaging experiments with antibodies against the glutamine synthetase, a marker protein for satellite glia cells. When we analyzed the subset of glutamine synthetase-positive cells, we found that more than 90% of those cells responded to TRPM3 agonists. We conclude that functional TRPM3 channels are expressed in satellite glia cells of dorsal root ganglia.

We next tested whether TRPM3 channels expressed in non-neuronal cells from dorsal root ganglia are also subject to inhibition by activated G_i-coupled receptors. To our surprise, we found that most non-neuronal cells did not show an inhibition of TRPM3 channels after activation of $GABA_B$ or somatostatin receptors. It appears therefore that TRPM3 channels in non-neuronal cells are less sensitive to inhibition by GPCRs compared to TRPM3 channels in neurons. The potential reasons for these functional differences will be discussed.

Nano-scale dynamics of voltage gated Ca^{2+} channels: an in vivo single molecule analysis

Tina Ghelani[1,2], Hylkje Geertseema[3], Ulrich Thomas[4], Martin Lehmann[5], Felix Ewers[3], Martin Heine[4], Stephan J. Sigrist[1,2]

[1]Faculty of Biology, Chemistry, Pharmacy, Freie Universität Berlin, Berlin, Germany
[2]NeuroCure Cluster of Excellence, Charité Universitätsmedizin Berlin, Berlin, Germany
[3]Faculty of Membrane Biochemistry; Freie Universität Berlin, Berlin, Germany
[4]RG Molecular Physiology, Leibniz-Institute for Neurobiology, Magdeburg, Germany
[5]Core Cellular Imaging Facility, Leibniz-Institute for Molecular Pharmacology (FMP), Magdeburg, Germany

Neurons communicate through Ca^{2+}-dependent neurotransmitter release at presynaptic active zones (AZs), where a precise Ca^{2+} channel organization, in nanodomains, via interaction with AZ molecules involved in synaptic vesicle fusion (SV) is assumed[1–3]. As a consequence of SV fusion, synaptic membranes readily rearrange, thus stipulating a dynamic and flexible localization of Ca^{2+} channels relative to SV release site[4]. Nevertheless, little is as yet known about how Ca^{2+} channel clusters are organized within their AZ nanodomains and how AZ protein interactions with Ca^{2+} channels influences its synaptic dynamics.

We use on locus-tagged voltage-gated Ca^{2+}channel- Cacophony[5] (Cac), and visualize their endogenous localization, numbers, and live dynamics employing different super-resolution microscopy techniques at *Drosophila* neuromuscular synapses. To probe whether interactions between Cac and AZ scaffold proteins might affect Cac nanodomain localization and mobility in vivo, we investigated Cac levels in mutants of AZ scaffold proteins known to interact with Cac at Drosophila AZs. Furthermore, to discern how these Cac and AZ scaffold protein interactions influence Cac levels and dynamics, ultimately affecting Cac function, we applied a presynaptic homeostatic potentiation (PHP) assay.

We, here, begin to quantify and mechanistically link live in vivo dynamics of synaptic voltage-gated Ca^{2+} -channels. We systematically exploit the genetic possibilities of the Drosophila system to mechanistically dissect voltage-gated Ca^{2+} channel dynamics at normal and plastically remodeling synapses.

Effect of solute carriers (SLC) on CA1 pyramidal cells, synaptic transmission and hippocampal network activity

Marco Rohde[1], Vanessa Ziesak[1], Andreas Birkenfeld[2], Rüdiger Köhling[1]

[1]Oscar Langendorff Institute of Physiology, Rostock University Medical Center, Gertrudenstraße 9, 18057 Rostock, Germany
[2]Section of Metabolic and Vascular Medicine, Medical Clinic III, Dresden University School of Medicine, Technische Universität Dresden, Germany; Paul Langerhans Institute Dresden of the Helmholtz Center Munich at University Hospital and Faculty of Medicine, TU Dresden, Dresden, Germany; Deutsches Zentrum für Diabetesforschung e.V. (DZD e.V.), Neuherberg, Germany; Diabetes and Nutritional Sciences, King's College London, London, UK

Since deficits of the sodium/citrate cotransport in neurons are associated with delayed brain development and epileptic encephalopathy of infantile patients, research into the SLC13A5 (sodium-dicarboxylate cotransporter solute carrier family 13 member 5, mammalian homolog of INDY: mINDY) becomes of increasing interest. We therefore analysed the effects of pharmacological inhibition and genetic ablation of SLC13A5 on active and passive membrane properties of CA1 pyramidal cells, on synaptic transmission of hippocampal Schaffer collateral-CA1 synapses and on hippocampal recurrent epileptiform discharges in an acute model of epilepsy.
Using intracellular recordings of murine CA1 pyramidal cells the SLC13A family inhibitor PF-06761281 (2 µM) had no effect on the membrane resistance, on the afterhyperpolarization (AHP) after trains of action potentials and on the voltage sag induced by hyperpolarizing current injections. However, in field potential recordings of the hippocampal CA1 region showing epileptic activity acutely induced by removal of magnesium, we found an increase of recurrent epileptic discharged as consequence of inhibition of SLC13A. Moreover, PF-06761281 augmented evoked synaptic transmission between Schaffer collaterals and CA1 pyramidal cells, which could also explain at least partly the pro-epileptic effect of SLC13A5 dysfunction. Although the underlying mechanisms are still unidentified, these results indicate that SLC13A5 modulate synaptic transmission and therefore dysfunction of solute carriers may be involved in the pathophysiology of epilepsy.

Alanine scanning mutagenesis of the rat P2X7 receptor highlights the requirement for lysine and aspartate in the first transmembrane domain

Marian Rupert[1], Anirban Bhattacharya[1], Hana Janouskova[1], Stanko Stojilkovic[3], Hana Zemkova[1]

[1]Institute of Physiology CAS, Cellular and Molecular Neuroendocrinology, Prague, Czech Republic
[2]Charles University in Prague, 1st Faculty of Medicine, Prague, Czech Republic
[3]National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Purinergic P2X receptors (P2XRs) are extracellular ATP-activated cation channels that are composed of two transmembrane domains (TM1 and TM2), a ligand-binding ectodomain, and intracellular N- and C-termini. There are seven types (P2X1 – P2X7) of receptor subunits. In the prolonged presence of agonist, the opening of P2X7R channel is followed by pore dilatation, which causes an increase in its permeability to larger organic cations. The P2X7R is involved in neurodegeneration, neuropathic pain, the release of inflammatory cytokines and is strongly deregulated in many tumors. P2X7Rs may affect neuronal cell death through their ability to regulate the processing and release of interleukin-1β;. The aim of our study was to investigate role of TM1 residues in the P2X7R functions using electrophysiology and measurement of fluorescent dye uptake, particularly Ethidium bromide (EtBr), by transfected cells. We substituted one by one all (22) residues in TM1 (from G27 to D48) of the rat P2X7R with alanine and expressed wild type (WT) and alanine mutants in HEK293 cells. Single point P2X7R mutants were exposed to agonists 2;,3;-O-(4-benzoyl-4-benzoyl)-ATP (BzATP) to stimulate EtBr uptake, which was measured after 100 s. Membrane currents evoked by BzATP were recorded using patch clamp technique. We identified two types of mutants affecting permeability of the channel pore. The TM1 mutants G27A, H34A, Y40A, F43A, L45A, and M46A showed significantly reduced trafficking to the cell surface, accompanied with reduced BzATP-stimulated dye uptake and membrane current amplitude. Two mutants, K30A and D48A, also showed reduced dye uptake and membrane current in response to BzATP application, but without changing significantly the plasma membrane expression. Substitution of K30 with arginine, but not glutamate, fully restored the receptor function. Homology model of P2X7R revealed that the K30 residue could interact with the cytoplasmic cap in receptor open state, and the D48 residue, located at the interface between TM1 and extracellular domain, could play a role in receptor sensitization after prolonged agonist application. These results indicate a critical role of charged residues in the TM1 domain for the P2X7R receptor function.

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Role of the Na^+-activated K^+ channel Slack (Slo2.2) for hearing function and noise vulnerability in mice

Pauline Schepsky[1], Anne Bausch[2], Robert Lukowski[2], Katharina Sorg[3], Dietmar Hecker[3], Bernhard Schick[3], Peter Ruth[2], Simone Kurt[1], Jutta Engel[1]

[1]Saarland University, Department of Biophysics and CIPMM, Homburg
[2]University of Tübingen, Department Clinical Pharmacy
[3]Saarland University, Department of Otorhino-laryngology

Slack (Slo2.2,*Kcnt1*) is a Na^+- and voltage-activated potassium channel that reduces neuronal excitability in response to neuronal activation and subsequent Na^+ influx. It is strongly expressed in neurons of the central auditory pathway, which operate at high firing rates.
Slack mRNA expression has also been reported in spiral ganglion neurons (SGNs), the peripheral auditory neurons in the cochlea that contact the inner hair cells and form the auditory nerve. The cellular and subcellular localization of Slack in the mammalian cochlea and auditory pathway remain to be elucidated because of the lack of specificity of various anti-Slack antibodies tested using wildtype and a systemic Slack-deficient ($Slack^{-/-}$) mouse (Lu*, Bausch* et al., J Neurosci 2015) as control.
Hearing function, which was determined by click and frequency-dependent auditory brainstem response (ABR) measurements and recordings of distortion product otoacoustic emissions (DPOAE), was not altered in 12 – 14 week-old $Slack^{-/-}$ mice compared with wildtype littermates.
To test whether Slack channels are required for reliable signal transmission under conditions when the auditory system is challenged by a mild noise trauma, we subjected $Slack^{-/-}$ mice and wildtype littermates of 8 weeks to band noise from 8 – 16 kHz at 100 dB SPL for two hours. Frequency-dependent ABR thresholds were determined before trauma and up to four weeks after trauma. After day 28, presynaptic ribbons and postsynaptic densities were co-immunolabeled in cochlear whole mounts using anti-CtBP2 and anti-HOMER antibodies. Recovery from noise trauma and the fate of ribbons in $Slack^{-/-}$ mice is currently being analyzed.
Supported by IRTG 1830/2

The auxiliary Ca^{2+} channel subunits $\alpha_2\delta2$ and $\alpha_2\delta3$ are required for proper $Ca_V2.1$ currents in cultured spiral ganglion neurons and for the development of endbulb of Held synapses

Friederike Stephani[1], Kerstin Blum[1], Jutta Engel[1]

[1]Saarland University, Department of Biophysics and CIPMM, Homburg, Germany

Spiral ganglion neurons (SGNs) connect hair cells with central auditory neurons and are therefore indispensable for proper auditory signal transmission. In the cochlea, SGNs form the postsynaptic part of the inner hair cell ribbon synapse, where Ca^{2+} release is mainly regulated via $Ca_V1.3$ channels. In the cochlear nucleus, auditory nerve fiber terminals form the endbulb of Held synapse at which transmitter release is mainly mediated by Ca^{2+} influx through $Ca_V2.1$ channels. Voltage-gated Ca^{2+} channels are composed of a pore-forming α_1 subunit and auxiliary subunits $\alpha_2\delta$ and β;, which both modulate amplitude and biophysical properties of the channel. SGNs express the $\alpha_2\delta$ subunits 1-3, with higher levels of $\alpha_2\delta2$ and 3. Notably, proper function and morphology of auditory nerve fiber synapses require the auxiliary Ca^{2+} channel subunit $\alpha_2\delta3$ (Pirone et al., J Neurosci 2014). To determine the role of $\alpha_2\delta3$ for SGNs, we analyzed Ca^{2+} currents in cultured SGNs isolated from $\alpha_2\delta3^{+/+}$ and $\alpha_2\delta3^{+/+}$ mice before (P5) and after the onset of hearing (P20). Deletion of $\alpha_2\delta3$ reduced the expression of $Ca_V2.1$ Ca^{2+} currents in SGNs isolated from hearing mice ($\alpha_2\delta3^{+/+}$) by more than 60 % leaving the total Ca^{2+} current unaltered. SGNs of neonatal mice expressed only a few $Ca_V2.1$ channels at the membrane, indicating a switch in the expression of Ca_V channels before and after the onset of hearing.
$\alpha_2\delta3$ not only controlled the size and morphology of endbulb of Held synapses (Pirone et al., 2014) in 5-week-old mice but also before the onset of hearing. In neonatal mice, where only few P/Q-type channels were present at the endbulbs yet, less boutons were identified at bushy cells in $\alpha_2\delta3^{-/-}$ mice compared with wildtype mice indicating that $\alpha_2\delta3$ not only determined the expression of $Ca_V2.1$ channels, but were required for proper development of the endbulb of Held synapse before up-regulation of $Ca_V2.1$ currents.

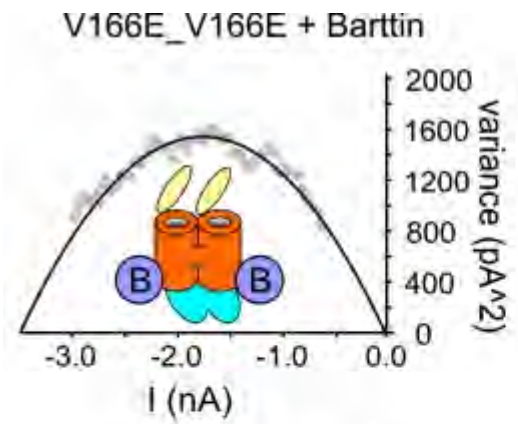
Ducky mice (du/du) with a truncation of the $\alpha_2\delta2$ subunit, regarded as $\alpha_2\delta2$ null mutants, show a severe phenotype. Their endbulbs of Held were smaller and reduced in number in 3-week-old du/du mice compared with their wild-type siblings. First recordings from cultured SGNs show a decrease of $Ca_V2.1$ currents by more than 80 % in SG neurons from the apical cochlea.
In summary, SGNs express different $\alpha_2\delta$ subunits and loss of either $\alpha_2\delta2$ or $\alpha_2\delta3$ cannot be fully compensated by another $\alpha_2\delta$ subunit. We hypothesize that both $\alpha_2\delta2$ and $\alpha_2\delta3$ play important roles in the development of the endbulb of Held synapse as well as in the regulation of $Ca_V2.1$ currents of SGNs.
Supported by SPP1608

In silico current prediction and noise analysis elucidates gating properties of heterodimeric rClC-K1 chloride channels

Stefan Thiemann[1], Birgit Begemann[1], Toni Becher[1], Martin Fischer[1]

[1]Medizinische Hochschule Hannover, Institut für Neurophysiologie, OE 4230, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

ClC-K chloride channels are expressed in Henle's loop of the kidney and in the stria vascularis of the inner ear and contribute to transepithelial transport processes that are essential for urinary salt balance and auditory signal transduction. The channels form dimers with each subunit exhibiting its own ion conductance pathway. The so called protopores are separately regulated by individual fast gates and both protopores together are opened and closed by a common slow gate. The nature of slow common gating, however, is a matter of speculation. Common gating might result from a precisely synchronized action of two protopore gates or from a conformational change of a unique structure that closed both pores simultaneously. Previous studies on heteroconcatamers of human hClC-1 and hClC-2 subunits displayed fast and slow gating for the individual pores but no common gating process. Since sequence homology of only 55% between hClC-1 and hClC-2 channels might impede building of a common gating structure, we here took advantage of heterodimers of WT and mutant V166E rClC-K1 subunits with almost 100% sequence identity but inverse voltage dependence of their subunit gating. Homoconcatamers of two identical covalently linked subunits, WT_WT rClC-K1 or V166E_V166E rClC-K1, served as controls for the analyses of the gating properties of the heteroconcatamer WT_V166E rClC-K1. YFP-labelled rClC-K1 homo- and heteroconcatamers were heterologously expressed in MDCKII cells to demonstrate by confocal imaging their interaction with CFP-labelled barttin, an accessory subunit that promotes trafficking and insertion of the channel complex into the plasma membrane. Patch-clamp current recordings in whole cell configuration and noise analyses were performed on HEK293T cells that transiently expressed the constructs. We firstly proved with homoconcatamers that the covalent linkage of subunits does not alter the gating properties of the channel. In order to characterize the gating mechanism of heteroconcatamers, current recordings were compared to *in silico* predictions of four different concatamer channel-models (A: common slow gate with V166E properties, B: common slow gate with WT properties, C: individual slow gates for WT and V166E pore, D: only one pore of the dimer is active). WT_V166E heteroconcatamers exhibit very similar current responses as V166E_V166E homoconcatamers. However, data distribution in noise analysis markedly differs from those of homoconcatamers (Figure). It turned out that the two fast gates act independently and with inverse voltage dependence. Notably, slow gating is realized by a common gate for both pores, owning the properties of the mutant V166E subunit. Single channel recordings of the rClC-K1 heteroconcatamer confirm the regulation of both pores by a common structure. In conclusion, fast protopore gates of rClC-K1 heteroconcatamers act independently. However, the slow gates of nearly identical subunits do not open and close separately. Instead, one of the subunits dominates the slow gate properties in the heterodimeric channel which suggests the formation of a common gating structure.


V166E_V166E + Barttin
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1200
800
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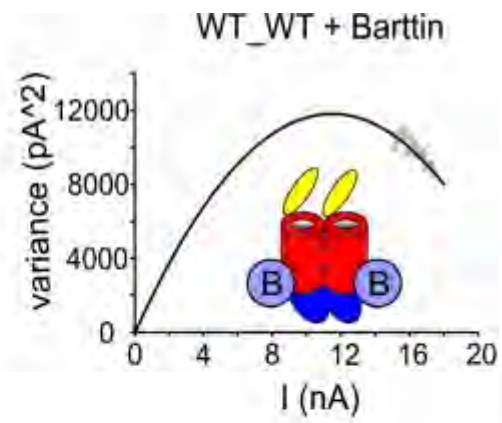
B
-3.0
-2.0
-1.0
0.0
I (nA)


WT_WT + Barttin
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B
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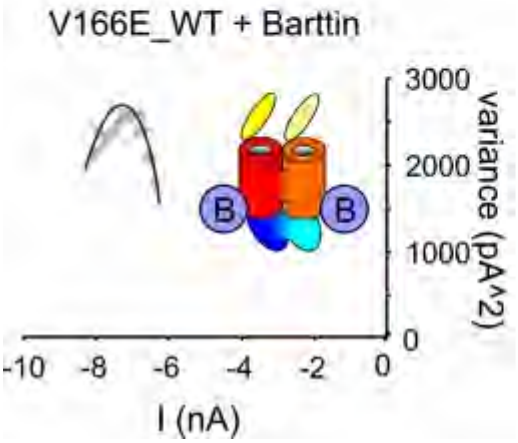
4
8
12
16
20
I (nA)


V166E_WT + Barttin
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1000
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B
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-8
-6
-4
-2
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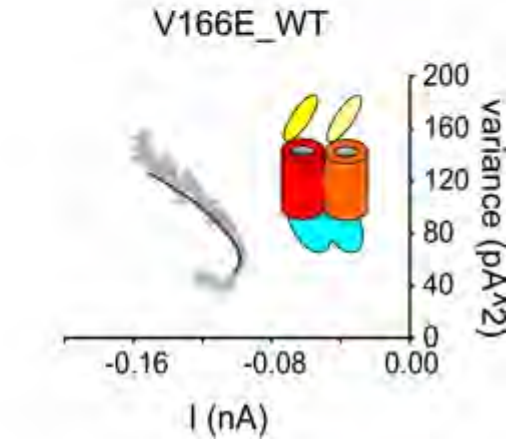
I (nA)


V166E_WT
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120
80
40
0
variance (pA^2)
-0.16
-0.08
0.00
I (nA)

Ionic channels involved in spontaneous and CRH-induced excitability and calcium signaling of mice corticotrophs

Hana Zemkova[1], Melania Tomic[2], Marek Kucka[2], Greti Aguilera[3], Stanko S Stojilkovic[2]

[1]Institute of Physiology, Czech Academy of Sciences, Department of Cellular and Molecular Neuroendocrinology, Videnska 1083, 14220 Prague, Czech Republic, [2]Sections on Cellular Signaling and [3]Endocrine Physiology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA

Corticotrophs are excitable cells in anterior pituitary gland that are using voltage-gated calcium influx for ACTH release and other cell functions. Previous studies have shown that these cells may exhibit both spontaneous large-amplitude spiking and pseudo-plateau bursting action potential waveforms, and spontaneous firing of action potentials is associated with fluctuations in intracellular calcium concentration, as well as that some corticotorphs are electrically silent. Although corticotrophs have been also reported to express the low threshold T-type and three types of high-threshold voltage-gated calcium channels, tetrodotoxin (TTX)-sensitive voltage-gated sodium channels, and several potassium-conducting channels, including calcium-activated potassium channel, to modulate action potential firing, the detailed mechanisms that underlie electrical activity of corticotrophs are poorly understood. Transgenic mice expressing the tdimer2(12) form of Discosoma red fluorescent protein under control of the proopiomelanocortin gene's regulatory elements are a useful model for studying corticotrophs. Using these mice, we studied the ion channels and mechanisms controlling corticotroph excitability. Corticotrophs were either quiescent or electrically active, with a 22-mV difference in the resting membrane potential between the two groups. In quiescent cells, CRH depolarized the membrane, leading to initial single spiking and sustained bursting; in active cells, CRH further facilitated or inhibited electrical activity and calcium spiking, depending on the initial activity pattern and CRH concentration. The stimulatory but not inhibitory action of CRH on electrical activity was mimicked by cAMP independently of the presence or absence of arachidonic acid. Removal of bath sodium silenced spiking and hyperpolarized the majority of cells; in contrast, the removal of bath calcium did not affect resting membrane potential but reduced CRH-induced depolarization, which abolished bursting electrical activity and decreased the spiking frequency but not the amplitude of single spikes. Corticotrophs with inhibited voltage-gated sodium channels fired calcium-dependent action potentials, whereas cells with inhibited L-type calcium channels fired sodium-dependent spikes; blockade of both channels abolished spiking without affecting the RMP. These results indicate that the background voltage-insensitive sodium conductance influences resting membrane potential, the CRH-depolarization current is driven by a cationic conductance, and the interplay between voltage-gated sodium and calcium channels plays a critical role in determining the status and pattern of electrical activity and calcium signaling.

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Poster Topic

T7: Synaptic Transmission, Pre- and Postsynaptic organization

T7-1A Expression of BDNF precursor protein and RNA transcript in individual hippocampal neurons demonstrated using Laser Capture Microdissection and qRT-PCR
Federico Jose Barreda Tomas, Heike Heilmann, Imre Vida, Agnieszka Muenster-Wandowski

T7-2A The relation between the different phases of early-phase synaptic plasticity and the underlying dynamics of AMPA-receptors
Moritz Becker, Christian Tetzlaff

T7-3A TOP3B: A novel candidate gene in juvenile myoclonic epilepsy?
Marwa Daghsni, Saida Lahbib, Mohamed Fradj, Lilia Kraoua, Faouzi Maazoul, Sonia Abdelhak, Ridha Mrad

T7-4A Rapid induction and sustained expression of presynaptic homeostatic plasticity at a mammalian CNS synapse
Igor Delvendahl, Katarzyna Kita, Martin Müller

T7-5A Direct measurement of glutamate release at Schaffer collateral synapses under low and high frequency activity
Céline D. Dürst, J. Simon Wiegert, Christian Schulze, Nordine Helassa, Katalin Török, Thomas G. Oertner

T7-6A From local to global signalling in rat olfactory bulb granule cell dendrites
Veronica Egger, Max Müller, S.Sara Aghvami

T7-7A Synaptic mechanisms underlying temporally precise information processing in the VNLL
Linda Fischer, Felix Felmy

T7-8A Relation between sodium signaling and ATP consumption
in mouse hippocampal neurons

Niklas J. Gerkau, Rodrigo Lerchundi, Jan Meyer, Christian Kleinhans, Marina Lantermann, Johannes Hirrlinger, Christine R. Rose

T7-9A In search of the synaptic vesicle tether at a sensory synapse
Kaspar Korbinian Maximilian Gierke, Sonja Kirsch, Tanja Müller, Craig Garner, Rainer Böckmann, Hanna Regus-Leidig, Johann Helmut Brandstätter

T7-10A Single synapse activity characterization reveals interdependences between release modes.
Andreas T Grasskamp, Meida Jusyte, Mathias A Böhme, Alexander M Walter

T7-1B The interplay between kinesin-3 and dynamic microtubules at presynapses specifies high precision delivery of synaptic cargo
Pedro Guedes-Dias, Jeffrey J Nirschl, Nohely C Abreu, Mariko K Tokito, Erika LF Holzbaur

T7-2B Role of Auxiliary Subunits in AMPA Receptor Trafficking in Hippocampal Neurons
Ali Harb, Nils Vogel, Walentina Frisch, Ali Shaib, Ute Becherer, Dieter Bruns, Ralf Mohrmann

T7-3B Presynaptic K^+ channels regulate spontaneous glutamate release through a specific association with Ca^{2+} channels in the hippocampal pyramidal neurons
Won-Kyung Ho

T7-4B Optogenetic characterization of excitatory inputs at spiny interneurons of the stratum oriens
Joaquin Isaac Hurtado Zavala, J. Simon Wiegert

T7-5B Proteomic alterations of GABAergic Interneurons following traumatic brain injury (TBI) in mouse neocortex.
Natascha Ihbe, Florie Le Prieult, Qi Wang, Ute Distler, Malte Sielaff, Stefan Tenzer, Serge Thal, Thomas Mittmann

T7-6B Freeze frame shots of synapses in action: Correlating presynaptic ultrastructure and function at the nanoscale
Cordelia Imig, Sünke L. Mortensen, Lydia Maus, Nils Brose, Benjamin H. Cooper

T7-7B Ca^{2+}-dependent Calmodulin-Unc13A interaction shapes structure, function, and short-term plasticity
Meida Jusyte, Mathias A. Boehme, Alexander M. Walter

T7-8B Uncovering the Role of Presynaptic GIT Proteins for Fast Auditory Signaling
Christian Keine, Samuel M. Young, Jr.

T7-9B Minimal input requirement for action potential generation in auditory brainstem nuclei
Nikolaos Kladisios, Linda Fischer, Felix Felmy

T7-10B Neuronal profilins as modulators of dendritic complexity and structural plasticity
Maximilian Klasmeier, Tania Meßerschmidt, Dorothea Hinz, Martin Korte, Martin Rothkegel

T7-1C Regulation of exocytosis by amisyn, a PI(4,5)P2 and syntaxin-binding protein
Ilona Kondratiuk, Shrutee Jakhanwal, Reinhard Jahn, Ira Milosevic

T7-2C Extracellular matrix ensures temporally precise high frequency synaptic transmission at the calyx of Held
Christoph Körber, Denise Harrach, Thomas Kuner

T7-3C How do glycinergic synapses transmit in the absence of the glycine transporter GlyT2?
Catharina Kurz, Sina Elena Brill, Dennis Julian Weingarten, Eckhard Friauf

T7-4C Neuronal calcium homeostasis: variations in an evolutionarily conserved molecular interplay between Neuroplastin/ Basigin and PMCAs.
Xiao Lin, Karl-Heinz Smalla, Thilo Kähne, Lennart Junge, Constanze Seidenbecher, Dirk

Montag, Eckart Gundelfinger, Rodrigo Herrera-Molina, Ulrich Thomas

T7-5C Examining the role of Complexins in adaptation processes at photoreceptor ribbon synapses
Uwe Thorsten Lux, Andreas Gießl, Katharina Pieger, Karsten Boldt, Kerstin Reim, Johann Helmut Brandstätter

T7-6C Resolving the Ultrastructural Organization of Synaptic Vesicle Pools at Hippocampal Mossy Fiber and Schaffer Collateral Synapses
Lydia S. B. Maus, Bekir Altas, Jeong-Seop Rhee, Nils Brose, Cordelia Imig, Benjamin H. Cooper

T7-7C Nogo-A signaling modulates synaptic transmission at a fast time scale
Kristin Metzdorf, Steffen Fricke, Stefan Haak, Martin Korte, Marta Zagrebelsky

T7-8C Do different Complexin isoforms act upon different SNARE complex types?
Jutta Meyer, Olaf Jahn, Nils Brose, Johann Helmut Brandstätter, Kerstin Reim

T7-9C Synaptic elimination and strengthening uncoupled: the impact of central L-type voltage-gated Ca^{2+} channels on circuit refinement of a sound source localization pathway
Nicolas Müller, Eckhard Friauf

T7-10C Rapid modulation of transsynaptically aligned glutamate receptor nanocluster rings during homeostatic plasticity
Paola Muttathukunnel, Martin Müller

T7-11C Presynaptic $GABA_A$ Receptors Modulate Glutamatergic Transmission at the Endbulb of Held
Jana Nerlich, Stefan Hallermann , Ivan Milenkovic

T7-1D Developmental easing of short-term depression in ‘winner’ climbing fibers
Christina Paetz, Simone Brachtendorf, Jens Eilers

T7-2D Phosphoinoside-dependent regulation of GABAergic Neurotransmission at inhibitory Postsynapses
Theofilos Papadopoulos

T7-3D SynTagMA: a new optogenetic tool to map active synapses
Alberto Perez-Alvarez, Brenna C Fearey, Ryan OToole, Ignacio Arganda-Carreras, Eric R Schreiter, J Simon Wiegert, Christian Schulze, Michael B Hoppa, Christine E Gee, Thomas G Oertner

T7-4D Synaptogenesis depends on axonal transport via lysosome-related vesicles
Astrid G. Petzoldt, Anela Vukoja, Ulises Rey, Christoph Ott, Dymtro Puchkov, Eric Reynolds, Martin Lehmann, Stephan J. Sigrist, Volker Haucke

T7-5D A role for Piccolo in the regulation of neurotransmitter release and presynaptic plasticity.
Eneko Pina, Carolina Montenegro-Venegas, Yulia Klyueva, Daria Davydova, Claudia Marini, Eckart D. Gundelfinger, Anna Fejtova

T7-6D Myosin XVI is a regulator of actin cytoskeleton dynamics in dendritic spines of Purkinje cells

Mona Katrin Roesler, Franco Lombino, Michaela Schweizer, Irm Hermans-Borgmeyer, Matthias Kneussel, Wolfgang Wagner

T7-7D Cognitive impairment and autistic-like behaviour in SAPAP4-deficient mice
Claudia Schob, Fabio Morellini, Ora Ohana, Lidia Bakota, Mariya V. Hrynchak, Michaela Schweizer, Markus Wöhr, Karl J. Vörckel, Craig C. Garner, Hans-Jürgen Kreienkamp, Stefan Kindler

T7-8D Building fast and resilient inhibitory synapses with Ca2+ nanodomains and microdomains
Henrique von Gersdorff, Dennis J. Weingarten, Nicolas Muller, Eckhard Friauf

T7-9D The Presynaptic Protein Mover is Heterogeneously Expressed across Brain Areas and Synapse Types
Rebecca Wallrafen, Thomas Dresbach

T7-10D A sequence of molecular events mediates the rapid addition of release site modules during presynaptic potentiation
Alexander Matthias Walter, Mathias Böhme, Anthony McCarthy, Christine Beuschel, Andrease Grasskamp, Desiree Laber, Meida Jusyte, Fabian Göttfert, David Owald, Stefan Hell, Stephan Sigrist

Expression of BDNF precursor protein and RNA transcript in individual hippocampal neurons demonstrated using Laser Capture Microdissection and qRT-PCR

Federico Jose Barreda Tomas[1,2], Heike Heilmann[1], Imre Vida[1,3], Agnieszka Muenster-Wandowski[1]

[1]Charité, Universitätsmedizin Berlin, Institute of Integrative Neuroanatomy, Virchowweg, 6, 10117 Berlin, Germany
[2]Bernstein Center for Computational Neuroscience, Berlin, Germany
[3]Neurocure Excellencecluster, Charité-Universitätsmedizin Berlin, Campus Mitte, Berlin, Germany

Brain-derived neurotrophic factor (BDNF) is synthesized as a glycosylated precursor (pro-BDNF) which is post-translationally converted to mature BDNF. It is a small (13kDa) protein of the neurotrophin family with wide and abundant expression in the central nervous system (CNS). BDNF plays an important role in its normal development, enhancing the survival and differentiation of specific neuronal populations (Barde, 1989). Although BDNF is known to be expressed in many glutamatergic (excitatory) neurons (Canals et al., 2001) and much is known about how its transcription is regulated, its precise cellular localization pattern in hippocampal neuronal populations is still a matter of debate.
Therefore, to investigate the cellular and subcellular distribution of BDNF precursor protein and RNA transcript we utilized immunofluorescent labeling and confocal microscopic analysis combined with Laser Capture Microdissection (LCM) and qRT-PCR in the rodent hippocampus. We used VGAT-YFP transgenic mouse and rat lines selectively expressing YFP-Venus in interneurons (INs) under the VGAT promotor. This allowed us to distinguish between glutamatergic and GABAergic cells.
Fluorescent immunolabeling revealed that the proBDNF protein localizes mainly to the somata in hippocampal neurons of both mice and rats. Strong immunofluorescent signal was observed in the pyramidal cell layer, as well as in scattered cell bodies of neuronal populations in the stratum radiatum and stratum oriens of the CA3 and CA1 areas. This specific somatic expression in the hippocampal neuropil indicates that BDNF precursor protein is not restricted to glutamatergic cells, but is also present in GABAergic INs. In fact, there was a high degree of convergence between the VGAT-positive interneuronal staining and the intense somatic labelling for proBDNF antibody across all hippocampal layers. Furthermore, the proBDNF staining showed a unique perinuclear distribution pattern, which overlaped extensively with perinuclear cisternae of rough endoplasmatic reticulum and perinuclear Golgi markers. The absent co-localization of proBDNF with post- and presynaptic markers in our study excludes the possibility of anterograde transport to the neuronal processes, as well as the retrograde transport to the soma via endocytosis and points to the endogenous synthesis of BDNF precursor protein in the somata of different neuron types. To confirm this assumption we further examined the relative distribution of the BDNF transcript in neurons in cell body- and neuropil layers of the hippocampus using LCM and qRT-PCR and found BDNF mRNA-transcripts in abundance in both glutamatergic and GABAergic neuron populations.
Our data demonstrate that endogenous expression of BDNF precursor protein and RNA transcript are primarily localized to the somatic compartment of two examined neuronal classes in the hippocampus. We provide substantial evidence for the presence of endogenous BDNF produced in interneurons, a concept that was previously controversial. These findings are of importance to our understanding of the development and regulation of the GABAergic system and shed new light on the mechanisms governing functional inhibitory signaling in the hippocampus.

The relation between the different phases of early-phase synaptic plasticity and the underlying dynamics of AMPA-receptors

Moritz Becker[1], Christian Tetzlaff[1,2]

[1]III. Institute of Physics – Biophysics, Georg-August University, Computational Neuroscience Group, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

[2]Bernstein Center for Computational Neuroscience, Am Faßberg 17, 37077 Göttingen, Germany

Large parts of excitatory synaptic transmission is mediated by AMPA-receptors in the postsynaptic density (PSD). Changes in their number, composition and properties are linked to long-term potentiation and depression. Therefore, AMPAR dynamics play an important role for memory and learning. The exact mechanisms and dynamics underlying activity-dependent AMPAR-trafficking are however still largely unknown [1].
In the current work, based on previous studies [2, 3], we developed and analyzed a theoretical model of AMPAR-trafficking in the synapse. For this, we divided the synapse into different compartments as the PSD or extrasynaptic membrane, such that we can describe the receptor dynamics in each compartment by a specific set of differential equations. Furthermore, we included mechanisms such as receptor clustering [4] and protein-protein interactions.
Our model is able to reproduce experimental findings [5] that relate potentiation and depression to AMPAR-trafficking and enables us to disentangle the different roles of AMPAR exocytosis [6], diffusion [7] and scaffold binding [8] at different points of time. Thus, our study indicates that AMPAR-trafficking is involved in different phases of synaptic plasticity, where several trafficking mechanisms act on different time scales. In summary, we have developed a compact theoretical model that enables to understand the relationships between processes of synaptic plasticity such as LTP and the underlying AMPAR-dynamics. Based on these results, next, we are able to investigate the link between AMPAR-trafficking and further complex synaptic plasticity processes as homeostatic plasticity [9].

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TOP3B: A novel candidate gene in juvenile myoclonic epilepsy?

Marwa Daghsni[1], Saida Lahbib[2], Mohamed Fradj[3], Lilia Kraoua[4], Faouzi Maazoul[4], Sonia Abdelhak[2], Ridha Mrad[1,4]

[1]University of Tunis El Manar, Faculty of Medicine of Tunis, LR99ES10 Laboratory of Human Genetics, 1007, Tunis, Tunisia. [2]Pasteur Institute of Tunis LR11IPT05, Biomedical Genomics and Oncogenetics Laboratory, 1002, Tunis, Tunisia.[3]Department of Neurology, Charles Nicolle Hospital, Boulevard du 9 Avril, 1006, Tunis, Tunisia.[4]Congenital and Hereditary Diseases Department, Charles Nicolle Hospital, 1006, Tunis, Tunisia.

Juvenile myoclonic epilepsy regroups seizures, severe cognition abnormalities and behavior impairments. These features could evolve over time and get worse especially when the encephalopathy is pharmaco-resistant. Thus, genetic studies should provide a better understanding of infantile epilepsy syndromes. Herein, we investigate the genetics of juvenile myoclonic epilepsy in a consanguineous family analyzing the copy number variations detected by over 700 K single nucleotide polymorphism (SNP) arrays. We have identified a 254 kb deletion in 22q11.2 region, including only the TOP3B gene, detected among the patient and her father. TOP3B gene coding for topoisomerase protein â, has been implicated in several neurological diseases such as schizophrenia and autism. In this study, we discuss the implication of 22q11.2 region in neurodevelopmental disorders and the association of TOP3B gene with epilepsy.

Rapid induction and sustained expression of presynaptic homeostatic plasticity at a mammalian CNS synapse

Igor Delvendahl[1,2], Katarzyna Kita[1,2], Martin Müller[1,2]

[1]University of Zurich, Institute of Molecular Life Sciences, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[2]Neuroscience Center Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Homeostatic mechanisms stabilize synaptic transmission at various synapses in different species. At the *Drosophila* neuromuscular junction, acute or prolonged glutamate receptor perturbation induces homeostatic potentiation of neurotransmitter release that precisely restores action potential-evoked excitatory postsynaptic current (EPSC) amplitudes to baseline levels. But it has remained elusive if presynaptic homeostatic plasticity can be rapidly induced at mammalian CNS synapses. Here we investigated if presynaptic mechanisms stabilize synaptic transmission upon glutamate receptor perturbation at cerebellar mossy fiber to granule cell synapses in acute mouse brain slices. Acute application of sub-saturating concentrations of a non-competitive glutamate receptor antagonist reduced miniature EPSC amplitudes, whereas action potential-evoked EPSCs were comparable to non-treated controls, translating into a significant increase in quantal content. Remarkably, this homeostatic potentiation of neurotransmitter release was evident within ~20 minutes after glutamate receptor perturbation. Repetitive stimulation revealed that the size of the readily-releasable pool (RRP) of vesicles was enlarged at synapses with impaired glutamate receptor function. By contrast, we did not detect marked differences in short-term plasticity, suggesting no major changes in release probability. A similar increase in quantal content and RRP size was observed at synapses of heterozygous GluA4 knockout mice as a model for sustained glutamate receptor perturbation. Correspondingly, we could demonstrate an increase in synaptic vesicle exocytosis at mossy fiber terminals of $GluA4^{+/-}$ mice using direct presynaptic recordings in combination with membrane capacitance measurements. Together, our results demonstrate that acute or prolonged glutamate receptor perturbation lead to homeostatic enlargement of RRP size at a mammalian CNS synapse that stabilizes synaptic transmission. Thus, presynaptic homeostatic plasticity may be an evolutionarily conserved form of synaptic plasticity securing robust synaptic function of excitatory synapses upon acute or sustained changes in glutamate receptor function.

Direct measurement of glutamate release at Schaffer collateral synapses under low and high frequency activity

Céline D. Dürst[1], J. Simon Wiegert[1], Christian Schulze[1], Nordine Helassa[2], Katalin Török[2], Thomas G. Oertner[1]

[1]Center for Molecular Neurobiology (ZMNH), Institute for Synaptic Physiology, Falkenried 94, 20251 Hamburg, Germany

[2] Cell Biology and Genetics Research Centre, Molecular and Clinical Sciences Research Institute, St George's, University of London, London SW17 0RE, UK

Information transmission at chemical synapses is a quantal process based on the release of transmitter-filled vesicles from the presynaptic terminal. In small mammalian presynaptic terminals of the central nervous system, it is unclear how many vesicles are released in response to a single action potential and how AMPARs report the release of glutamate. To address this question, we expressed the genetically encoded glutamate sensor, iGluSnFR, in CA3 pyramidal cells of rat hippocampal slice cultures and performed two-photon glutamate imaging on individual Schaffer collateral boutons. We can detect the fusion of a single vesicle and localize the fusion site on the bouton with high precision. Under physiological conditions, Schaffer collateral synapses typically released only a single vesicle, switching to multivesicular release when extracellular calcium is high. Statistical analysis of response amplitudes allowed us to extract the three synaptic parameters defining synaptic efficacy: the number of synaptic vesicles (n), the release probability (p_r) and the quantal size (q).

iGluSnFR enables visualization of glutamate release from presynaptic terminals at frequencies up to ~10 Hz. However, to resolve glutamate dynamics during high-frequency bursts, faster indicators are required. Here, we report the development of an ultrafast (iGlu$_u$) variant, which presents 5-fold faster kinetics at synapses. When we stimulate cells at 100 Hz, we find that depression of iGlu$_u$ responses during 100 Hz trains correlates with depression of postsynaptic EPSPs, whereas recovery from depression involved both, pre- and postsynaptic changes.

From local to global signalling in rat olfactory bulb granule cell dendrites

Veronica Egger[1], Max Müller[1], S.Sara Aghvami[1,2]

[1]Regensburg University, Regensburg Center of Neuroscience, Universitätsstr. 31, 93040 Regensburg, Germany
[2]School of Electrical and Computer Engineering, University of Tehran, 14395-515 Tehran, Iran

The inhibitory axonless olfactory bulb granule cells (GCs) form reciprocal dendrodendritic synapses with mitral and tufted cells, the principal neurons of the olfactory bulb, via large spines. GC dendrites are highly excitable in multiple ways: Synaptic inputs to individual GC spines can generate Na^+ spikes within the spine head, and stronger activation results in globally propagating signals that encompass both low-threshold Ca^{2+} spikes (LTS) and Na^+ spikes.
To investigate dendritic integration we implemented a holographic two-photon uncaging system which allows simultaneous photostimulation of multiple spines in 3D. We uncage glutamate at sets of spines on GC dendrites in acute juvenile rat brain slices while recording the membrane potential from the soma and two-photon Ca^{2+} imaging within one focal plane. Although GC resting potentials are hyperpolarized, we find that the threshold for global GC Na+ spiking is attained at similar numbers of simultaneously activated spines (9 ± 2) as in cortical pyramidal cells, whereas activation of 5 ± 2 spines suffices to elicit Ca^{2+} signals in dendrites remote from the stimulated spines, possibly corresponding to the LTS (n = 27 GCs). Surprisingly, the putative LTS does not follow an all-or-none rule but decreases with distance (n = 8 GCs). In the subthreshold regime, EPSPs summate on average linearly at the soma, with occasional supra- and sublinear summation. Ca^{2+} signals in individual spines (n = 20 in 10 GCs) and in the dendrite (n = 14) increase with the total number of activated spines. Summation of Ca^{2+} signals evoked by global somatically evoked Na^+ spikes and local spine inputs is sublinear for perfect coincidence and supralinear for EPSP-AP sequences (pre before post, $\Delta t \geq 10$ ms, n = 9 spines), as predicted by simulations. The sublinearity results from the suppression of the local spine spike by the global Na^+ spike.
In conclusion, GCs turn out to be yet more excitable than previously thought, and postsynaptic spine Ca^{2+} entry is tuned to the overall level of excitation. Thus the GC spines - who according to our previous findings can operate as independent mini-neurons – do sense the general activation state of their ‘mother GC’ already at subthreshold levels. Funded by the BMBF (01GQ1502).

Synaptic mechanisms underlying temporally precise information processing in the VNLL

Linda Fischer[1,2], Felix Felmy[1]

[1]University of Veterinary Medicine Hannover, Foundation, Institute of Zoology, Bünteweg 17, 30559 Hannover, Germany

[2]Hannover Graduate School for Veterinary Pathobiology, Neuroinfectiology, and Translational Medicine (HGNI)

A hallmark of neurons in the ventral nucleus of the lateral lemniscus (VNLL) is their extremely precise temporal discharge with very low, invariant latencies. Driven by amplitude modulated sound stimulation these neurons follow on average best modulation frequencies of about 200 Hz with a small modulation bandwidth. These described firing properties suggest that VNLL neurons preserve time information on the stimulus onset and the envelope structures during ongoing stimulation. Therefore, these neurons might play an important role in processing of sound transients as they occur in speech. How precise onset firing and the specific tuning of firing frequencies are implemented on a cellular level in VNLL neurons is unclear. One hypothesis would be that the bi-exponential decay of synaptic excitation promotes these features.

Here, we examine the functional role of these decay components in order to understand the neurons' firing properties. To this end, we performed *in vitro* whole-cell voltage-, current- and dynamic-clamp recordings in VNLL neurons of 19-30 days old *Meriones unguiculatus*. Quantitative description of synaptic transmission, based on successive pharmacological isolation, reveals that AMPARs mediate the fast and NMDARs the slow excitatory postsynaptic current (EPSC) decay component, while KainateRs are not involved. Determining of short-term plasticity shows onset facilitation at stimulation frequencies above 50 Hz. Moreover, NMDARs generate build-up currents at high frequency train stimulations associated with increased EPSC decay times and EPSC amplitude depression. Dynamic-clamp recordings were used to test whether VNLL neurons' output depends on the observed short-term plasticity and the different synaptic current components. Our data reveals that action potential (AP) generation in VNLL neurons depends on the stimulation frequency and the receptor composition. Close to AP threshold facilitation can trigger transient supra-threshold excitation. At higher stimulation intensities the NMDARs boost AP firing during ongoing stimulation, counterbalancing synaptic depression. Interestingly, there is a breakdown of firing at 400 Hz stimulation frequency, when using dynamic-clamp technique, whereas during current injection, cells follow faithfully this stimulation frequency, indicating an effect on spike generation by sustained depolarisations. From the synaptic conductance and dynamic-clamp recordings it follows that faithful cycle-by-cycle information transfer requires at least two synchronised endbulb synapses at frequencies above 50 Hz.

Taken together, our study shows that EPSCs in VNLL neurons are mediated by AMPAR and NMDAR and display frequency dependent facilitation and depression. This data indicates a mechanism by which VNLL neurons sustain temporally precise output that is on average limited by an interplay between facilitation, depression and spike generation to below 400 Hz.

Relation between sodium signaling and ATP consumption in mouse hippocampal neurons

Niklas J. Gerkau[1], Rodrigo Lerchundi[1], Jan Meyer[1], Christian Kleinhans[1], Marina Lantermann[1], Johannes Hirrlinger[2,3], Christine R. Rose[1]

[1]Heinrich Heine University Düsseldorf, Institute of Neurobiology, Universitätsstr. 1, 40225 Düsseldorf, Germany
[2]Carl-Ludwig-Institute for Physiology, Faculty of Medicine, University of Leipzig, Liebigstrasse 27, D-04103 Leipzig, Germany
[3]Department of Neurogenetics, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Strasse 3, D-37075 Göttingen, Germany

Excitatory activity resulting in opening of voltage- and ligand-activated channels is accompanied by sodium influx into neurons, generating transient elevations in the intracellular sodium concentration. Depending on the temporal and spatial patterns of activity, such activity-related sodium signals can be locally restricted to the site of influx or be global and encompass the entire cell. Sodium export upon global sodium increases is largely mediated by the Na^+/K^+-ATPase (NKA) and therefore ultimately depends on an intact energy metabolism. For recovery from local sodium increases, fast lateral diffusion dominates over extrusion, operating efficiently even during short periods of energy deprivation (Mondragao et al., J Physiol, 2016). Although sodium will eventually be extruded by the NKA, this indicates that recovery from local sodium signals does not require significant local ATP consumption.
In the present study, we tested this hypothesis by performing sodium imaging with the sodium indicator SBFI (sodium-binding benzofurane isophthalate) in CA1 pyramidal neurons of acute mouse hippocampal tissue slices. In addition, we employed FRET imaging of the genetically-encoded nanosensor Ateam1.03^{YEMK}, specifically expressed in hippocampal neurons, to monitor intracellular ATP levels during different patterns of activity.
Induction of recurrent network activity by removal of magnesium and addition of bicuculline resulted in global neuronal sodium oscillations, exhibiting peak amplitudes of 10-20 mM. Sodium oscillations were accompanied by a slow, steady decrease in ATP levels in their somata and apical dendrites, which persisted as long as the activity persisted. Bath application of glutamate for 10 seconds induced a transient sodium increase by in the same range as detected in neuronal somata and apical dendrites. At the same time, a delayed transient decrease in ATP levels in both compartments was observed. Finally, we performed a local puff application of glutamate onto a spiny dendrite, which resulted in a transient sodium signal by about 15 mM that was locally restricted to the stimulated dendritic region. In contrast to global sodium signals, such local dendritic sodium increases were not accompanied by a detectable change in ATP levels in the stimulated dendritic region.
Taken together, our results show that global sodium increases in neurons are accompanied by a decrease in cellular ATP levels, indicating activation of the NKA and consumption of cellular ATP for export of sodium. Localized sodium influx, in contrast, apparently does not require a local increase in ATP consumption. This suggests that sodium loads generated locally are removed by fast lateral diffusion and therefore do not result in local activation of NKA. Intracellular spread of sodium from activated to non-activated regions might thus serve a homeostatic function by accelerating the re-establishment of low intracellular sodium and by lowering local energy requirements.

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In search of the synaptic vesicle tether at a sensory synapse

Kaspar Korbinian Maximilian Gierke[1], Sonja Kirsch[2], Tanja Müller[1], Craig Garner[3], Rainer Böckmann[2], Hanna Regus-Leidig[1], Johann Helmut Brandstätter[1]

[1]FAU-Erlangen Nürnberg, Department of Biology, Animal Physiology, 91058 Erlangen, Germany

[2]FAU-Erlangen Nürnberg, Department of Biology Computational Biology, 91058 Erlangen, Germany

[3]German Center for Neurodegenerative Diseases, Charité - Universitätsmedizin Berlin, 10117 Berlin, Germany

At conventional chemical synapses, the presynaptic active zone (AZ) is the hotspot for synaptic vesicle (SV) exocytosis. Before SVs are fusion competent, they are docked close to calcium channels and primed for release. In contrast to conventional chemical synapses where SVs are directly bound to the AZ, sensory ribbon-type synapses possess a presynaptic organelle, the synaptic ribbon (SR), tethering SVs up to several hundred nanometers away from the release site. To date it is not known how the tethering of SVs to the SR is achieved. Recent results from conventional chemical synapses indicate that the AZ protein Piccolo is involved in the organization of SV pools[1,2]. In the current study, we explore how Piccolino, the C-terminally truncated splice variant of Piccolo[3] interacts with SVs at the photoreceptor ribbon synapse.

First, we ultrastructurally analyzed the distribution of SVs at rod photoreceptor ribbon synapses in a Piccolino knock-out (KO) rat. We found that the density of ribbon-associated SVs in Piccolino KO rod photoreceptors was decreased by about 50% when compared to WT synapses. This finding indicates an involvement of Piccolino in SV tethering and prompted us to investigate this idea in more detail. Next, we analyzed whether Piccolino spans the predicted tether extension of 30-40nm. With immunoelectron microscopy we were able to show that an antibody directed against the N-terminus of Piccolino localized further away from the synaptic ribbon (24,5 nm +-4,5 nm), than an antibody directed against the C-terminus (11,8 nm+-2,5 nm). Searching for a possible synaptic tethering mechanism, we identified an alpha-helical ALPS (**a**mphiphatic **l**iquid **p**acking **s**ensor) motif at the N-terminus of Piccolino. Subsequent molecular dynamics simulations showed that the Piccolino ALPS motif readily binds to curved membranes of a SV like composition.

From our data we conclude that Piccolino displays features that are a prerequisite for a tethering factor. Electrophysiological and biochemical experiments are under way to corroborate our initial observations and provide a detailed mechanistic model of Piccolino's function at photoreceptor ribbon synapses.

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Single synapse activity characterization reveals interdependences between release modes.

Andreas T Grasskamp[1,2], Meida Jusyte[1,3], Mathias A Böhme[1], Alexander M Walter[1,3]

[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Molecular and Theoretical Neuroscience, Charitéplatz 1/Virchowweg 6, 10117 Berlin, Germany

[2]NeuroCure Cluster of Excellence, Charité Universitätsmedizin Berlin, Germany

[3]Einstein Center for Neurosciences, Berlin, Germany

Communication between nerve cells happens via neurotransmitters stored in synaptic vesicles (SVs) that are liberated by membrane fusion at specialized structures termed active zones (AZs). This exocytosis of SVs can be synchronously triggered by action potentials (AP) that induce influx of Calcium into the presynapse through voltage-gated channels. However, it can also occur spontaneously, without an explicit stimulus. So far, it is not entirely elucidated whether there is a function of spontaneous release. It has long been seen as a non-regulated, unavoidable “byproduct” of the AP inducible release machinery. Additionally, only few results exist on whether both release mechanisms are dependent on each other, which could explain factors of synaptic functionality like SV pool composition. The dependence of release modes on presynaptic AZ-structure and on each other can be studied using postsynaptically expressed calcium indicators like GCaMP, which reports Ca^{2+} influx through neurotransmitter receptors. We investigated this at neuromuscular junctions of *Drosophila* larvae, whose synapses display a large heterogeneity in structure and activity, likely due to differences in maturation. We could show that both spontaneous and evoked activity depend on the synaptic abundance of the release site generating protein Unc13A, and indirectly on the cytomatrix protein BRP [1]. Recently, it has also been a controversial issue whether synapses show preference for either release mode. We now show that AZs (IHC stained for BRP) engaging in AP evoked release are more likely to release SVs spontaneously, and vice-versa. This implies a common pool of SVs operating in both release modes and partly resolves the issue of synaptic release mode preference. Furthermore, we show that unlike expected for truly spontaneous and independent events, the frequency of spontaneous activity at individual AZs decreased after AP stimulation. This observation further solidifies the notion that the vesicles used in both transmission modes (spontaneous and evoked) are derived from a shared pool.

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The interplay between kinesin-3 and dynamic microtubules at presynapses specifies high precision delivery of synaptic cargo

Pedro Guedes-Dias[1], Jeffrey J Nirschl[1], Nohely C Abreu[1], Mariko K Tokito[1], Erika LF Holzbaur[1]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6085 USA

Neurons in the central nervous system establish thousands of en passant synapses along their extensive axonal arbors. Robust and reliable neurotransmission is dependent on the replenishment of presynapses with new synaptic vesicles in a timely and spatially-precise manner. However, the mechanisms specifying the local delivery of synaptic vesicle precursors (SVPs) to presynaptic sites remain unclear. By employing live-cell microscopy and single-molecule reconstitution assays, we found that delivery of SVPs to en passant synapses in hippocampal neurons occurs with high precision and is specified by an interplay between the kinesin-3 KIF1A motor and presynaptic microtubules. We identified presynaptic sites as hotspots of dynamic microtubules rich in GTP-tubulin, and found that KIF1A binds weakly to GTP-tubulin and competes with EB proteins for binding to the microtubule lattice. The enrichment of dynamic microtubules at the presynapse effectively defines a localized SVP unloading zone and ensures a supply rate of SVPs at the presynapse that is in tune with the estimated synaptic vesicle lifetime. Additionally, we identified a human disease-causing mutation within KIF1A loop 11 that reduces the differential binding of KIF1A to the GTP microtubule lattice. Expression of this mutation specifically disrupts SVP delivery to presynapses and reduces presynaptic strength in hippocampal neurons. Together, we show that microtubule dynamics and organization along the axon provide a spatial code that specifies presynaptic delivery of KIF1A-SVPs and controls presynaptic strength in hippocampal neurons.

Role of Auxiliary Subunits in AMPA Receptor Trafficking in Hippocampal Neurons

Ali Harb[1,2], Nils Vogel[2], Walentina Frisch[2], Ali Shaib[3], Ute Becherer[2], Dieter Bruns[2], Ralf Mohrmann[1]

[1]Institute of Physiology, Medical Faculty, Otto-von-Guericke University, 39120, Magdeburg, Germany
[2]Institute of Molecular Neurophysiology, University of Saarland, CIPMM Homburg, Germany.
[3]Max Planck Institute for Experimental Medicine, Department of Molecular Neurobiology, Göttingen, Germany.

AMPA-type glutamate receptors (AMPARs) are present at most glutamatergic synapses throughout the vertebrate central nervous system and dominate excitatory synaptic transmission. A dynamic recruitment of extrasynaptic AMPARs to synaptic sites is believed to underlie synaptic homeostasis as well as plasticity. AMPARs associate with different types of auxiliary subunits that in part modulate channel function, facilitate their forward trafficking from the ER towards the plasma membrane, and support synaptic recruitment. Two prominent auxiliary subunits from different families, TARPγ8 and CKAMP44a, have been shown to govern AMPAR function in hippocampal neurons. Yet, the role of these two types of auxiliary subunits in local recycling of dendritic receptors has remained unclear. Using live-cell imaging, we show here that the basal turnover of AMPA-type glutamate receptor subunit (GRIA) 1-containing receptors via recycling endosomes is strictly regulated by the abundance of AMPAR-associated TARPγ8 or CKAMP44a. In pulse-chase experiments, a HaloTag-marked GRIA1-subunit was used to selectively label surface receptors with a membrane-impermeable fluorescent ligand, and endocytosis-rate under different conditions was estimated based on the progressive fluorescence decline on the plasma membrane. Overexpression of either auxiliary subunit prolonged the lifetime of extrasynaptic GRIA1 on the surface by reducing the rate of constitutive endocytosis. AMPAR surface expression generally remains constant under basal conditions, as endo- and exocytosis rates stay balanced. To study potential changes in the rate of AMPAR-delivery to the plasma membrane, we employed a superecliptic pHluorin (SEP)-tagged GRIA1-variant, whose pH-dependent, N-terminal fluorophore can report exocytosis of AMPAR-containing transport organelles by means of a local fluorescence increase. SEP-GRIA1-containing intracellular compartments were identified in dendrites by application of ammonium chloride solution, which neutralizes the acidic lumen of the organelles and unquenches SEP. Normalizing the frequency of detected fusion events to the number of intracellular SEP-GRIA1 storage compartments, we found that the insertion rate of SEP-GRIA1-containing AMPARs was dramatically reduced in neurons that overexpressed TARPγ8 or CKAMP44a. In line with a diminished endocytosis rate of AMPARs under these conditions, we also observed a reduced pool of GRIA1-containing receptors in dendritic recycling endosomes. Taken together, our novel data indicate that association with auxiliary subunits protects AMPARs from rapid turnover and stabilizes the surface receptor pool.

Presynaptic K^+ channels regulate spontaneous glutamate release through a specific association with Ca^{2+} channels in the hippocampal pyramidal neurons

Won-Kyung Ho[1]

[1]Seoul National University College of Medicins, Department of Physiology, 103 Daehak-ro, 03080 Seoul, Korea (South)

Spontaneous neurotransmitter release has important functions, including regulation of dendritic protein synthesis and stability of synaptic networks. Stochastic opening of presynaptic Ca^{2+} channels is suggested to cause vesicle fusion, but the mechanism of how it is regulated is poorly understood. The role of K^+ channels on presynaptic Ca2+ at basal state is thought to be attributable to the changes in resting membrane potential (RMP), but direct experimental evidence is lacking. In the present study, we found a significant disparity between the effect of K^+ channels on RMP and that on spontaneous glutamate release, and presented evidence for a novel mechanism that enables a specific K+ channel to regulate a specific Ca^{2+} channel in presynaptic terminals. Blockade of M-type K^+ currents (I_M) using linopirdine increased frequency of miniature excitatory postsynaptic currents (Fmini) by 2.1 fold in pyramidal cells of CA1 hippocampus (CA1-PCs), while it depolarized RMP of pyramidal cells of CA3 hippocampus, which are presynaptic cells for CA1-PCs, by 2.8 mV. In hippocampal autaptic cultured neurons (ACNs) where RMP was maintained at -70 mV under voltage clamp condition, linopirdine induced a similar increase in F_{mini} (1.7 fold). In contrast, depolarizing RMP in ACNs by 10 mV in ACNs by injecting currents increased F_{mini} to a smaller extent (1.4 fold). Similar disparity between the effects of K+ channel block on RMP and F_{mini} was also observed when A-type K^+ channels (I_A) was blocked using 4-AP. These results suggest that effect of K^+ channel blockade on spontaneous glutamate release is more powerful than that expected by its effect on RMP depolarization. Alternative hypothesis to explain such powerful effects of K^+ channel blockade on Ca^{2+} channel opening is a close coupling between a specific K+ channel and a specific Ca^{2+} channel, so that the effect of a specific K^+ channel on an opening of specific Ca2+ channels is more powerful than its effect on global RMP. To examine this hypothesis, we tested the effects of linopirdine and 4-AP on Fmini in the presence of various Ca2+ channel blockers, and found that linopirdine effects were abolished in the presence of nimodipine, an L-type specific blocker, while 4-AP effects were abolished in the presence of w-Agatoxin, a P/Q type-specific blocker, indicating that I_M and I_A specifically regulate L-type and P/Q type Ca2+ channel, respectively. Our study reveals a specific association between K^+ and Ca^{2+} channels in the presynaptic terminals, which allows K^+ channels to control Ca^{2+} channel activity strongly with a high specificity.

Optogenetic characterization of excitatory inputs at spiny interneurons of the stratum oriens

Joaquin Isaac Hurtado Zavala[1], J. Simon Wiegert[1]

[1]ZMNH, Synaptic Wiring and information processing, Falkenried 94, 20251 Hamburg, Germany

In the CA1 region of the hippocampus, inhibitory interneurons are part of a local microcircuit formed by a diverse population of neurons that include principal CA1-pyramidal cells as well as a heterogeneous population of inhibitory neurons. Although inhibitory interneurons comprise the minority of neurons of the CA1 region, they have a profound impact on hippocampal network activity. Inhibitory neurons thus control the flow of information from different proximal and distant brain areas, regulate the excitability of neurons by the release of GABA, modulate synaptic transmission and control the timing of neuronal firing precisely shaping entrainment and retrieval of ensemble activity. Different types of interneurons are highly specialized, which is reflected by the huge diversity within and between inhibitory classes (even in a confined region of the hippocampus), regarding their morphology, connectivity, neurophysiology and biochemical composition. Interestingly, in the CA1 area a sparse quantity of inhibitory interneurons, confined in the stratum oriens and the alveolus bear dendritic spines, that otherwise are rarely observed in other cortical inhibitory interneurons. Dendritic spines at these interneurons, like in excitatory pyramidal neurons, receive glutamatergic excitatory input and might serve as the smallest units of information storage. A major fraction of these synaptic spines receives input from local CA1 pyramidal cells. Thus, plasticity at these synapses may directly regulate the recruitment of spiny interneurons in local circuits of CA1. Yet the mechanisms by which excitatory synaptic connections from CA1 pyramidal neurons to spiny interneurons of the stratum oriens are regulated still remains elusive. We aim to elucidate at the synaptic level how spiny interneurons of the CA1 stratum oriens are recruited by principal CA1 neurons and how synaptic plasticity mechanisms regulate the long-term connectivity between these cells. Using ChR2, we first confirmed that spiny interneurons of the CA1 stratum oriens receive direct excitatory synaptic input from CA1 cells in organotypic hippocampal mouse cultures. Local photostimulation of ChR2-expressing CA1-pyramidal neurons and ChR2-expressing axon collaterals terminating at spiny interneurons of the CA1-SO elicited postsynaptic currents. On the other hand, photostimulation of the CA3 stratum pyramidale (SP) evoked indirect synaptic currents, whereas photostimulation of CA3 local axons near CA1 OLMs did not evoke a response, indicating a low prevalence of direct inputs from CA3 neurons. Despite the fact that some interneurons of the CA1 stratum oriens are long known to be exceptionally spiny, the temporal structural dynamics of these spiny synapses remain poorly understood. Therefore, we chronically monitored synaptic spine dynamics at interneurons in stratum oriens in slice culture and in vivo. In summary, understanding dynamic changes of excitatory connections from CA1 pyramidal neurons onto local interneurons may provide us with a better concept of how interneurons adjust the recruitment of specific neuronal assemblies on short and long time scales.

Proteomic alterations of GABAergic Interneurons following traumatic brain injury (TBI) in mouse neocortex.

Natascha Ihbe[1], Florie Le Prieult[1], Qi Wang[1], Ute Distler[2], Malte Sielaff[2], Stefan Tenzer[2], Serge Thal[3], Thomas Mittmann[1]

[1]Institute of Physiology, University Medical Center of the Johannes Gutenberg University, Mainz, Germany
[2]Institute for Immunology, University Medical Center of the Johannes Gutenberg University, Mainz, Germany
[3]Clinic for Anesthesiology, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

Traumatic Brain Injury (TBI) is one of the leading causes for mortality in industrialized countries. Even though advances in intensive care units led to a reduced mortality, patients still suffer from severe physical and cognitive outcomes (Werner and Engelhard, 2007). This reflects the need for substantial investigations of the time course of neuronal alterations shortly after the injury.
Here, we focused on the early transhemispheric diaschisis post-TBI, which is suggested to be mediated by an imbalance in strength of glutamatergic, excitatory vs. GABAergic, inhibitory neurotransmission (Imbrosci et al, 2014). In accordance, we recently disclosed an impairment of GABAergic inhibition with no changes in glutamatergic EPSCs in the contralateral cortex early after TBI induction (Le Prieult et al., 2017). Here we hypothesized that these changes in function of GABA interneurons in the cortex contralateral to the lesion are accompanied by specific changes in protein expression in these neurons.
To test this hypothesis, we used a transgenic mouse line expressing the Green Fluorescent Protein (GFP) under the control of the glutamic acid decarboxylase 67 (GAD67) promotor and induced a TBI with an established Controlled Cortical Impact (CCI) model in the primary motor and somatosensory cortex at postnatal day 19-21 under anesthesia in vivo.
Single interneurons located in the primarily undamaged, contralateral cortex were identified by their GFP expression and isolated by Fluorescence-Activated Cell Sorting (FACS) at 1, 3 and 7 days post lesion. Using proteomic analysis by Mass Spectrometry (MS) we demonstrate that GABAergic interneurons derived from the contralateral cortex reacted in a diverse manner to an ipsilateral cortical injury showing dynamic processes over the selected time window. We detected an overrepresentation of proteins linked to structural and molecular functions in GABAergic interneurons at 1 and 7 days post-TBI, while 3 days post-TBI Gene Ontology (GO) analysis revealed rather protein enrichments in cellular components related to neuron projection including dopaminergic and glutamatergic synapses.
In summary, our experiments do not only provide a proof of the concept that GABAergic interneurons can be identified, successfully isolated and further analyzed through transgenic GFP-labeling, FACS and proteomic analysis. Furthermore, we disclosed TBI-induced specific changes of protein expression in these GABAergic interneurons in the somatosensory cortex of mice after TBI. This potentially allows us to detect so far unknown therapeutic targets to mediate the beneficial and/or maladaptive processes in the cortical circuits occurring during functional recovery after TBI in the contralesional cortex.

Freeze frame shots of synapses in action: Correlating presynaptic ultrastructure and function at the nanoscale

Cordelia Imig[1], Sünke L. Mortensen[1], Lydia Maus[1], Nils Brose[1], Benjamin H. Cooper[1]

[1]Max Planck Institute of Experimental Medicine, Department of Molecular Neurobiology, Hermann-Rein-Str. 3, 37075 Göttingen, Germany

Neurons communicate with each other at synaptic contact sites, where neurotransmitter-filled synaptic vesicles (SVs) fuse in response to an action potential at structurally and molecularly specialized presynaptic active zone (AZ) release sites. Electron microscopy (EM) is a powerful tool for probing ultrastructure-function relationships by revealing the structural organization of synapses at subcellular resolution, however, conventional EM preparation methods have thus far provided limited insight into (i) how differences in the ultrastructural architecture of synaptic release sites contribute to, or reflect, the functional properties of distinct synapse types, or (ii) how activity-dependent structural and molecular remodelling changes the ultrastructural organization of synapses to support, or indeed shape, their functional plasticity characteristics. To address these questions, we have designed an experimental approach to resolve activity-dependent changes in synaptic ultrastructure during the induction of defined and physiologically-relevant activity states in the context of an intact neuronal circuit. Our methodology combines mouse genetics, organotypic hippocampal slice culture, high-pressure freezing (HPF), automated freeze-substitution (AFS), and electron tomography (ET) to determine presynaptic architecture with nanoscale precision and to enable optogenetic stimulation of distinct synapse types in living brain slices immediately prior to rapid cryo-fixation. In the present study, we focus on two well-characterized glutamatergic hippocampal synapse types with profoundly different morphological and functional characteristics, i.e. Schaffer collateral (SC) and mossy fiber (MF) synapses. To relate optogenetic stimulation conditions as assessed and standardized by electrophysiology with the optogenetic stimulation of slices in the HPF instrument, both experiments must be conducted in identical physiological buffer conditions. In the past, this has posed a considerable technical challenge, since most tissue preparations require external cryoprotectants in the liquid media to achieve adequate ultrastructural preservation. We have circumvented this problem by establishing an experimental workflow, which allows organotypic slices to undergo rapid cryo-fixation with excellent morphological preservation in artificial cerebrospinal fluid without additional cryoprotection. Our goal is to use this methodological approach to establish whether distinct synaptic short-term plasticity states, i.e. synaptic short-term depression (STD) and short-term facilitation (STF), that are characteristic for hippocampal SC and MF synapses, respectively, are reflected by stereotypic changes in the spatial organization of morphologically distinct SV pools (i.e., docked/primed, tethered) at individual presynaptic AZs.

Ca^{2+}-dependent Calmodulin-Unc13A interaction shapes structure, function, and short-term plasticity

Meida Jusyte[1,2], Mathias A. Boehme[1], Alexander M. Walter[1]

[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Molecular and Theoretical Neuroscience, Chariteplatz 1 , 10117 Berlin , Germany

[2]Einstein Center for Neurosciences Berlin
Charitéplatz 1, 10117 Berlin

Communication between neurons occurs at synapses, where synaptic vesicles (SVs) undergo docking (membrane attachment) and molecular priming (gaining fusion competence) into a readily releasable pool (RRP) at specialized presynaptic regions referred to as active zones (AZs). In response to presynaptic Ca2+-influx triggered by incoming action potentials (APs), SVs of the RRP fuse with presynaptic active zone (AZ) membranes with high temporal and spatial precision. (M)Unc13s (Mammalian homolog of uncoordinated protein 13) are evolutionarily conserved multi-domain proteins, previously shown to mediate docking and priming of SVs as well as to regulate the RRP and short-term plasticity (STP). Preceding investigations of the Ca2+-dependent interaction of Calmodulin (CaM) with (M)Unc13 have shown, that its loss leads to slower replenishment and reduced RRP sizes upon repetitive stimulation. Here we aimed to investigate, how a disruption of CaM binding to Unc13A (induced by two point mutations) affects synaptic structure, function and short-term plasticity at the Drosophila neuromuscular junction (NMJ) using a combination of super-resolution STED microscopy and electrophysiology. While the localization of Unc13A to the AZ is not impaired by a loss of the Unc13A-CaM, reduced AZ sizes in Unc13-CaM mutant synapses could be observed with the aid of STED microscopy. Basic electrophysiological experiments revealed higher AP-evoked responses and reduced spontaneous activity upon loss of Unc13A-CaM. Further, the mutation induced synaptic short-term depression and we could show that these effects are highly dependent of extracellular Ca^{2+}-levels. In order to determine the parameters which modulate these changes in STP, we performed fluctuation analysis (FA) and could observe a higher number of release sites (N), elevated initial release probability (pvr) and lower quantal sizes (q) in Unc13A-CaM mutants. Moreover, FA upon repeated stimulation suggests a tendency on the number of N utilized for release upon repetitive synaptic activation. Thus, the interaction of Unc13A with CaM is a positive regulator of AZ size and modulates synaptic function via initial pvr, N and q. In addition, its role in STP might be governed by the number of N contributing to release during prolonged AP-evoked activity.

Uncovering the Role of Presynaptic GIT Proteins for Fast Auditory Signaling

Christian Keine[1], Samuel M. Young, Jr.[2]

[1]Department of Anatomy and Cell Biology, Carver College of Medicine, University of Iowa, Iowa City, USA

[2]Department of Otolaryngology, Iowa Neuroscience Institute, Aging Mind Brain Initiative, University of Iowa, Iowa City, USA

To encode sounds, the auditory system relies on temporally precise action potentials (AP) and rapid changes in AP firing rates in the auditory brainstem. However, the timing and efficacy of AP firing critically depends on the tight regulation of neurotransmitter release at the presynaptic terminal, from a limited number of fusion competent synaptic vesicles (SVs), the readily releasable pool (RRP). Therefore, to understand how sound is encoded it is imperative to determine the molecular mechanisms that control SV release dynamics. A critical synapse for the first stages of binaural sound processing is the calyx of Held/MNTB synapse located in the auditory brainstem. The calyx of Held/MNTB synapse has extraordinary fidelity and reliability of synaptic transmission up to kilohertz firing rates which provides fast synaptic inhibition to several auditory nuclei involved in sound localization. Recently, we identified at the calyx of Held that the G protein-coupled receptor kinase-interacting proteins (GITs) control synaptic strength by regulating SV release probability. However, the GIT proteins' role in enabling temporally precise and sustained auditory signaling is unknown. To elucidate their roles, we used Cre recombinase expressing viral vectors in conjunction with a $GIT1^{fl/fl}$ $GIT2^{-/-}$ mouse line to ablate both GIT isoforms in the calyx of Held right after birth. To mimic in vivo conditions, we performed all experiments at 37C with in vivo like release probability on functionally adult synapses (P20-P25). Using afferent fiber stimulation frequencies similar to sound-evoked firing rates (300 Hz and 500 Hz), we found that ablation of both GIT isoforms resulted in a two-fold increase in initial release probability with no change in RRP size and slightly slower rate of RRP recovery. Importantly, this effect was developmentally independent, as GIT ablation after hearing onset exhibited a similar phenotype. Despite the robust change in the initial SV release probability, however, the reliability and temporal precision of AP spiking in MNTB neurons was not affected. These results suggest that the late steps in SV life cycle controlling initial release probability are not critical for information transfer at the calyx of Held-MNTB synapse.

Minimal input requirement for action potential generation in auditory brainstem nuclei

Nikolaos Kladisios[1], Linda Fischer[1], Felix Felmy[1]

[1]University of Veterinary Medicine Hannover, Institute of Zoology, Bünteweg 17, 30559 Hannover, Germany

The number of ascending excitatory axons that generate post-synaptic action potentials vary between auditory brainstem nuclei according to their biophysical specializations. Developmental synaptic refinement further modifies the innervation pattern and the EPSC shape. Relaying nuclei, like the medial nucleus of the trapezoid body (MNTB) and the ventral nucleus of the lateral lemniscus (VNLL), are innervated by one or two fibers with calyceal terminals, promoting fairly reliable information transfer. Nuclei integrating binaural cues, such as the medial superior olive (MSO) and the dorsal nucleus of the lateral lemniscus (DNLL), receive a substantially larger number of axons. A comparative inquiry into the minimal input requirement for ongoing supra-threshold excitation is still lacking.

Here, we addressed this issue by recording juvenile (postnatal day 9/10) and mature (postnatal day 26-90) neurons of the VNLL, MNTB, MSO and DNLL in acute brain slices of the Mongolian gerbils in current-, voltage- and dynamic-clamp. First, we quantified the current strength-stimulus duration relationships of the neurons of each nucleus. Next, we determined the single fiber IV-relationships of AMPA- and NMDA-receptor mediated currents and their respective conductance and kinetics. Furthermore, we investigated the synaptic short-term depression at various stimulation frequencies, at physiologically relevant calcium concentration (1.2 mM). By combining the single fiber EPSC time course at resting potential with the steady-state depression, we gained a first estimate of the minimum number of input fibers required for ongoing activity.

Our results indicate that sustained supra-threshold excitation in juvenile VNLL neurons is achieved with three to four inputs; in contrast, a single one is enough for mature cells. In MNTB, one input marginally produces failures in mature, but none in juvenile neurons. Depending on the stimulation frequency, juvenile MSO neurons require up to 10 and 25 fibers and adult cells between 5 and 12. The surprisingly excitable cells of the DNLL seem to receive four to eight and two to four fibers in juvenile and adult neurons respectively. Finally, we used dynamic-clamp to validate our predictions. We introduced varying AMPAR and NMDAR conductances of previously recorded stimulation trains of 10-300 Hz and extracted the threshold current for onset and steady-state ongoing excitation. So far, in juvenile neurons the results coincide with the estimations and reveal a small functioning role of NMDARs in spike generation.

Neuronal profilins as modulators of dendritic complexity and structural plasticity

Maximilian Klasmeier[1], Tania Meßerschmidt[1], Dorothea Hinz[1], Martin Korte[1], Martin Rothkegel[1]

[1]TU Braunschweig, Cellular Neurobiology, Spielmannstr. 7 , 38106 Braunschweig, Germany

In neuronal cells actin dynamics are crucially involved in processes of neuronal differentiation, dendritic and axonal maintenance and in processes of synaptic plasticity of dendrites, axons and synapses related to learning events in the adult nervous system. These tasks are fulfilled with the help of a still increasing number of factors tightly regulating actin polymerization and F-actin structure in space and time. These microfilament modulators mediate signals from the pre- and postsynaptic membrane to the actin cytoskeleton and by this means change the function and the structure of specific neuronal compartments. In this context profilins most likely play a specific but so far largely unexplored role in regulating the microfilament system. In the central nervous system (CNS), two isoforms of profilin, PFN 1 and PFN 2a, are co-expressed quite in contrast to most other cells in the mammalian body and known to be recruited to dendritic spines in an activity-dependent manner.

Recent studies focusing on the cellular role of profilin 1 and 2a in neuronal and glial cells are showing that both isoforms possess overlapping as well as isoform specific functions. Furthermore, the findings received by knocking out only one of the isoforms could not exclude that each isoform could compensate the loss of the other one.

Here, we were interested in the role of PFN1 and PFN2 for neuronal function and structure and the underlying molecular mechanisms in murine hippocampal neurons. For this purpose, we generated AAV vectors encoding *pfn1* and *pfn2* specific single guiding RNAs (sgRNAs) and the Cre recombinase under the control of the human synapsin promoter. In combination with a Cre dependent CRISPR/Cas9 mice these vectors enable a simultaneous knock out of both isoforms by genome editing in a cell type specific manner. In this study, neuronal dissociated and organotypic cultures were prepared and either transduced or transfected with the appropriate vectors. In order to shed light on the role of the profilins in modulating neuronal structure and function, eight days post transduction/transfection the neurons were analyzed with respect to neuronal morphology, dendritic complexity as well as spine density and morphology.

Our results show that the viability of the neurons is not affected by the loss of both profilins. Preliminary data of Sholl analysis of transfected neurons indicates an alteration of dendritic complexity and spine density is also modulated by profilins. Taken together our results suggest a crucial role of both profilins for the maintenance and plasticity of the neuronal morphology.

Regulation of exocytosis by amisyn, a PI(4,5)P2 and syntaxin-binding protein

Ilona Kondratiuk[1], Shrutee Jakhanwal[2], Reinhard Jahn[2], Ira Milosevic[1]

[1]European Neuroscience Institute, Göttingen, Germany
[2]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Higher functions of the brain, for example learning and memory, are mediated by fast and precisely coordinated neurotransmitter release through the process of regulated exocytosis. Intense research in the past three decades has identified numerous proteins involved in exocytosis, including the Sec1/Munc18 (SM) protein family (Munc18, Munc13), synaptotagmins that sense calcium, and the SNARE complex proteins: synaptobrevin-2/VAMP-2, syntaxin-1 and SNAP-25 that mediate membrane fusion. While the key exocytic proteins are highly conserved through evolution, the regulation of exocytosis has advanced and requires more proteins in the higher organisms, such as vertebrates.
In addition to the core set of exocytic machinery, exocytosis is regulated by complexin, tomosyn and amisyn (STXBP6), cytosolic proteins that bind the SNARE complex. Amisyn is reported to be an important negative regulator of exocytosis, yet little is known about this brain-enriched protein. We found that, in addition to the C-terminal SNARE motif that interacts with syntaxin-1 and forms 'fusion-inactive' SNARE complex, amisyn contains an N-terminal pleckstrin homology (PH) domain. The PH domain of amisyn is phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) specific, and it mediates its interaction with the plasma membrane. Given that amisyn is a conserved protein present only in vertebrates, it makes it necessary to characterize it better.
We have generated amisyn knock-out mice to study amisyn-dependent processes at the vertebrate neurons and neurosecretory cells. We found that amisyn is important for the priming of secretory vesicles and the size of vesicle pools, but not fusion kinetics. Curiously, the inhibition is not due to amisyn's SNARE motif binding to syntaxin-1, but the full-length protein is needed for the proper control of exocytosis. The mechanisms of amisyn-dependent inhibition and its implication to neurotransmission and higher brain functions will be discussed.

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Extracellular matrix ensures temporally precise high frequency synaptic transmission at the calyx of Held

Christoph Körber[1], Denise Harrach[1], Thomas Kuner[1]

[1]Heidelberg University, Department of Functional Neuroanatomy, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany

In the mammalian brain, a small fraction of the neurons is surrounded by a special form of extracellular matrix, the so called perineuronal nets (PNNs). PNNs are a complex meshwork mainly composed of hyaluronan as a basic element, tenascin-R and chondroitin sulfate proteoglycans (CSPGs) that bind to hyaluronan and consist of glycosaminoglycan side chains (GAGs) bound to a core protein. Most of the neurons described so far that bear PNNs were also found to express parvalbumin and the Kv3.1 potassium channel subunit, suggesting they are fast-spiking interneurons. However, the physiological function of PNNs in synaptic transmission remains elusive.
In the medial nucleus of the trapezoid body (MNTB) of the auditory brainstem, all principal neurons are surrounded by PNNs. MNTB principal neurons receive their main excitatory input from a single calyx of Held synapse, a giant axo-somatic synapse that comprises 300-700 individual active zones and has evolved as a model system for synaptic transmission in recent years. We removed PNNs from MNTB neurons by chondroitinase (ChABC) treatment and examined the effects on synaptic transmission. ChABC treatment led to faster synaptic short-term depression (STD). However, this effect was prevented by addition of cyclothiazide and kynurenic acid to the extracellular solution. These results are suggestive of a role for PNNs in the effective clearing of glutamate from the synaptic cleft in order to prevent postsynaptic glutamate receptors from desensitization and/or saturation. We thus propose that PNNs are necessary for ensuring fast glutamate clearance during high frequency firing thereby maintaining high fidelity signal transmission.

How do glycinergic synapses transmit in the absence of the glycine transporter GlyT2?

Catharina Kurz[1], Sina Elena Brill[1], Dennis Julian Weingarten[2], Eckhard Friauf[1]

[1]TU Kaiserslautern, AG Tierphysiologie, Erwin-Schrödinger-Str. 13, 67663 Kaiserslautern, Germany
[2]Vollum Institute, Oregon Health & Science University, 3181 SW Sam Jackson Park Rd, Portland, OR 97239, USA

Glycine is the major inhibitory neurotransmitter in the spinal cord and the brainstem. Reuptake of released glycine from the synaptic cleft into the presynaptic neuron is achieved by the neuronal glycine transporter 2 (GlyT2). Here we assessed the role of GlyT2 in the auditory brainstem, where synaptic transmission is reliable and relatively indefatigable even during sustained high-frequency activation (Krächan/Fischer et al, 2017 J Physiol). In particular, the glycinergic synapses between the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO) are extraordinarily resilient. To investigate the role of GlyT2 at these MNTB-LSO inputs, we performed whole-cell patch-clamp recordings in brainstem slices of mice lacking GlyT2 (KO) and wild-type mice (WT) at postnatal day 11 ± 1. After having placed a stimulation electrode in the fiber tract from the MNTB, we gradually increased the stimulation intensity and determined the number of MNTB fibers converging onto single LSO neurons and the fiber strength. To do so, we determined amplitude changes in evoked inhibitory postsynaptic currents (eIPSCs). A single WT neuron received an average of 4 fibers, with a mean strength of 300 pA. The input number in KOs was reduced 4-fold (1 fiber) and the single fiber strength was 6 fold lower (50 pA), implying a massively disturbed microcircuit. We characterized several synaptic parameters with maximal fiber stimulation at 50 Hz. The readily releasable pool in KOs was 12-fold lower (20 vs 240 vesicles), but the release probability was 2-fold higher (26 vs 13%). By introducing interstimulus intervals of 30 ms-10 s after 50-Hz stimulation bursts for 2 s, we assessed eIPSC recovery from short-term depression. Preliminary results indicate that recovery in KOs can be described with a single exponential, while WTs exhibit a double exponential recovery. In addition, weighted τ was 2-fold slower (5.5 vs 2.5 s). Interestingly, at gaps of 10 s, eIPSCs of KO synapses showed an overshoot to 120% compared to the 1st eIPSC, which was not present at WT synapses. This could be due to changes in vesicle recruitment or pool sizes. In a separate set of experiments, we investigated spontaneous IPSPs (sIPSCs).The sIPSC frequency was reduced 4-fold in KOs (2 vs 8 s^{-1}) and sIPSC amplitudes were decreased 1.5-fold (60 vs 100 pA). Quantal size did not differ between WTs and KOs (52 vs 51 pA). Preliminary data indicate an increased sIPSC decay time in KOs, yet an unchanged rise time, implying a change in receptor kinetics. In addition, the reduced readily releasable pool size and the slowed recovery from depression point to disturbed neurotransmitter delivery. Possible glycine sources for the slowly replenished vesicles in KOs include de novo synthesis, large vesicle stores, or an unknown reuptake mechanism. In conclusion, GlyT2 is essential for proper synapse formation and reliable high-frequency transmission of MNTB-LSO synapses as a means to ensure neurotransmitter recycling and subsequent vesicle replenishment.

Neuronal calcium homeostasis: variations in an evolutionarily conserved molecular interplay between Neuroplastin/ Basigin and PMCAs.

Xiao Lin[1], Karl-Heinz Smalla[1], Thilo Kähne[2], Lennart Junge[1], Constanze Seidenbecher[1], Dirk Montag[1], Eckart Gundelfinger[1], Rodrigo Herrera-Molina[1], Ulrich Thomas[1]

[1]Leibniz Institute for Neurobiology, Neurochemistry and Molecular Biology, Brenneckestr. 6, 39118 Magdeburg, Germany

[2]Otto von Guericke University, Institute for Biochemistry and Cell Biology, Leipziger Strasse 44, 39120 Magdeburg, Germany

Single nucleotide polymorphisms that correlate with altered expression of Neuroplastin, a single pass transmembrane glycoprotein of the Immunoglobulin superfamily, have been linked to intellectual performance and schizophrenia in humans. Elimination of Neuroplastin expression causes synaptic deficits and impaired learning and memory in mice (Bhattacharya et al., 2017). To unravel molecular mechanisms underlying these phenotypes we have approached the synaptic interactome of Neuroplastin by using mass spectrometry. This led us to identify plasma membrane calcium ATPases (PMCAs) as prominent binding partners (Korthals et al., 2017; Herrera-Molina et al., 2017; see also Schmidt et al., 2017; Gong et al., 2018). Structure-function analyses in cell lines and primary neurons point to a pivotal role of the transmembrane domain of Neuroplastin in this interplay and in the regulation of calcium homeostasis. Moreover, the interaction is evolutionarily conserved between mammals and *Drosophila*, where reduction of the Neuroplastin homolog Basigin entails a 1:1 reduction of PMCAs at neuromuscular junctions, causing severe phenotypes in the pre- and postsynaptic compartments. In Neuroplastin-deficient mouse brains, however, levels of the four PMCA paralogs are differentially affected with little reduction in PMCA2 contrasting with significant reduction of PMCA1 and 3 and almost complete loss of PMCA4. The reason for this variation and its functional impact on different types of neurons remains currently unclear, but redundancy may be relevant here. In fact, a robust upregulation of Basigin is observed in the absence of Neuroplastin. We therefore currently assess how Basigin, can stabilize PMCAs in a splice isoform- and/or cell type-specific manner.

Examining the role of Complexins in adaptation processes at photoreceptor ribbon synapses

Uwe Thorsten Lux[1], Andreas Gießl[2], Katharina Pieger[1], Karsten Boldt[3], Kerstin Reim[4], Johann Helmut Brandstätter[1]

[1]Animal Physiology, Department of Biology, FAU Erlangen-Nürnberg, 91058 Erlangen, Germany
[2]Department of Ophthalmology, University Hospital Erlangen, 91054 Erlangen, Germany
[3]Institute for Ophthalmic Research, Core Facility for Medical Bioanalytics, University of Tübingen, 72076 Tübingen, Germany
[4]Department of Molecular Neurobiology, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany

Aim: Complexins (Cplxs) are small soluble proteins that bind to the assembled SNARE complex and are essential for the regulated release of neurotransmitter at chemical synapses. The two structurally unique Cplxs 3 and 4 are the only Cplxs present at the highly specialized retinal ribbon synapses (Reim et al., 2005). The results of our recent study suggest that in addition to their role in synaptic vesicle exocytosis, the Cplxs 3 and 4 may also be involved in light adaptation processes at photoreceptor ribbon synapses (Babai et al., 2016). Here we started to analyze the interactome of the Cplxs 3 and 4 to shed light on their putative function in light adaptation.
Methods: Tandem affinity purification (TAP) tag screen with the Cplxs 3 and 4 as baits and analysis of putative interaction partners with immunocytochemistry, bimolecular fluorescence complementation assay (BiFC) and *in situ* proximity ligation assay (PLA) for light-dependent interaction.
Results: In our TAP tag screen, we found several G-Protein subunits as putative interactors of the two Cplxs 3 and 4. Amongst them the G-protein subunit beta 1 (GNB1), also known as Transducin beta chain 1. Immunofluorescence stainings in mouse retinae showed that after light adaptation the three rod photoreceptor Transducin subunits α, β and γ translocated from the outer segments to the synaptic terminals of the photoreceptors where they co-localized with Cplx 4. Strong PLA signals in the photoreceptor synaptic layer indicate a putative *in vivo* interaction of Cplx 4 and the rod photoreceptor Transducin subunits in the light-adapted retina. Furthermore, in BiFC-Experiments we were able to demonstrate an interaction between the γ-subunit of rod photoreceptor Transducin and the Cplxs 3 and 4.
Conclusions: From these data, we propose that the putative action of the Cplxs 3 and 4 in light adaptation processes at photoreceptor ribbon synapses may be regulated by Transducin.

Resolving the Ultrastructural Organization of Synaptic Vesicle Pools at Hippocampal Mossy Fiber and Schaffer Collateral Synapses

Lydia S. B. Maus[1], Bekir Altas[1,2], Jeong-Seop Rhee[1], Nils Brose[1], Cordelia Imig[1], Benjamin H. Cooper[1]

[1]Max Planck Institute for Experimental Medicine, Molecular Neurobiology, Hermann-Rein-Str. 3, 37075 Goettingen, Germany
[2]Department of Pharmacology, University of Maryland School of Medicine, 670 West Baltimore St., Baltimore, MD 21201, USA

Synaptic neurotransmission occurs with high spatiotemporal precision at presynaptic active zones (AZs) to rapidly propagate information between neurons in the CNS. Molecular components at the AZ are involved in docking and priming synaptic vesicles (SVs) at the presynaptic membrane to generate a pool of fusion-competent, readily releasable (RRP) vesicles that can rapidly fuse and release their contents into the synaptic cleft upon calcium influx. Despite similarities in the composition of the molecular release machinery at AZ release sites, synapses can exhibit strikingly different morphological and functional properties. Paradigmatic and well-characterized examples of this type of functional heterogeneity are the Schaffer collateral (SC) and mossy fiber (MF) synapses of the mammalian hippocampus. Although both synapse types use glutamate as their neurotransmitter, they differ drastically in their ultrastructural architecture, transmitter release properties, short- and long-term plasticity characteristics, and in the mechanisms that modulate synaptic efficacy. Despite being functionally well characterized, MF ultrastructure has not previously been scrutinized at a level of resolution permitting the accurate discrimination of morphologically and functionally distinct SV pools at AZ release sites. Consequently, the extent to which such differences in structural organization contribute to or reflect distinct functional properties of SC and MF synapses remains unclear.

We have addressed this by using a combination of hippocampal organotypic slice culture, high-pressure freezing, freeze substitution, and 3D-electron tomography to preserve synaptic ultrastructure in a near-native state and resolve the spatial organization of SV pools with nanoscale precision. This experimental approach, which permits a direct comparative analysis of SC and MF synapses in the same organotypic slice, revealed that at 14 days *in vitro* MF synapses harbored fewer morphologically docked SVs per AZ area than SC synapses. However, in more mature slices frozen at 28 days *in vitro*, the spatial density of docked SVs at MF and SC synapses was highly comparable indicating that the different transmitter release characteristics of these two synapse types are likely not due to docked SV availability. Consistent with our previous work on SC synapses, we found the number of morphologically docked SVs in MF synapses to be in close agreement with RRP estimates from electrophysiological studies. Interestingly, we found that MF synapses exhibit considerable heterogeneity in docked SV size and that they possess a distinct pool of non-docked but possibly tethered membrane proximal SVs that may play a role in rapidly refilling SV release sites during sustained activity, thereby contributing to MF short-term facilitation properties. Our analysis also revealed that in contrast to SC synapses, MFs frequently harbored dense-core vesicles (DCVs) docked at AZ release sites, indicating that DCVs may fuse at AZs in this synapse type. Both SV and DCV docking was completely abolished in Munc13-deficient MF synapses, demonstrating that both vesicle types require Munc13-priming factors for fusion at AZ release sites. Our data provides novel insight into how differences in the ultrastructural architecture of MF and SC synapses at individual presynaptic AZs could contribute to their distinct functional properties and corresponding short- and long-term plasticity characteristics.

Nogo-A signaling modulates synaptic transmission at a fast time scale

Kristin Metzdorf[1], Steffen Fricke[1], Stefan Haak[1], Martin Korte[1], Marta Zagrebelsky[1]

[1] Zoological institute, Div. Cellular Neurobiology, TU Braunschweig, Germany

In the mature brain a certain degree of stability underlies the proper function of the neuronal network and is required for the long-term storage of information. On the other hand, learning and memory processes relay on changes in the morphology and strength of synaptic connections in response to neuronal activity (plasticity). Among the molecules crucial for maintaining the balance between the plasticity and stability of synaptic contacts the neurite growth inhibitor Nogo-A has been identified as an important molecule promoting stability. Indeed, in the adult hippocampus Nogo-A restricts both functional and structural plasticity signaling via two inhibitory domains, Nogo66 and Nogo-delta20 binding to the Nogo 66 receptor 1 (NgR1) and the sphingosine 1-phosphate receptor 2 (S1PR2) respectively. However, the cellular and signaling processes mediating this function of Nogo-A are still largely unknown.

Here we show that a Nogo-A loss-of-function approach in primary hippocampal neurons results within minutes in a significant increase in the amplitude of calcium transients. Interestingly, while blocking the S1PR2 results reproduces the increase in the amplitude of the calcium influx, no changes were observed after blocking the NgR1 suggesting a Nogo-delta20/S1PR2 specific effect. Furthermore, we show that Nogo-A neutralization strengthen excitatory while restricting inhibitory synaptic transmission. Interestingly, calcium signaling has been shown to affect $GABA_A$ receptor lateral movement. In line with this, blocking Nogo-A signaling via the S1PR2 signaling pathway, decreases surface $GABA_A$ receptors by increasing their lateral motility.

Taken together our results describe a new function of Nogo-A signaling in acutely modulating the strength of synaptic transmission at a fast time scale.

Do different Complexin isoforms act upon different SNARE complex types?

Jutta Meyer[1,2], Olaf Jahn[3], Nils Brose[1], Johann Helmut Brandstätter[4], Kerstin Reim[1]

[1] Department of Molecular Neurobiology, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany
[2] Göttingen Graduate School for Neurosciences, Biophysics and Molecular Biosciences, University of Göttingen, Germany
[3] Proteomics Group, Max Planck Institute of Experimental Medicine, 37075 Göttingen, Germany
[4] Animal Physiology, Department of Biology, FAU Erlangen-Nürnberg, 91058 Erlangen, Germany

SNARE-proteins are the key elements of the complex multi-step process that controls membrane fusion in eukaryotic cells. At neuronal synapses, SNARE function is regulated by multiple auxiliary proteins to achieve the high speed and spatial precision of synaptic vesicle fusion. Of particular importance among these SNARE regulators are the Complexins (Cplxs), which were originally identified as stoichiometric components of the exocytotic SNARE complex formed by Syntaxin-1, SNAP25 and Synaptobrevin-2. In mammals, Cplxs form a family of four isoforms (Cplx1-Cplx4). Cplx1 and Cplx2 are expressed in almost all neuron types of the brain, while Cplx3 and Cplx4 are preferentially expressed in retinal photoreceptors and bipolar cells. All four Cplxs share a short (~30 aa) conserved central α-helix that is necessary for SNARE complex binding of Cplx1. The high degree of conservation in this central α-helix suggests that probably all Cplxs exert their function via an interaction with SNARE complexes, raising the question whether different Cplxs act upon different SNARE complex types. To address this aspect we developed an affinity purification approach with short Cplx-derived peptides covering the central α-helical SNARE-binding domain, based on the observation that such peptides retain the high affinity of Cplx holoproteins to fully assembled SNARE complexes. In initial experiments, we incubated immobilized Cplx peptides with synaptosomal fractions of mouse cortices. By quantitative mass spectrometry (MS) numerous proteins were detected, which did not show any preference for a specific Cplx isoform. However, we also found several proteins that appeared to be specifically enriched by one Cplx peptide. From these results we concluded that the Cplx peptides are suitable tools to gain quantitative insight into the protein network that the SNARE fusion machinery is embedded in. Building on this experience, we next applied this experimental workflow to analyze the Cplx-SNARE interactome of the retina. In this approach, eluted proteins were first analyzed by immunodetection of the canonical SNARE proteins to show that our method is capable of enriching SNARE complex constituents from homogenates of mouse retina. To further proof the principle of our approach and to identify retina-specific molecules interacting directly with Cplx or indirectly via bound SNARE complexes, quantitative MS was performed. Interestingly, we found many proteins of the phototransduction cascade, including transducin beta chain 1 and other subunits, which corroborates the results from an independent TAP tag screen (Brandstätter lab Erlangen). Our results demonstrate, that it will be possible with our approach to address retina-specific questions, e.g. as to whether and which alternative SNARE complexes exist in ribbon synapses and how Cplxs contribute to their unique release efficacy. Moreover, our approach is a powerful tool to complement other screening methods as TAP tag or Y2H strategy to identify Cplx effectors and interactors.

Synaptic elimination and strengthening uncoupled: the impact of central L-type voltage-gated Ca^{2+} channels on circuit refinement of a sound source localization pathway

Nicolas Müller[1], Eckhard Friauf[1]

[1]Animal Physiology Group, Department of Biology, University of Kaiserslautern, Germany

Circuit refinement by synaptic elimination and strengthening is crucial for proper functioning of sensory circuits. The glycinergic auditory projection from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) is involved in sound source localization and is functionally refined between postnatal days (P)5-9 in mice. Initially, supernumerous MNTB fibers innervate postsynaptic LSO neurons in a topographically imprecise manner. Subsequently, activity-dependent refinement yields elimination of supernumerous fibers, resulting in precise topography. Remaining MNTB fibers are developmentally strengthened due to addition of release sites to MNTB fibers and an increased quantal size. Changes in the number of release site are associated with a presynaptic locus due to their location on presynaptic terminals, while changes in the quantal size are mainly associated with a postsynaptic locus due to changes in the number of postsynaptic receptors. While the impact of peripheral spontaneous activity on auditory circuit refinement becomes increasingly clear, virtually nothing is known about on-site molecules involved in MNTB-LSO refinement. Here, we investigated the impact of on-site Ca^{2+} channel signaling on MNTB-LSO circuit refinement. For this purpose, we employed mice with a brainstem-specific lack of L-type voltage gated calcium channels 1.2 ($Ca_V1.2$ cKO) or 1.3 ($Ca_V1.3$ cKO). To address synaptic elimination and strengthening, we recorded from LSO neurons at P10-12 in acute brainstem slices and gradually recruited MNTB fibers by electrical stimulation with stepwise increasing stimulation intensities. This allowed us to quantify the number of converging MNTB fibers and the single fiber strength. In both $Ca_V1.2$ and $Ca_V1.3$ cKOs, we found a normal number of MNTB fibers, providing evidence for normal synaptic elimination. MNTB single fiber strength was also normal in $Ca_V1.2$ cKOs, but reduced by ~30% in $Ca_V1.3$ cKOs. The reduction in synaptic strength may be due to a presynaptic locus and/or a postsynaptic locus. The number of released vesicles (quantal content), the release probability and the number of release sites was normal in $Ca_V1.3$ cKOs, arguing against a presynaptic locus. The quantal size was reduced by ~30%, implying that the reduced synaptic strength is manifested postsynaptically. Since elimination and strengthening overlap during development and impairments always affected both processes, it appeared that elimination and strengthening have a common molecular mechanism at the MNTB-LSO projection. Surprisingly however, we found evidence that elimination and strengthening are uncoupled processes. In summary, we show that on-site calcium signaling of neither $Ca_V1.2$ nor $Ca_V1.3$ have impact on synaptic elimination, while on-site $Ca_V1.3$ contributes to synaptic strengthening.

Rapid modulation of transsynaptically aligned glutamate receptor nanocluster rings during homeostatic plasticity

Paola Muttathukunnel[1,2], Martin Müller[1,2]

[1]University of Zurich, Institute of Molecular Life Sciences, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[2]Neuroscience Center Zurich

Subtle changes in the organization of synaptic proteins may have profound effects on synaptic transmission and animal behavior. The *Drosophila* neuromuscular junction (NMJ) has emerged as a powerful model system to dissect the sub-synaptic molecular architecture of presynaptic active zones using super-resolution light microscopy approaches. However, little is known about a corresponding postsynaptic organization and its relationship to presynaptic architecture. Using stimulated emission depletion microscopy, we here uncovered that postsynaptic glutamate receptors (GluRs) are organized in ring-like arrays composed of ~6 sub-diffraction GluR 'nanoclusters' at the *Drosophila* NMJ. Interestingly, postsynaptic GluR nanocluster rings aligned with rings formed by the C-termini of the presynaptic cytomatrix protein Bruchpilot (Brp), suggesting transsynaptic co-alignment. Genetic perturbation of the auxiliary GluR subunit neto or the adaptor protein ankyrin resulted in more pronounced or less distinct receptor rings, respectively. Specifically, we detected a predominant decrease in GluR fluorescence intensity outside the rings in *neto*^{109} mutants and a slight decrease in GluR cluster number within the rings. Conversely, postsynaptic *ankyrin* RNAi expression led to an increase in GluR nanocluster number that masked the GluR rings. Increased GluR fluorescence intensity towards the ring perimeter after *ankyrin* knock down indicates that the clusters are mainly added outside of the rings. Interestingly, we also observed a significant increase in Brp-ring diameter upon postsynaptic *ankyrin* RNAi expression. Finally, we revealed rapid modulation of transsynaptically aligned Brp-GluR rings during homeostatic plasticity induced by GluR perturbation. Application of the GluR antagonist philanthotoxin-433 (PhTX) for 30 minutes resulted in a pronounced, scaled increase of GluR fluorescence intensity within the nanoring. Moreover, PhTX treatment induced a significant increase in receptor cluster number within the ring. Additionally, we revealed a slight, but significant increase in both Brp-fluorescence intensity and Brp-cluster number without marked differences in Brp-ring diameter. Together, our findings provide evidence for transsynaptic nanomodule rings that undergo rapid changes during synaptic plasticity.

Presynaptic $GABA_A$ Receptors Modulate Glutamatergic Transmission at the Endbulb of Held

Jana Nerlich[1], Stefan Hallermann [1], Ivan Milenkovic[1]

[1]Carl Ludwig Institute for Physiology, Faculty of Medicine, University of Leipzig, Germany

Spherical bushy cells (SBCs) in the ventral cochlear nucleus integrate acoustically driven excitatory input from the auditory nerve with non-primary glycinergic and GABAergic inhibitory inputs to precisely encode the temporal structure of sounds. The inhibitory conductance shows an activity-dependent build-up with high-pass filter properties for large and well-timed excitatory inputs. GABA increases the overall inhibitory strength particularly during ongoing synaptic activity when GlyR currents undergo depression, and provides slow, tonic-like inhibition. However, it is not known whether the functional role of GABA may extend beyond the SBC inhibition by modulating the endbulb of Held terminal through presynaptic GABAAR. To examine whether the endbulb of Held expresses functional $GABA_AR$ and GlyR, whole cell recordings from the terminals were performed in acute brainstem slices from P13-15 gerbils. Puff-application of GABA (10 ms) evoked a prominent chloride conductance at the endbulb while glycine had no effect. Gramicidin perforated patch recordings revealed a depolarizing chloride gradient within the terminal (E_{Cl}=-28mV; estimated intraterminal $[Cl^-]$=40 mM). Activation of $GABA_AR$ decreased the amplitude, prolonged the rise time and shortened the falling time of presynaptic APs evoked by electrical stimulation of auditory nerve fibers. The magnitude of this effect depended on the depolarization level. The functional role of $GABA_AR$ at the endbulb was assessed with whole cell recordings from SBCs upon stimulation of AN fibers. To segregate the effects of pre- and postsynaptic $GABA_AR$, each SBC was held at the experimentally-determined reversal potential for $GABA_AR$ ($V_{hold}=E_{GABA}$). This enabled to record isolated EPSCs while GABA-puff elicited a membrane current only in the endbulb terminal. All recordings were done under 3µM CGP55845 to block $GABA_BR$. Brief application of GABA transiently reduced EPSC amplitudes and this effect was blocked by the $GABA_AR$ antagonist SR95531. Such reduction of glutamate release also persists at P25 endbulbs, suggesting that the modulation is not constrained to immature synapses. Puff application of glycine did not affect EPSCs, consistent with the lack of GlyR at the endbulb terminal. The present results suggest that $GABA_AR$ can modulate the strength of glutamatergic transmission at the endbulb of Held-SBC synapse.

Developmental easing of short-term depression in ‘winner’ climbing fibers

Christina Paetz[1], Simone Brachtendorf[1], Jens Eilers[1]

[1]Carl-Ludwig-Institute for Physiology, Department II - Neurophysiology, Liebigstr. 27, 04103 Leipzig, Germany

Cerebellar climbing fibers (CFs) undergo a substantial pruning during the first three postnatal weeks. As a result, the innervation of Purkinje cells, their main targets in the cerebellum, switches from multiple- to single-CF innervation. The associated strengthening of the remaining 'winner' CF is thought to be guided by long-term potentiation (LTP) (Bosman et al., 2008; Ohtsuki and Hirano, 2008). In contrast, 'loser' CFs were proposed to be weakened by presynaptic long-term depression (LTD), ultimately leading to their elimination (Ohtsuki and Hirano, 2008). It remained unclear whether LTP of winner CFs is pre- (Ohtsuki and Hirano, 2008) or postsynaptically (Bosman et al., 2008) expressed and whether corresponding changes in paired-pulse depression (PPD) occur in the pruning period: an increase of PPD would accompany presynaptic LTP, while postsynaptic LTP would not affect PPD. We, therefore, analyzed the developmental profile of CF-PPD in the first three weeks after birth [postnatal (p) day 3-21], dividing the animals into three age groups p3-p8, p9-p12, and p13-p21.
Igsf9-eGFP mice, which allow visually-guided stimulation of GFP-labeled CFs (Pätz et al., 2018), were anesthetized by inhalation of isoflurane. Slice preparation and paired-pulse experiments were performed as described previously (Pätz et al., 2018). Excitatory postsynaptic currents (EPSCs) evoked by CFs were classified as originating from winner CFs when their peak amplitude exceeded -360 pA (at a holding potential of -75 mV) or from loser CFs otherwise. We found a developmental easing of PPD in winner but no change in loser CFs. Winner CFs showed PPD values of 0.15±0.012 at p3-p8 (n=19), 0.21±0.017 at p9-p12 (n=47), and 0.34±0.02 at P13-p21 (n=61), the latter reaching statistical significance compared to both other age groups ($P \leq 0.001$***, Kruskal-Wallis test with $P<0.05$ for all pairwise comparisons, Dunn's test). The easing was well described by a Hill equation with start and end PPD values of 0.12 and 0.36, respectively, the inflexion point at p12 and a Hill coefficient of 5.36. Loser CFs, which were only found at sufficient numbers up to p12, showed PPD values of 0.270±0.069 at p3-p8 (n=14), 0.21±0.014 at p9-p12 (n=19) and 0.32±0.049 at p13-p21 (n=6).
Taken together, our data unexpectedly reveal that maturation of winner CFs is associated with an easing of PPD, denoting a decrease in the release probability and that PPD in loser CFs remains unchanged. Thus, neither pre- nor postsynaptic LTP appears to persistently contribute to the maturation of winner CFs, nor presynaptic LTD to elimination of loser CFs. We propose a simpler scenario in which synapses on dendrites (harboring the growing winner CF) and somata (harboring loser CFs and to-be-disintegrated synaptic contacts of the winner CF) are characterized by different release probabilities.

Phosphoinoside-dependent regulation of GABAergic Neurotransmission at inhibitory Postsynapses

Theofilos Papadopoulos[1]

[1]Universitätsmedizin Göttingen, Molecular Biology, Humboldtallee 23, 37073 Göttingen, Germany

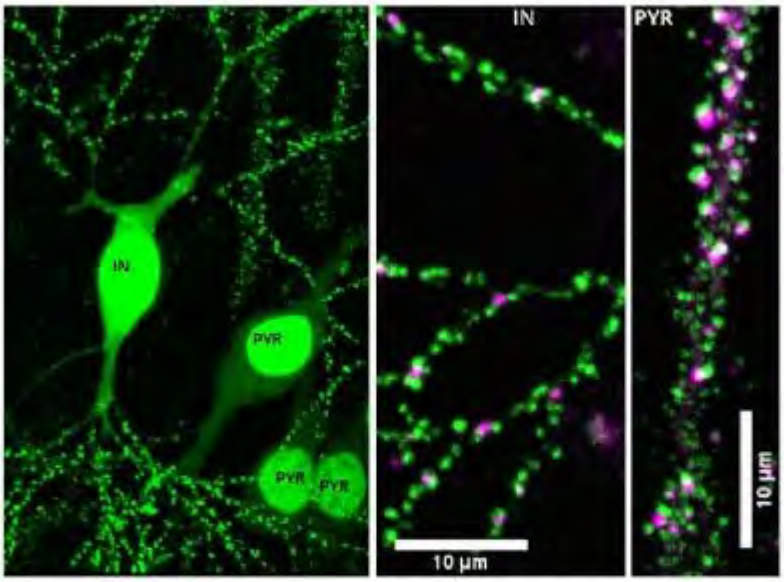
The formation of neuronal synapses and the dynamic regulation of their efficacy depend on the proper assembly of the postsynaptic neurotransmitter receptor apparatus. Receptor recruitment to inhibitory GABAergic postsynapses requires the scaffold protein gephyrin and the guanine nucleotide exchange factor collybistin (Cb). In vitro, the pleckstrin homology domain of Cb binds phosphoinositides, particularly phosphatidylinositol 3-phosphate (PI3P). In this work, we used a membrane-permeant PI3P derivative, time-lapse confocal imaging, electrophysiology, as well as knockdown and overexpression of PI3P-metabolizing enzymes in neurons. We provide the first in cellula evidence that PI3P located at early/ sorting endosomes regulates the postsynaptic clustering of gephyrin and GABAA receptors and the strength of inhibitory postsynapses. Our results show that an endosomal pool of PI3P, generated by the class III phosphatidylinositol 3-kinase, is important for the Cb-mediated recruitment of gephyrin and GABAA receptors to developing inhibitory postsynapses and thus the formation of postsynaptic membrane specializations. Furthermore, we provide first evidence that small Rho-like GTPases, particularly TC10, modulate the phosphoinositide-specificity of Cb and suggest a new model for the role of the TC10/Cb-interaction in the assembly of the gephyrin scaffold at postsynaptic membranes.

SynTagMA: a new optogenetic tool to map active synapses

Alberto Perez-Alvarez[1], Brenna C Fearey[1], Ryan OToole[2], Ignacio Arganda-Carreras[3], Eric R Schreiter[4], J Simon Wiegert[1], Christian Schulze[1], Michael B Hoppa[2], Christine E Gee[1], Thomas G Oertner[1]

[1]Institute for Synaptic Physiology, ZMNH, University-Medical Center Hamburg-Eppendorf, Germany
[2]Dept. of Biological Sciences, Darmouth College, Hanover, NH, USA
[3]Ikerbasque, Basque Foundation for Science, Bilbao, Spain
[4]Janelia Research Campus, HHMI, Ashburn, VA, USA

Excitatory synapses show remarkable differences in plastic properties and organelle machinery. In the hippocampus, only a small set of dendritic spines contain a specialized endoplasmic reticulum (ER) structure, the spine apparatus (SA), and show mGluR-dependent long term depression. In order to study a potential link between the SA and synaptic activity, we developed a method to reliably monitor all active connections over time. Laser scanning microscopes allow monitoring synaptic activity with high spatial and temporal resolution (i.e. calcium imaging) but are limited to the simultaneous recording of one or few synapses. To tackle this impediment, we employed CaMPARI, a fluorescent genetically encoded calcium integrator that irreversibly photoconverts from green to red in presence of high calcium concentrations upon violet light (PC light) illumination. We have targeted it to either presynaptic boutons or postsynaptic densities to develop **SynTagMA^{pre}** or **SynTagMA^{post}** (**Syn**aptic **Tag** for **M**apping **A**ctivity). For the SynTagMA^{post} variants, we found that negative feedback control of expression was necessary to achieve selective postsynaptic targeting (see also BC Fearey et al.). Upon pairing extracellular synaptic stimulation with PC light, the SynTagMA^{post} signal photoconverted in a subset of highly active synapses (see C Schulze et al. for analysis method). Therefore, using this approach we 'freeze' the state of the synapses in the few seconds immediately before the photoconversion. On the other hand, SynTagMA^{post} completely turns over within 2 hours. This feature will help us map repeatedly the active synapses and also follow changes in the pattern of functional connections. We expect that this will help us find an explanation to why most spines are only transiently visited by the ER. In summary, SynTagMA will enable us to simultaneously study across thousands of synapses in living tissue, the relationship between synaptic calcium transients, the spine apparatus and intracellular calcium stores (figure).



Green: SynTagMA
Magenta: Spine apparatus (synaptopodin-mCerulean)
IN: Interneuron
PYR: Pyramidal neuron

Synaptogenesis depends on axonal transport via lysosome-related vesicles

Astrid G. Petzoldt[1,3], Anela Vukoja[1,2], Ulises Rey[1,3], Christoph Ott[1,2], Dymtro Puchkov[1,2], Eric Reynolds[1,3], Martin Lehmann[1,2], Stephan J. Sigrist[1,3], Volker Haucke[1,2]

[1]Freie Universität Berlin, Institut für Biologie, Takustr. 6, 14195 Berlin, Germany
[2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), 13125 Berlin, Germany
[3]NeuroCure Cluster of Excellence, Charité Universitätsmedizin Berlin, 10117 Berlin, Germany

Neuronal function relies on the construction and maintenance of synapses, established by a continuous transport of synaptic material from the soma through the axon to the synaptic terminal. To date, the biological origin of the transporting synaptic precursor organelles have not been fully revealed. Here we show by live imaging in Drosophila larvae that both synaptic vesicle (SV) and active zone (AZ) proteins co-traffic with lysosomal markers and furthermore, that loss of the small GTPase Arl8, a lysosomal adaptor connectin lysosomes to kinesin motors, results in a decreased of synaptic proteins at the synaptic terminal and an accumulation of transport vesicles in the neuronal soma ([1], [2], [3]). These findings imply that SV as well as AZ proteins are cargos of presynaptic lysosome-related vesicles (PLVs), which ate distinct from derivative mature lysosomes and responsible anterograde delivery of synaptic material for presynaptic biogenesis. This hypothesis is strengthened by the finding that genetic upregulation of Arl8 causes an increase in neurotransmission combined with increased AZ protein levels at the synaptic terminal. In summary, lysosome related organelles might constitute a basic component of presynaptic transport vesicles and thus could connect the similar phenotypes of neuronal defects and lysosomal dysfunction in human diseases.

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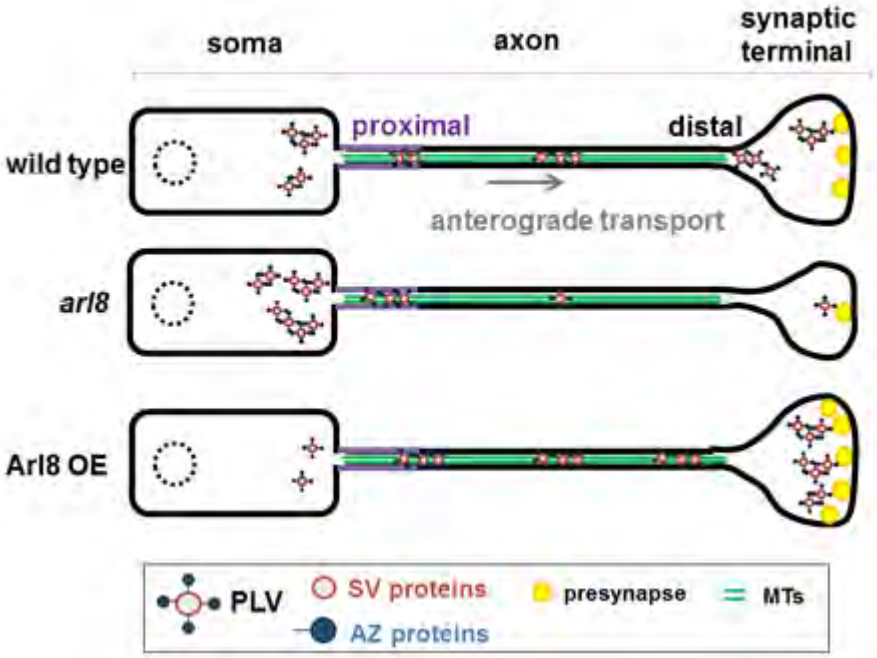
soma
axon
synaptic terminal
wild type
proximal
distal
anterograde transport
arl8
Arl8 OE
PLV
SV proteins
AZ proteins
presynapse
MTs

A role for Piccolo in the regulation of neurotransmitter release and presynaptic plasticity.

Eneko Pina[1,2], Carolina Montenegro-Venegas[1], Yulia Klyueva[2], Daria Davydova[1], Claudia Marini[1], Eckart D. Gundelfinger[1], Anna Fejtova[2,3]

[1]Leibniz Institute Neurobiology Magdeburg, Neurochemistry and MolecularBiology Department,brenneckstraße, 6, 39118 Magdeburg, Germany. [2]RG Presynaptic Plasticity. [3]Molecular Psychiatry, Dept. of Psychiatry and Psychotherapy, FAU Erlangen-Nürnberg, University Hospital Erlangen, Germany. brenneckstraße, 6, 39118 Magdeburg, Germany.

The active zone (AZ) is the region of the presynaptic membrane where the neurotransmitter release takes place. This region is characterized by a dense meshwork of proteins called the cytomatrix at the active zone (CAZ). Proteins of the CAZ define the release sites, localize voltage-gated calcium channels (VGCC) within the presynaptic membrane and coordinate the exo- endocytotic events during synaptic vesicle cycle to achieve fast and reliable neurotransmission. Bassoon (Bsn) and Piccolo (Pclo) are highly homologous large CAZ proteins implicated in these processes. We demonstrated the importance of Bsn as a specific regulator for the localization of the Cav2.1 to the plasma membrane via molecular interaction with the RIM-binding proteins (RBPs) (Davydova et al., 2014). The role of Pclo in the recruitment and localization of VDCC is still unclear. We have used Pclo mutant mice, in which the exon 14 was deleted and the expressed Pclo levels were decreased to <5% in comparison with WT (Mukherjee et al., 2010). Using whole-cell voltage clamp recordings of mature hippocampal WT and Pclo mutant neurons, we observed that the absence of Pclo alters the AMPA-mediated miniature excitatory postsynaptic currents (mEPSC). Furthermore, we have identified, by in vitro experiments, RBP2 as a binding partner of Pclo, which could potentially function as a link to VGCC. Functional analysis showed that, while the VGCC recruitment and contribution to synaptic transmission are not affected in hippocampal synapses of Pclo mutant neurons, the SV recycling is impaired. Further, we observed Pclo is indispensable for normal presynaptic scaling in response to global neuronal network activity silencing. Pclo is necessary for the inactivity-induced regulation of SV recycling, and it is not for the inactivity-induced changes of VGCC-mediated presynaptic Ca2+ influx. Taken together, our data suggest a new role for Pclo in the regulation of SV recycling and homeostatic presynaptic scaling.

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Myosin XVI is a regulator of actin cytoskeleton dynamics in dendritic spines of Purkinje cells

Mona Katrin Roesler[1], Franco Lombino[1], Michaela Schweizer[2], Irm Hermans-Borgmeyer[3], Matthias Kneussel[1], Wolfgang Wagner[1]

[1]Center for Molecular Neurobiology, Department for Molecular Neurogenetics, Falkenried 94, D-20251 Hamburg, Germany

[2]Center for Molecular Neurobiology, Morphology Core Facility, Falkenried 94, D-20251 Hamburg, Germany

[3]Center for Molecular Neurobiology, Transgenic Animal Unit, Falkenried 94, D-20251 Hamburg, Germany

Regulation of actin cytoskeleton dynamics in dendritic spines is important for synaptic development and function. Moreover, genetic evidence implicates altered regulation of the neuronal actin cytoskeleton in autism spectrum disorder (ASD), a disease characterized by social and communication deficits. *MYO16* is an ASD-linked gene that encodes the heavy chain of the actin-based cytoskeletal motor myosin XVI. Interestingly, the 'WAVE regulatory complex' (WRC) is an identified interaction partner of myosin XVI. The WRC is a known regulator of dendritic spine actin and comprises subunits encoded by ASD-linked genes (*CYFIP1, NCKAP1*). It is unknown, however, whether also myosin XVI plays a role for actin cytoskeleton regulation in the dendritic spines. In mice, *Myo16* was shown to be expressed in cerebellar Purkinje cells, a neuronal cell type important for motor learning, social cognition and vocalization. Alterations of the cerebellum and Purkinje cells have been associated with ASD, and with ASD-like phenotypes in mice. Nevertheless, compared to hippocampal neurons, surprisingly little is known about actin cytoskeleton dynamics in the dendritic spines of Purkinje cells. Here, we address this issue and define myosin XVI as a novel component of the regulatory machinery influencing the actin cytoskeleton at postsynaptic sites. Using CRISPR/Cas9, we generated a *Myo16* knockout mouse. Fluorescence recovery after photobleaching (FRAP) analyses of GFP-actin in cultured Purkinje cells shows that both *Myo16* knockout and Purkinje cell-specific *Myo16* knockdown accelerate the rate of actin filament turnover in spines. Similarly, dominant negative inhibition of the WRC and pharmacological inhibition of its downstream effector Arp2/3 accelerate actin filament turnover in Purkinje cell spines. While Arp2/3 is known to drive the formation of a branched actin meshwork, formins mediate the polymerization of linear filaments. We find that WRC and Arp2/3, but also formin activity is required to maintain the normal size of the dynamic actin filament pool in Purkinje cell spines. Taken together, our data suggest that myosin XVI may act as an activator of the postsynaptic WRC-Arp2/3 pathway in the dendritic spines of Purkinje cells.

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Cognitive impairment and autistic-like behaviour in SAPAP4-deficient mice

Claudia Schob[1], Fabio Morellini[2], Ora Ohana[2], Lidia Bakota[3], Mariya V. Hrynchak[3], Michaela Schweizer[2], Markus Wöhr[4], Karl J. Vörckel[4], Craig C. Garner[5], Hans-Jürgen Kreienkamp[1], Stefan Kindler[1]

[1]University Medical Centre Hamburg-Eppendorf, Institute for Human Genetics, Martinistr. 52, 20251 Hamburg, Germany

[2]University Medical Centre Hamburg-Eppendorf, Centre for Molecular Neurobiology Hamburg, Martinistr. 52, 20251 Hamburg, Germany

[3]University of Osnabrück, Department of Neurobiology, D-49076 Osnabrück, Germany

[4]Philipps-University of Marburg, Behavioral Neuroscience, Experimental and Biological Psychology, Faculty of Psychology, D-35032 Marburg, Germany

[5]German Centre for Neurodegenerative Diseases (DZNE), c/o Charité University Medical Centre, D-10117 Berlin, Germany

In humans, genetic variants of *DLGAP1-4* have been linked with neuropsychiatric conditions, including autism spectrum disorder (ASD). While these findings implicate the encoded postsynaptic proteins, SAPAP1-4, in the etiology of neuropsychiatric conditions, underlying neurobiological mechanisms are unknown. To assess the contribution of SAPAP4 to these disorders, we characterized SAPAP4-deficient mice. Our study reveals that loss of SAPAP4 triggers profound behavioural abnormalities, including cognitive deficits combined with impaired vocal communication and social interaction, phenotypes reminiscent of ASD in humans. These behavioural alterations of SAPAP4-deficient mice are associated with dramatic changes in synapse morphology, function and plasticity, indicating that SAPAP4 is critical for the development of functional neuronal networks and that mutations in the corresponding human gene, *DLGAP4*, may cause deficits in social and cognitive functioning relevant to ASD-like neurodevelopmental disorders.

Building fast and resilient inhibitory synapses with Ca2+ nanodomains and microdomains

Henrique von Gersdorff[1], Dennis J. Weingarten[2], Nicolas Muller[2], Eckhard Friauf[2]

[1]Oregon Health and Science University, Vollum Institute, 3181 SW Sam Jackson Park Road, 97239 Portland, USA

[2]Animal Physiology Group, University of Kaiseslautern, Kaiserslautern, Germany

Excitatory synapses in the auditory brainstem can process sound information with exquisite temporal precision. However, less is known about how inhibitory synapses shape the processing of sound signals and how they develop after hearing onset. We have recently investigated synapses of the lateral superior olive (LSO) in the mouse auditory brainstem. Using whole-cell patch clamp recordings in acute brainstem slices we characterized inputs from the medial nucleus of the trapezoid body (MNTB) and the cochlear nucleus (CN) to principal neurons of the LSO. The MNTB-LSO synapse is glycinergic and CN-LSO synapse is glutamatergic. We used electrical afferent fiber stimulation to elicit EPSCs and IPSCs. Recordings were done at 36°C from pre-hearing mice at postnatal day P10-12 and young adults at P28-34. Using high-frequency stimulation at 50, 100 and 200 Hz the synaptic parameters could be determined. Like the calyx of Held synapse in the MNTB after hearing onset, CN-LSO synapses showed an increase in their number of readily releasable vesicles (RRP). However, MNTB-LSO glycinergic synapses showed a rapid form of short-term depression followed by robust facilitation. Surprisingly, the RRP of MNTB-LSO glycinergic synapses dropped from 600 vesicles at P10-12 to below 300 vesicles at P28-34. To counteract this reduced number of vesicles and rapid synaptic depression, these synapses developed a robust frequency-dependent vesicle replenishment not present in pre-hearing synapses. The slow Ca2+ buffer EGTA and the K+-channel blocker TEA had little effect on the extent of replenishment in young synapses. These immature synapses seem to exhibit active zones with docked vesicles tightly coupled to Ca2+ channels (Ca2+ nanodomain triggered exocytosis). However, mature synapses showed a two-fold higher vesicle replenishment with increased Ca2+ and a drop to ~50% of steady-state IPSC amplitude compared to P10-12 under EGTA. Mature synapses were thus surprisingly sensitive to EGTA. This was in stark contrast to previous findings in the developing calyx of Held synapse. In summary, mature MNTB-LSO glycinergic synapses develop a remarkably fast vesicle replenishment, specialized for faithful and sustained steady-state inhibition during high-frequency activity, using both Ca2+ nanodomain and microdomain mediated exocytosis.

The Presynaptic Protein Mover is Heterogeneously Expressed across Brain Areas and Synapse Types

Rebecca Wallrafen[1], Thomas Dresbach[1]

[1]Institute of Anatomy and Embryology, Synaptogenesis, Kreuzbergring 36, 37075 Göttingen, Germany

The assembly and function of presynaptic nerve terminals relies on evolutionarily conserved proteins. A small number of presynaptic proteins occurs only in vertebrates. These proteins may add specialized functions to certain synapses, thus increasing synaptic heterogeneity.
We show that the vertebrate-specific synaptic vesicle protein Mover is differentially distributed in the forebrain and cerebellum of the adult mouse. Using a quantitative immunofluorescence approach, we compare the expression of Mover to the expression of the general synaptic vesicle marker Synaptophysin in sixteen brain areas. We find that Mover is particularly abundant in the septal nuclei, ventral pallidum, amygdala and hippocampus. Within the hippocampus, Mover is predominantly associated with excitatory synapses. Its levels are low in layers that receive afferent input from the entorhinal cortex, and high in layers harboring intra-hippocampal circuits. In contrast, Mover levels are high in all nuclei of the amygdala, and Mover is associated with inhibitory synapses in the medioposterior amygdala.
Our data reveal a striking heterogeneity in the abundance of Mover on three levels, i.e. between brain areas, within individual brain areas and between synapse types. This distribution suggests a role for Mover in providing specialization to subsets of synapses, thereby contributing to the functional diversity of brain areas.

A sequence of molecular events mediates the rapid addition of release site modules during presynaptic potentiation

Alexander Matthias Walter[1], Mathias Böhme[1], Anthony McCarthy[1], Christine Beuschel[2], Andrease Grasskamp[1], Desiree Laber[3], Meida Jusyte[1], Fabian Göttfert[4], David Owald[3], Stefan Hell[4], Stephan Sigrist[2]

[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Molecular and Theoretical Neuroscience, Chariteplatz 1, 10117 Berlin, Germany

[2]Freie Universität Berlin, Institute for Biology/Genetics, 14195 Berlin, Germany

[3]Institut für Neurophysiologie, Charité Universitätsmedizin, 10117 Berlin, Germany

[4]Department of Nanobiophotonics, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany

Synaptic transmission is mediated by action-potential evoked neurotransmitter release at presynaptic active zones (AZs) followed by postsynaptic neurotransmitter detection. Plastic changes in transmission strength maintain functionality during perturbations and enable memory formation. Postsynaptic plasticity regulates neurotransmitter receptors, but the presynaptic plasticity mechanisms directly regulating the neurotransmitter release apparatus remain largely enigmatic. Here we describe a hierarchical sequence of molecular events reorganizing AZ nano-architecture to boost transmitter release within minutes during homeostatic plasticity of Drosophila neuromuscular synapses. Super-resolution microscopy revealed that individual AZs displayed a modular architecture of discrete SV release sites. Within minutes of triggering plasticity, AZs added such release modules to promote release. This fast scaling requires cognate transport machinery and a discrete subset of AZ scaffold proteins. Preventing plastic release site addition at central synapses impaired short-term memory, suggesting learning-induced plasticity also utilizes this mechanism. Thus, an intricate molecular sequence rapidly integrates AZ release site modules to enhance transmitter release and enable adaptive animal behavior.

Poster Topic

T8: Synaptic Plasticity, LTP, LTD

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T8-2A Superresolution of PSD95 Remodeling after Induction of Long-term Potentiation
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T8-3A TGF-β family member activin modulates hippocampal CA1 synaptic plasticity in a frequency-dependent fashion.
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T8-5A Human Autoantibodies against the AMPA Receptor Subunit GluA2 Induce Receptor Reorganization and Memory Dysfunction
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Carolina Montenegro Venegas, Bianka Goetze, Santosh Pothula, Franziska Greifzu, Eneko Pina, Anil Annamneedi, Karl-Friedrich Schmidt, Eckart D. Gundelfinger, Siegrid Löwel, Anna Fejtova

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Zeeshan Mushtaq, Raiko Stephan, Jan Pielage

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Anu G. Nair, Martin Müller

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Gloria Quiceno, Elke Edelmann, Volkmar Leßmann

T8-4C Mechanisms of protein trafficking in dendritic synapse-to-nucleus communication
Sebastian Samer, Rajeev Raman, Katarzyna Grochowska, Anna Karpova, Michael R. Kreutz

T8-5C Role of a novel TrkB agonist antibody in modulating the structure and function of murine hippocampal neurons
Charlotte Tacke, Jia Xie , Peter S. DiStefano, Marta Zagrebelsky , Martin Korte

T8-6C Increased spine dynamics in the visual cortex of PSD-95 knockout mice: Chronic two-photon imaging of neuronal morphology in the awake brain
Anja Tippmann, Bettina Joachimsthaler, Cornelius Schwarz, Oliver Schlüter, Siegrid Löwel

T8-1D Acute stress promotes metaplasticity in the ventral subiculum in rats by NMDA receptor- and β-adrenergic receptor-mediated mechanisms
Monique von Cramon, Julia C. Bartsch, David Gruber, Uwe Heinemann, Joachim Behr

T8-2D *Sox11* - a novel activity-dependent gene with dentate gyrus-specific expression
Julia von Wittgenstein, Fang Zheng, Marie-Theres Wittmann, Elli-Anna Balta, Fulvia Ferrazzi, Maria J. Valero-Aracama, Arif B. Ekici, Andrè Reis, Christian Alzheimer, D. Chichung Lie

T8-3D Long-term potentiation in an innexin-based electrical synapse
Georg Welzel, Stefan Schuster

T8-4D Stress affects the dynamics of hippocampal CA1 synapses and CA1-dependent learning and memory
Ghabiba Weston, Tommaso Carlo Caudullo, Tim Phillip Castello-Waldow, Alon Chen, Alessio Attardo

T8-5D The anesthetic state of the hippocampus and its effect on spine dynamics
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T8-6D Homeostatic regulation of mossy fiber LTP by TGF-β family member activin
Fang Zheng, Christian Alzheimer

Understanding the relationship between long-term synaptic dynamics and neuronal activity in hippocampal CA1

Tim Phillip Castello-Waldow[1,2], Ghabiba Weston[1,2,3], Alon Chen[1,4], Alessio Attardo[1]

[1]Max-Planck-Institute for Psychiatry, Munich, Germany
[2]Ludwig-Maximilians University, Munich, Germany
[3]Graduate School of Systemic Neurosciences GSN-LMU, Munich Germany
[4]Weizmann Institute of Science, Rehovot, Israel

It is believed that memories are stored in the brain in groups of neurons showing concerted activity patterns and that the continuous remodeling of synaptic connections between neurons endows our brains with the ability to acquire, store and recall new information. However, it is unclear how changes in connectivity lead to the emergence of activity patterns able to store information. The mouse hippocampus is a brain structure essential for episodic memory and spatial navigation and hence it has always been a very important model system to study the relationship between connectivity, activity and the ability to learn and remember. However, due to its location deep in the brain, investigations of synaptic structural plasticity have been performed mostly ex vivo, thus missing for the larger part the dynamic aspects of structural connectivity.

To solve this hurdle we used longitudinal deep-brain 2-photon imaging to investigate the relationship between neuronal activity and structural excitatory synaptic connectivity in live mice.

To visualize principle neurons and their spines (as a proxy for excitatory synapses) we used the Thy1-eGFP transgenic mouse line, in which a cytoplasmic GFP labels a sparse, random subset of pyramidal neurons. Additionally, to label neurons active during a defined time window, we crossed this line with another transgenic line in which the promoter of the immediate early gene Arc drives the expression of the red fluorescent protein tdTomato upon Tamoxifen injection.

We tracked thousands of dendritic spines from hundreds of basal dendrites of CA1 pyramidal neurons during a week of baseline; we then induced high neuronal activity by housing these mice in an enriched environment (EE) overnight. Finally, we tracked the same dendrites for one more week after EE. This allowed us to compare the excitatory synaptic dynamics of CA1 pyramidal neurons which became active upon EE with neighboring CA1 pyramidal neurons in the same animals which did not become active. Importantly, in addition to studying the dynamic changes after EE, we also compared retrospectively the excitatory synaptic dynamics before EE.

Overall we found a significant correlation between the persistence of connectivity and the probability of becoming active upon EE. Moreover, we found that a single episode of EE has a significant effect on the stabilization of excitatory connectivity.

Our data offer insight, for the first time in live animals, on the relationship between long-term connectivity and activity in hippocampal CA1.

Superresolution of PSD95 Remodeling after Induction of Long-term Potentiation

Valérie Clavet Fournier[1,2,3], Waja Wegner[2,3], Katrin Willig[2,3]

[1]Research Group Optical Nanoscopy in Neuroscience, Center for Nanoscale Microscopy and Molecular Physiology of the Brain, University medical center, Göttingen, Germany
[2]Max Planck Institute of Experimental Medicine, Göttingen, Germany
[3]Göttingen Graduated School for Neuroscinces, Biophysics, and Molecular Biosciences, Göttingen, Germany

Brain function is controlled by synapses, which are the fundamental information processing units within the neuronal circuit. The post-synaptic side of excitatory synapses is characterized by the post-synaptic density (PSD). It was shown by electron microscopy (EM) that the PSD can be perforated or macular (non-perforated) and that it can change in size and shape dependent on the synaptic activity. Moreover, EM studies have observed an increase in the proportion of perforated PSD compare to macular PSD following the induction of long-term potentiation (LTP)[1]. However, EM cannot image the dynamic of the morphological change of PSDs. The postsynaptic density protein 95 (PSD95) is an abundant scaffold protein found in the PSD of excitatory synapses where it is essential for the glutamatergic transmission and synaptic plasticity. PSD95 stabilizes the post-synaptic ionotropic receptors in the synapse, therefore increasing synaptic strength[2]. Recently, our laboratory discovered with the help of superresolution in vivo STED (Stimulated Emission Depletion) microscopy that some PSD95 assemblies appear ring-like at the nanoscale[3]. However, the function of these ring-like PSD95 assemblies and the mechanism that cause this structure is still unknown. Here, we demonstrate using STED microscopy that the number of these perforated PSD95 increase after a LTP stimulation. Furthermore, these PSD95 structures co-localize with the presynaptic active zone in the hippocampal and cortical neuronal cell culture. We will show the correlation of the increase in size of PSD95 and the active zone protein Bassoon. Moreover we will show that the assemblies of PSD95 are also associated with the activated CaMKII and the postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which suggest a role of this specific structure of PSD95 in the mechanism of learning and memory. To confirm this hypothesis, we will also perform live cell imaging of PSD95 while inducing chemical LTP stimulation to observe structural changes of PSD95 assemblies.

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TGF-β family member activin modulates hippocampal CA1 synaptic plasticity in a frequency-dependent fashion.

Marc Dahlmanns[1], Fang Zheng[1], Christian Alzheimer[1]

[1]Friedrich-Alexander-University Erlangen-Nürnberg, Institute of Physiology and Pathophysiology, Universitätsstraße 17, 91054 Erlangen, Germany

Activin is a member of the TGF-β family with multiple regulatory functions in various tissues and organs including the brain. In addition to its established role in neurodevelopment and neuroprotection, activin is increasingly recognized as a modulator of GABA- und glutamatergic synapses, which, at the behavioral level, has an impact on cognitive functions and affective behavior. We have previously shown that disruption of activin signaling reduced long-term potentiation (LTP) in the hippocampal CA1 region after theta-burst stimulation. Elaborating on this finding, we now investigated systematically the effects of activin signaling on short- and long-term synaptic responses to a broad spectrum of physiological stimulation patterns. We performed field potential recordings in stratum radiatum of area CA1 using hippocampal slices from adult wild type mice and transgenic mice expressing dominant-negative activin receptor IB (dnActRIB) in a forebrain-specific fashion. A comparison between the two groups showed that disruption of activin signaling diminished LTP at the Schaffer collateral-CA1 synapse after high-frequency stimulation (HFS, 100 Hz for 1 sec). By contrast, long-term depression (LTD) after low-frequency stimuli (LFS, 1 Hz for 15 min) was markedly enhanced in dnActRIB hippocampi. Moreover, mutant slices failed to display the characteristic depotentiation seen in control slices, when a train of 5 Hz stimuli was delivered shortly after HFS. Interestingly, close examination of field responses during the plasticity-inducing stimulation protocols revealed striking differences between wild type and dnActRIB slices in the lower frequency band (1 - 10 Hz), with a peak at 5 Hz, whereas frequencies ≥20 Hz yielded responses that were indistinguishable between groups. As a side note independent of activin signaling, we found that the pharmacologically isolated fiber volley reflecting action potential firing of Schaffer collaterals showed a dramatic decline during LFS that did not fully recover afterwards and might thus represent a presynaptic contribution to LTD. Interestingly, fiber volley depression was strongly attenuated, when extracellular Ca^{2+} was reduced to 0.2 mM (with compensatory elevation of Mg^{2+}). In summary, our results demonstrate that activin receptor signaling affects all forms of hippocampal synaptic plasticity, including LTD, LTP and depotentiation. Moreover, activin tunes also short-term changes in synaptic responsiveness during repetitive stimulation with a maximum effect in the 5 Hz range. Thus, activin is well poised to optimize the performance of excitatory synapses in the hippocampus, with particular emphasis on plastic changes that are thought to underlie memory functions and behavioral flexibility.

Characteristics of low repeat spike timing-dependent LTP at Schaffer collateral-CA1 synapses of the hippocampus

Elke Edelmann[1,2], Efrain Cepeda-Prado[1], Gloria Quiceno[1], Babak Khodaie[1], Swantje Beythien[1], Volkmar Lessmann[1,2]

[1]Otto-von-Guericke Universität Magdeburg, Institut für Physiologie, Leipziger Strasse 44, 39112 Magdeburg, Germany
[2]Center of Behavioral Brain Sciences (CBBS), Magdeburg, Germany

Synaptic plasticity is believed to represent a cellular mechanism for learning and memory formation in the brain. It can be induced by a plethora of paradigms that often rely on high frequency and high repeat synaptic stimulation protocols. Here we chose to focus on spike timing–dependent plasticity (STDP) to investigate long-lasting changes in synaptic transmission in single postsynaptic CA1 pyramidal neurons of the hippocampus. STDP is induced after repetitive, nearly coincident (i.e. within tens of milliseconds) action potential (AP) firing in pre- and postsynaptic neurons. Different types of STDP stimulation protocols have been identified across synapses that commonly employ 30 – 100 repeats of stimulation. In this study we set out to identify the cellular and molecular mechanisms of t-LTP induced by very low numbers (i.e. 3-6) of repeated synaptic stimulation.

We examined the impact of a novel STDP paradigm consisting of only six repeats (6x) of either 1 presynaptically stimulated EPSP paired with 1 postsynaptic AP (1:1) or a 1EPSP/4AP (1:4) paradigm at Schaffer collateral-CA1 synapses in acute mouse hippocampal slices. Our data indicate that even with such a few number of repeats both STDP paradigms induced robust t-LTP, comparable in magnitude to recently published high repeat protocols (Edelmann et al. Neuron, 2015). Presynaptically expressed t-LTP was induced with a 6x 1:1 stimulation that was dependent on NMDA receptors (Rs) and L-type Ca^{2+} channels. However, this 6x 1:1 paradigm did not require the presence of brain-derived neurotrophic factor (BDNF). In contrast, the 6x 1:4 paradigm induced a postsynaptically expressed t-LTP, which did not require activation of NMDARs or L-type Ca^{2+} channels, but was completely blocked by intracellular application of BAPTA. Further experiments demonstrated that the 6x 1:4 t-LTP depends on the incorporation of GluA1-containing AMPARs into the postsynaptic membrane. Interestingly, the 6x 1:1 protocol was inhibited in the presence of dopamine (D)1 like R antagonists, while the 6x 1:4 paradigm depended on the combined activity of D1 and D2 Rs.

Our results suggest that various types of synaptic plasticity can be activated by subtle differences in STDP protocols in the same CA1 neuron. The induction and expression mechanisms of these different types of t-LTP are regulated by distinct signaling pathways that depend on the number, pattern and the temporal correlation between pre- and postsynaptic firing of APs.

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Human Autoantibodies against the AMPA Receptor Subunit GluA2 Induce Receptor Reorganization and Memory Dysfunction

Holger Haselmann[1,2], Francesco Mannara[3], Christian Werner[4], Jesus Planagumà[3], Lars Schmidl[1], Federico Miguez-Cabello[3,5], Mar Petit-Pedrol[3], David Soto[3,5], Sören Doose[4], Josep Dalmau[3,6], Stefan Hallermann[7], Christian Geis[1,2]

[1]University Hospital Jena, Department of Neurology, Am Klinikum 1, 07477 Jena, Germany

[2]Center for Sepsis Control and Care (CSCC), Jena University Hospital, Am Klinikum 1, 07747 Jena, Germany

[3]Institut d'Investigacions Biomediques August Pi i Sunyer (IDIBAPS), Hospital Clinic, Universitat de Barcelona, 08036 Barcelona, Spain

[4]Department of Biotechnology and Biophysics, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[5]5Laboratori de Neurofisiologia, Departament de Biomedicina, Facultat de Medicina i Ciencies de la Salut, Institut de Neurociencies,
Universitat de Barcelona, 08036 Barcelona, Spain

[6]Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104, USA

[7]Ludwig-Institute for Physiology, Medical Faculty, University of Leipzig, Liebigstrasse 27, 04103 Leipzig, Germany

AMPA receptors are essential for fast excitatory transmission in the central nervous system. Autoantibodies to AMPA receptors have been identified in humans with autoimmune encephalitis and severe defects of hippocampal function. Here, combining electrophysiology and high-resolution imaging with neuronal culture preparations and passive-transfer models in wild-type and GluA1 knock-out mice we analyze how specific human autoantibodies against the AMPA receptor subunit GluA2 affect receptor function and composition, synaptic transmission, and plasticity. Anti-GluA2 antibodies induce receptor internalization and a reduction of synaptic GluA2 containing AMPARs followed by compensatory ryanodine receptor-dependent incorporation of synaptic non-GluA2 AMPARs. Furthermore, application of human pathogenic anti-GluA2 antibodies to mice impairs long-term synaptic plasticity *in vitro* and affects learning and memory *in vivo*. Our results identify a specific immune-neuronal rearrangement of AMPA receptor subunits providing a novel framework to explain disease symptoms.

Mechanisms of spike timing-dependent LTP along the longitudinal axis of Schaffer collateral -CA1 synapses in the mouse hippocampus

Babak Khodaie [1,3], Elke Edelmann[1,2,3], Volkmar Leßmann[1,2,3]

[1]Otto-von-Guericke University, Magdeburg, Institute of Physiology, Leipziger Str. 44, 39112 Magdeburg , Germany
[2]Center for Behavioral Brain Sciences (CBBS), Magdeburg
[3]OVGU ESF-founded International Graduate School ABINEP
39120 Magdeburg, Germany

Memory information is processed by the hippocampus co-operatively with cortical and subcortical brain regions. Despite previous concepts, which considered the hippocampus as a homogenous structure, recent studies revealed a genetical, morphological and functional diversity along the dorso-ventral axis of the hippocampus. Furthermore, CA1 pyramidal cells (CA1 PCs) are heterogeneous along the longitudinal axis of the hippocampus with respect to neuromodulatory as well as excitatory input and output fibers. Previous studies also described a distinct expression of plasticity-related receptors in the CA1 area across dorsal (DH), intermediate (IH) and ventral (VH) parts of the hippocampus. N-methyl-D-aspartate (NMDA) receptor (R) GluN2B subunit expression shows a very distinct gradient in the stratum radiatum of the CA1 region along the dorso-ventral axis (DH< IH< VH). Moreover, basal electrophysiological and firing properties of CA1 PCs differ along the dorso-ventral axis, showing a more negative resting membrane potential in dorsal CA1 PCs compared to VH and IH, as well as higher glutamate release probability in VH CA1 PCs compared to DH. Here we set out to investigate possible differences in induction and expression properties of spike timing-dependent LTP (t-LTP) along the dorso-ventral hippocampal axis using whole cell patch clamp recordings from postsynaptic CA1 PCs. Recordings were performed in acute hippocampal slices from P28-P36 C57Bl6/J mice. Two STDP protocols, 1) a canonical (1 presynaptic action potential (AP) followed by 1 postsynaptic AP stimulation with 6 repeats (6x 1:1) at 0.5 Hz, and 2) a burst protocol (6x 1:4) were used to induce t-LTP at Schaffer collateral-CA1 synapses. For burst STDP protocols our preliminary data showed a slight difference in potentiation magnitude along the dorso-ventral axis. In addition, the canonical protocol revealed a significantly higher t-LTP in DH compared to VH CA1 PCs. It is well described that elevation of intracellular calcium governs LTP induction. This process is mainly mediated by glutamate receptors or voltage gated calcium channels. Therefore, we next determined that for both low repeat t-LTP protocols (6x 1:1 and 6x 1:4) Ca^{2+} permeable channels are critically required for calcium elevation and subsequent t-LTP induction. Furthermore we asked whether the mechanisms of t-LTP induction in CA1 PCs are regulated differentially along the dorso-ventral hippocampal axis. Our preliminary results in IH CA1 PCs show that 6x 1:4 t-LTP is reduced but not blocked in the presence of NMDAR inhibitors and does not depend on activation of L-type voltage gated calcium channels (VGCC). However, inhibition of calcium release from endoplasmic reticulum by a Ryanodine receptor blocker significantly reduced this type of t-LTP. Together, these results suggest an intricate interplay between Ca^{2+} influx and subsequent Ca^{2+} induced Ca^{2+} release from internal stores to trigger 6x 1:4 t-LTP in the IH. Released Ca^{2+} following Ryanodine receptor activation will govern downstream cascades of t-LTP induction. In conclusion, our data suggest, a t-LTP paradigm-dependent gradient in t-LTP strength along the longitudinal axis of the hippocampus. However, whether the graded t-LTP serves a function in distinct capabilities of memory formation in dorsal vs. ventral hippocampus remains to be investigated.

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Investigation of synaptic mechanisms underlying behavioral tagging

Monique Klausch[1], Volkmar Leßmann[1,2], Elke Edelmann[1,2]

[1] Institute of Physiology, Otto-von-Guericke University Magdeburg, 39120 Magdeburg, Germany
[2] Center for Behavioral Brain Sciences Magdeburg, Germany

Cellular correlates and mechanisms for learning and memory can be tested as long-lasting changes in synaptic plasticity, termed long-term potentiation (LTP) for an increase and long-term depression for a decrease in synaptic transmission. Long-lasting enhancement of excitatory postsynaptic potentials measured as an increased slope of field-EPSPs in extracellular field potential recordings can be observed after LTP induction by high frequency stimulation (HFS) of hippocampal Schaffer-Collateral (SC)-CA1 synapses.
Behaviorally relevant neuromodulators such as dopamine (DA) and norepinephrine (NE) can affect the probability to induce LTP by either direct modulation or “priming” of synapses by proactive neuromodulation. At the systemic level, secretion of such neuromodulators in novelty exposure was shown to facilitate memory formation of a weakly learned task (e.g. behavioral tagging hypothesis; Moncada et al., Neural Plasticity 2015). Since the underlying cellular and molecular mechanisms of behavioral tagging are not fully understood, we established field potential recordings in acutely isolated hippocampal slices from 8- 15 weeks old male C57BL/6J mice in the CA1 region. We observed robust and stable early (e-) LTP in response to a 3x 100 Hz (for 1 s) stimulation protocol (30 s interval). To better understand the role of DA and NE in behavioral tagging relevant synaptic processes, we established a subthreshold LTP (1x 100 Hz (for 1 s)) protocol to induce weak e- LTP. This weak LTP protocol was converted to robust LTP in the presence of bath applied DA. In an attempt to mimic one type of novelty induced behavioral tagging at the synaptic level, we next tried to determine the required duration and time course of DA application that was able to prime synapses for successful LTP. Our data suggest that a 10-min pre-stimulation by bath applied DA, 60 and 45 minutes before but not after HFS, can facilitate robust LTP induced by the subthreshold stimulation protocol. Overall, it is the aim of our experiments to better understand synaptic mechanisms of neuromodulation that might underly learning phenomena such as behavioral tagging.
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Tumor necrosis factor modulates hippocampal synaptic plasticity through intracellular calcium stores

Dimitrios Kleidonas[1], Maximilian Lenz[1], Nicola Maggio[2,3,4,5], Andreas Vlachos[1]

[1]Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany

[2]Department of Neurology and Sagol Center for Neurosciences, Sheba Medical Center, 52621, Ramat Gan, Israel

[3]Department of Neurology, The Chaim Sheba Medical Center, 52621, Tel HaShomer, Israel

[4]Talpiot Medical Leadership Program, The Chaim Sheba Medical Center, 52621, Tel HaShomer, Israel

[5]Department of Neurology and Neurosurgery, Sackler Faculty of Medicine and Sagol School of Neuroscience, Tel Aviv University, 52621, Tel Aviv, Israel

The role of the pro-inflammatory cytokine tumor necrosis factor (TNF) in synaptic plasticity has long been identified. Yet, it remains unclear how TNF asserts its pleiotropic effects on neural plasticity. Moreover, the neuronal targets through which TNF modulates the ability of neurons to express plasticity remain not well-understood. Here we employed hippocampal tissue slices to test for the effects of TNF on synaptic plasticity at hippocampal Schaffer collateral CA1 synapses. Using extracellular recordings, whole-cell patch-clamp recordings, and immunostainings we demonstrate that TNF modulates the ability of neurons to express synaptic plasticity in a dose-dependent manner: high concentrations of TNF impair synaptic plasticity, whereas low concentrations of TNF improve plasticity. These metaplastic effects of TNF are modulated by intracellular calcium stores and are not observed in synaptopodin-deficient preparations, which show deficits in neuronal calcium store-mediated synaptic plasticity. In line with these findings, TNF triggers changes in synaptopodin expression. These results provide new important insight on the role of TNF in synaptic plasticity by identifying TNF as a mediator of metaplasticity, which acts through intracellular calcium stores and neuronal synaptopodin. (supported by German-Israeli-Foundation; GIF G-1317-418.13/2015)

Intracellular Zn^{2+} signaling facilitates mossy fiber input-induced heterosynaptic potentiation of direct cortical inputs in hippocampal CA3 pyramidal cells

Suk Ho Lee[1], Kisang Eom[1], Won Kyung Ho[1]

[1]Seoul Nat'l Univ. College of Medicine, Physiology, 103 Daehak-ro, Jongno-gu, 110-799 Seoul, Korea (South)

Repetitive action potentials (APs) in hippocampal CA3 pyramidal cells (CA3-PCs) backpropagate to distal apical dendrites, and induce calcium and protein tyrosine kinase (PTK)-dependent downregulation of Kv1.2, resulting in long-term potentiation of direct cortical inputs and intrinsic excitability (LTP-IE). When APs were elicited by direct somatic stimulation, only a narrow window of distal dendritic [Ca^{2+}] allowed LTP-IE because of Ca2+-dependent co-activation of PTK and protein tyrosine phosphatase (PTP), which renders non-MF inputs incompetent in LTP-IE induction. High frequency MF inputs, however, could induce LTP-IE at high dendritic [Ca^{2+}] out of the window. We show that MF input-induced Zn^{2+} signaling inhibits postsynaptic PTP, and thus enables MF inputs to induce LTP-IE at wide range of [Ca^{2+}]. Extracellular chelation of Zn^{2+} or genetic deletion of vesicular zinc transporter abrogated the privilege of MF inputs for LTP-IE induction. Moreover, the incompetence of somatic stimulations was rescued by inhibition of PTP or supplement of extracellular zinc, indicating that MF input-induced increase in dendritic [Zn^{2+}] facilitates induction of LTP-IE by inhibiting PTP. Consistently, high frequency MF stimulations induced immediate and delayed elevations of [Zn^{2+}] at proximal and distal dendrites, respectively. These results indicate that MF inputs are uniquely linked to the regulation of direct cortical inputs owing to synaptic Zn^{2+} signaling.

Denervated neurons compensate for a defect in excitatory synaptic scaling by adjusting their intrinsic excitability

Maximilian Lenz[1], Christos Galanis[1], Dimitrios Kleidonas[1], Andreas Vlachos[1]

[1]Department of Neuroanatomy, Institute of Anatomy and Cell Biology, Faculty of Medicine, University of Freiburg, 79104 Freiburg, Germany

It is well accepted that neurons respond to denervation with structural and functional changes that partially compensate for the loss of input. In previous work, we were able to demonstrate that neurons, which lose part of their input after traumatic injury increase their excitatory synaptic strength in a homeostatic manner. Here, we employed denervation experiments in entorhino-hippocampal tissue cultures to test for compensatory changes in intrinsic excitability. Using whole-cell patch-clamp recordings, we find that input-output properties of denervated dentate granule cells remain stable, while excitatory synaptic strength increases 3 days post lesion (dpl). Under conditions of pharmacological inhibition of glutamatergic neurotransmission, which prevents compensation through excitatory synaptic scaling, the intrinsic cellular properties of denervated neurons are changed in a homeostatic manner. Strikingly, structural and functional properties of GABAergic synapses remain stable in this condition. We conclude that denervated neurons adjust their excitatory synaptic strength while maintaining their inhibitory and intrinsic excitability set-points. Under conditions in which glutamatergic neurotransmission fails to compensate for the loss of input, homeostatic intrinsic plasticity is recruited. These results reveal that denervated neurons recruit (and balance) a repertoire of distinct homeostatic mechanisms in a highly coordinated manner. (supported by DFG)

Learning-induced transformation of spiking pattern through nonlinear dendritic processing in vivo

Xiang Liao[1], Meng Wang[1], Ruijie Li[1], Ran Ding[1], Xiaowei Chen[1]

[1]Third Military Medical University, Brain Research Center, Gaotanyan Street No.30, Shapingba District, 400038 Chongqing, China

Cortical circuits modify their response patterns through learning to meet animal's behavioral needs. However, the specific changes on the level of single neurons remain poorly understood. Here, using two-photon Ca2+ imaging combined with whole-cell recordings in mouse auditory cortex in vivo, we demonstrate the de novo induction of complex spike bursts in a subpopulation of neurons by an associative learning task. Such bursts are promoted by N-methyl-D-aspartate (NMDA) receptor-mediated 'depolarizing waves'. These depolarizing waves are invariably associated with large-amplitude Ca2+ transients present throughout the dendrites, revealing the existence of global dendritic NMDA spikes. Thus, we demonstrate that the auditory associative learning is associated with a reliable transformation of neurons from a regular spiking to a bursting mode through mechanisms involving nonlinear dendritic signal processing.

Neuroplastin-plasma membrane Ca^{2+} ATPases complexes: Are they new players in Ca^{2+} signaling and synaptic plasticity?

Ayse Malci[1,2], Michael Naumann[3], Eckart D. Gundelfinger[2,4], Constanze I. Seidenbecher[2,4], Rodrigo Herrera-Molina[2]

[1]Otto-von-Guericke-University Magdeburg, Faculty of Natural Sciences, ESF Graduate School ABINEP
[2]Leibniz Institute for Neurobiology, Department of Neurochemistry and Molecular Biology, Magdeburg, Germany
[3]Institute of Experimental Internal Medicine, Otto von Guericke University, Magdeburg, Germany
[4]Center for Behavioral Brain Sciences (CBBS) Magdeburg, Germany

Neuroplastins, type 1 transmembrane proteins with extracellular Ig-like domains, play a crucial role in synapse formation and stabilization. Our lab had shown that in Neuroplastin-deficient mutant mice synaptic plasticity is reduced. Also, the protein levels of the four plasma membrane Ca^{2+} ATPases (PMCA) were found to be reduced. We and others have shown that Neuroplastin binds PMCA to regulate Ca^{2+}-extruding activity in the plasma membrane. Thus, we are interested in the role of Neuroplastin-PMCA-modulated synaptic Ca^{2+} signaling in activity-induced plastic changes of synapses. Ca^{2+}-dependent extracellular signal-regulated kinases (ERK) activation is well-known to be important for synaptic plasticity, Thus, we hypothesize that Neuroplastin-PMCA-modulated Ca^{2+} signals may affect ERK activation during synaptic plasticity. Our preliminary results show that ERK phosphorylation and PMCA abundance are drastically altered in brain samples derived from Neuroplastin-deficient mice. Furthermore, we monitored by immunoblot, STED microscopy as well as FLIM/FRET-based biosensors the activation of ERK and the dynamics of Ca^{2+} signals in synapses of cultured hippocampal neurons. We assessed the contribution of Neuroplastin and PMCA to the activity-dependent plastic mechanisms using overexpression and extracellular peptides targeting Neuroplastin and PMCA. We observed that pharmacological inhibition of PMCA activity in electrically stimulated neurons alters normal activation of ERK. Altogether, considering Ca^{2+}-dependent ERK activation as a prominent and essential mechanism for activity-dependent synaptic plasticity, we propose Neuroplastin as a main partner of PMCA in the regulation of synaptic Ca^{2+} during plastic changes of synapses.

Bassoon is required for normal presynaptic homeostatic scaling and ocular dominance plasticity

Carolina Montenegro Venegas[1,5], Bianka Goetze[2,5], Santosh Pothula[3], Franziska Greifzu[2], Eneko Pina[3], Anil Annamneedi[1], Karl-Friedrich Schmidt[2], Eckart D. Gundelfinger[1], Siegrid Löwel[2], Anna Fejtova[3,4]

[1]AG Chemical Synapses, Department of Neurochemistry and Molecular Biology, Leibniz Institute of Neurobiology, Magdeburg
[2]Department of Systems Neuroscience, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Georg-August-Universität, Göttingen
[3]RG Presynaptic Plasticity, Leibniz Institute for Neurobiology, Magdeburg
[4]Molecular Psychiatry, Department of Psychiatry and Psychotherapy, University Hospital, FAU Erlangen -Nuremberg
[5]Co-first authors

Bassoon (Bsn) is a large scaffolding protein of the presynaptic cytomatrix, involved in the structural and functional organization of neurotransmitter release. Neuronal homeostatic plasticity is a mechanism by which neurons regulate their own excitability and synaptic strength to keep network activity in the physiological range independently of synaptic input. Main synaptic homeostatic processes are regulation of the availability of postsynaptic glutamate receptors, regulation of presynaptic release probability of neurotransmitter release and of the number of releasable presynaptic vesicles. While molecular players contributing to postsynaptic homeostatic adaptation are understood quite well, little is known about presynaptic molecular players. Monocular deprivation-induced ocular dominance plasticity (MD-ODP) in the primary visual cortex (V1) is an established in vivo paradigm, where inactivity-driven homeostatic reconfiguration of cortical network plays a key role.

Here we explore the role of Bassoon in homeostatic synaptic plasticity. To study the role of Bsn in this process we tested inactivity-induced homeostatic scaling in cultured hippocampal neurons using electrophysiology and imaging of synaptic vesicle recycling at individual synapses with a synaptophysin-pHluorin-based reporter. Interestingly, our data indicate that MD-ODP is absent in adult constitutive *Bsn knock-out* mice.

Moreover, we found that Bassoon deletion leads to a defect in homeostatic adaptation of neurotransmitter release at excitatory synapses leaving homeostatic rearrangements of postsynaptic receptor apparatus unaffected. In vivo experiments in a conditional mouse model revealed that selective removal of Bassoon from cortical excitatory synapses also prevented adult MD-ODP. Taken together, our results put homeostatic scaling of neurotransmitter release machinery as an important mechanism contributing to activity-dependent modulation of cortical circuit plasticity and Bassoon as an indispensable molecular player in this process.

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Microtubule-dependent control of synaptic maintenance at the Drosophila NMJ

Zeeshan Mushtaq[1], Raiko Stephan[2], Jan Pielage[1]

[1]Technical University of Kaiserslautern, Department of Biology, Erwin-Schroedinger strasse 13, D-67663 Kaiserslautern, Germany

[2] Friedrich Miescher Institute for Biomedical Research, Maulbeerstr. 66, CH-4058 Basel

The remarkable dynamics of the nervous system rely on the precise formation, maintenance and plasticity of synapses. The interaction between synaptic cell adhesion molecules and the underlying cytoskeleton controls the assembly and maintenance of synapses. Regulation of the microtubule cytoskeleton contribute to the formation of axonal branches, maintenance of axonal transport and to the organization and function of synapses. The importance of the microtubule cytoskeleton for these processes is underscored by the observation that mutations in microtubule-associated proteins are frequent causes of neurodegenerative diseases. Despite recent advances our understanding of the regulatory mechanisms controlling microtubule dynamics at the synapse remain limited. Using the Drosophila neuromuscular junction (NMJ) as a model system we aim to identify novel microtubule regulators contributing to synaptic plasticity and function. In a first screen, we identified the kinetochore-associated protein NudE as an essential regulator of axonal transport and synapse stability in larval motor neurons. Loss of NudE results in a perturbation of synaptic microtubule stability and in defects in synaptic maintenance. By combining genetic interaction experiments with a correlative analysis of markers of microtubule integrity we identified a sequence of events demonstrating a local requirement of microtubules for the control of synaptic stability. We now aim to identify the molecular events linking defects in microtubule organization to defects in synapse stability and ultimately neurodegeneration.

Probing the dynamics of presynaptic homeostatic potentiation at the *Drosophila* neuromuscular junction

Anu G. Nair[1], Martin Müller[1]

[1]University of Zurich, Institute of Molecular Life Sciences, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Neurotransmission at chemical synapses is under active control of homeostatic mechanisms. These mechanisms contribute to the overall stability of neural network function by conferring synapses with the ability to respond to perturbations. Electrophysiology-based genetic screens at the *Drosophila* neuromuscular junction (NMJ) have identified several genes involved in presynaptic homeostatic potentiation (PHP), a form of homeostatic plasticity that is characterized by the upregulation of presynaptic release upon neurotransmitter receptor perturbation. Though several molecular players have been implicated in PHP, surprisingly little is known about the temporal dynamics of this form of synaptic plasticity. The ability to experimentally delineate PHP dynamics is partly hampered by currently employed experimental perturbations to induce PHP. While genetic receptor perturbation does not allow studying PHP on short time scales, recordings with the currently used glutamate receptor antagonist Philanthotoxin-433 (PhTx), are confounded by secondary factors, such as irreversibility or activity-dependent receptor blockade.

In this study, we therefore sought to develop an experimental protocol that allows assessing the time course of PHP induction and reversal at the *Drosophila* NMJ. To this end, we tested the allosteric glutamate receptor antagonist Gyki-53655 that has been shown to inhibit mammalian AMPA receptors. We found that Gyki-53655 application (10 µM) for ten minutes significantly reduced the amplitude of spontaneous miniature EPSPs (mEPSPs) with respect to controls, indicating a block of *Drosophila* glutamate receptors. Interestingly, the amplitude of action potential (AP)-evoked EPSPs was similar to untreated NMJs. The resulting increase in quantal content (EPSP amplitude/mEPSP amplitude) suggests that Gyki application induces a homeostatic increase in release within ten minutes. Gyki washout for 15 minutes resulted in mEPSP amplitudes and EPSP amplitudes that were similar to untreated controls, indicating that both, receptor inhibition and PHP are reversible within only 15 minutes. In contrast to PhTX, we find no evidence that Gyki-induced receptor blockade is activity dependent. Together, these two properties of Gyki-dependent receptor inhibition make it well-suited to further explore the dynamics of PHP induction and reversal, as well as the underlying molecular mechanisms with high temporal resolution.

The role of Dopamine in different types of hippocampal spike timing-dependent plasticity

Gloria Quiceno[1], Elke Edelmann[1,2], Volkmar Leßmann[1,2]

[1]Institute of Physiology, Otto-von-Guericke University Magdeburg
[2]Center for Behavioral Brain Sciences,
Magdeburg

With associative synaptic plasticity implicated in learning and memory formation, there has been an emphasis on investigating long-term potentiation (LTP) and long-term depression (LTD). These bidirectional changes result from either a long-lasting enhancement, LTP, or a long-lasting decrease, LTD, of synaptic transmission. To understand activity dependent synaptic plasticity, it is important to examine the role of different activation patterns and effects of neuromodulatory transmitters involved in LTP or LTD. Presently, Dopamine (DA) is indicated as one of the most significant modulators in hippocampal synaptic plasticity as well as hippocampus dependent learning. DA activates 2 different families of dopamine receptors, the low affinity D1 like receptor (R) family (D1 and D5 Rs), and the high affinity D2 like receptor family (D2, D3 and D4 Rs). To better understand the functional role of DA in synaptic plasticity accessed at single cell level, spike timing-dependent plasticity (STDP) approaches were used. STDP is elicited by nearly time coincident spiking of pre- and postsynaptic neurons. Causal pairings (pre- before post) leads to timing-dependent (t-)LTP, while anti-causal pairings results in t-LTD. To investigate dopaminergic modulation of STDP in CA1 region whole cell patch clamp recordings in acute hippocampal slices of mice (P25-P42) were used. Two different types of STDP protocols, a canonical consisting of 1 presynaptically stimulated EPSP paired with 1 postsynaptic AP (1EPSP/1AP) and a burst (1EPSP/4AP) protocol, with short positive time delays, similar low pairing frequencies and different number of repeats were used to pharmacologically investigate the specific contribution of D1 like or D2 like Rs. Our results reveal that depending on the type and pattern of t-LTP induction, different members of DA like Rs are specifically co-/activated and involved in t-LTP at Schaffer collateral (SC)-CA1 synapses.
Together our data demonstrates that different types of dopamine modulated t-LTP can be induced at SC-CA1 synapses. In respect of underlying signaling mechanisms, however, different types of t-LTP recruit separate and discrete molecular mechanisms triggered by dopaminergic action. Since dopamine gates several types of hippocampus dependent learning, the underlying synaptic mechanisms might resemble pathways activated by our induction protocols.

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Mechanisms of protein trafficking in dendritic synapse-to-nucleus communication

Sebastian Samer[1], Rajeev Raman[1], Katarzyna Grochowska[1,2], Anna Karpova[1], Michael R. Kreutz[1,2]

[1]Leibniz Institute for Neurobiology, RG Neuroplasticity, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]Leibniz Group "Dendritic Organelles and Synaptic Function", Center for Molecular Neurobiology (ZMNH), University Medical Center Hamburg-Eppendorf, Hamburg 20251, Germany

In order to adjust to external stimuli and enable plastic remodeling of network activity, neurons need to change their transcriptional profile. Gene expression is orchestrated by integration of different signals that reach the nucleus on different time scales and along different routes. Electrochemical Ca^{2+}-signals trigger a fast transcriptional response upon synaptic activity, but they need to be complemented by delayed and sustained signaling through messenger proteins to elicit sufficient remodeling of the neuronal structure.

The synapto-nuclear messenger Jacob can encode even more specific information, such as the subcellular origin of the NMDAR activation. It acts as hub for protein interaction: upon activation of synaptic GluN2B-containing NMDAR, Jacob dissociates from the receptor complex, binds the phosphorylated MAP-kinase ERK and travels to the nucleus, where it causes a sustained activation of the transcription factor *cAMP-response-element-binding protein* (CREB). Other binding partners, such as importin-α and the molecular motor dynein enable the translocation of the complex, whereas α-internexin protects phosphorylation sites during transport. Even though, the components of the signalosome are known, it is still unclear how they interact with each other to precisely regulate the movement of the complex and enable the initiation of the transport process.

We therefore investigated the mechanisms that enable Jacob to leave the spine and to translocate to the nucleus. These do not only comprise interaction of the proteins in the complex with each other but also with components of the cytoskeleton, which need to be reorganized to create transport routes and initiate the transport process. One potential route we examined were microtubules, which temporarily invade dendritic spines upon synaptic activity. We show that the synapto-nuclear translocation of Jacob is governed by the molecular motor dynein and an interplay of members of the importin-α and -β subfamilies. Interaction of importin-α and -β, which can be found in the postsynaptic density, assemble and stabilize the components that are required for the transport of the Jacob complex. They compete with other binding partners of Jacob, such as caldendrin, which are activated upon Ca^{2+} influx and prime the complex to leave the spine, likely through close interaction with the cytoskeleton. We further elucidated the role of single components of the cytoskeleton in the transport process and show that an intact F-actin meshwork as well as reorganization of microtubules are crucial. This suggests a regulation of the transport process in dependence of cytoskeletal dynamics.

Role of a novel TrkB agonist antibody in modulating the structure and function of murine hippocampal neurons

Charlotte Tacke[1], Jia Xie [2], Peter S. DiStefano[3], Marta Zagrebelsky [1], Martin Korte[1]

[1]TU Braunschweig, Cellular Neurobiology, Spielmannstr. 7, 38106 Braunschweig, Germany

[2]Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037

[3]Zebra Biologics, Concord, MA 01742

BDNF-mediated signaling via its high affinity receptor, TrkB, is crucial in sculpting and maintaining the architecture and function of neuronal networks of the central nervous system. Aberrant BDNF/TrkB signaling has been associated with several severe neurological disorders. Therefore, the possibility to modulate BDNF/TrkB signaling is highly interesting as a potential therapeutic strategy for a multitude of neurological disorders. However, the therapeutic efficacy of BDNF is limited by its poor pharmacological properties. Among the possible approaches to circumvent the poor drug-like properties of BDNF is the development of highly selective, small-molecule TrkB agonists. While, when tested both *in vivo* and *in vitro* the TrkB agonists currently available have been shown to exert positive effects in different disease models, the analysis of their pharmacological properties failed to show changes in TrkB activation and downstream signaling suggesting that their specificity and mechanism of action are still largely unclear and indicating a need for analyzing the effects of new TrkB agonist approaches.

Here we take advantage of a new, recently described fully human TrkB agonist antibody (ZEB85) shown to mimic BDNF both in potency and activity. Indeed, ZEB85 was shown to promote maintenance of dendritic arbors of axotomized adult mouse retinal ganglion cells (RGCs) in explant cultures, a well-established assay known to depend on BDNF/TrkB signaling. In the current study we investigate the effects of ZEB85 in modulating the development, maintaining the mature structure and promoting plasticity processes of hippocampal murine neurons. A 24h application of ZEB85 to primary hippocampal cultures results in a significant increase in dendritic spine density as well as in the number of cFOS positive neurons compared to a control antibody. Moreover, treatment with ZEB85 leads to a significantly higher neurite length and complexity in developing hippocampal neurons (DIV7).

These results indicate that, *in vitro* ZEB85 indeed reproduces the typical BDNF effects on the architecture and activation state of hippocampal neurons. Current experiments, using hippocampal cultures derived from BDNF knockout mice, investigate the potential ability of ZEB85 to rescue the effects of a lack of BDNF on the structure and plasticity of excitatory and inhibitory neurons of different brain regions.

Increased spine dynamics in the visual cortex of PSD-95 knockout mice: Chronic two-photon imaging of neuronal morphology in the awake brain

Anja Tippmann[1], Bettina Joachimsthaler[2], Cornelius Schwarz[2], Oliver Schlüter[3,4,5], Siegrid Löwel[1,5]

[1]University of Göttingen, JFB Institute for Zoology and Anthropology, Systems Neuroscience, Göttingen, Germany
[2]Werner Reichardt Centre for Integrative Neuroscience, Hertie Institute for Clinical Brain Research, Systems Neurophysiology,Tübingen, Germany
[3]Department of Psychiatry and Psychotherapy c/o European Neuroscience Institute Göttingen, Germany
[4]Department of Neuroscience, University of Pittsburgh, USA
[5]Sensory Collaborative Research Center 889, Universität Göttingen,Germany

The postsynaptic signaling scaffold PSD-95 is present in the majority of excitatory synapses in the brain and hypothesized to influence their function and stability (Cane et al., 2014). We previously showed that adult PSD-95 knockout (KO) mice have 9x more AMPA-silent synapses in the primary visual cortex (V1) than wildtype littermates (WT), retain a lifelong and juvenile ocular dominance plasticity and experience-induced network changes happen faster compared to WT mice (Huang et al., 2015). Thus, PSD-95 KO mice display enhanced cortical plasticity but the neuronal circuits are less stable suggesting that dendritic spines may be more dynamic in V1 of the KOs.
To this end, we chronically imaged spine dynamics of V1-neurons in awake head-fixed PSD-95 KO and WT mice (>P145) using two-photon microscopy through a cranial window (Joachimsthaler et al., 2015). Nerve cell morphology was visualized with LifeAct-GFP via AAV-injections into V1; LifeAct labels the F-actin of neurons, which is the major cytoskeletal component of dendritic spines (Hotulainen and Hoogenraad, 2010).
Mice were thoroughly habituated to the head fixation under the two-photon microscope for at least 3 weeks. We repeatedly imaged the same dendrites and spines of layers 2/3 and 5 pyramidal neurons over eight consecutive days and recorded changes of dendritic spine numbers and dynamics (gained/lost spines). Our results clearly show that PSD-95 KO mice have a higher spine turnover rate, reduced numbers of stabile spines, and higher numbers of both eliminated and new spines compared to PSD-95 WT mice. Notably turnover rates of PSD-95 WT mice were similar to values previously published for visual cortex of anesthetized mice (Holtmaat et al., 2005).

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Acute stress promotes metaplasticity in the ventral subiculum in rats by NMDA receptor- and β-adrenergic receptor-mediated mechanisms

Monique von Cramon[1,2], Julia C. Bartsch[2,3,4], David Gruber[2], Uwe Heinemann[2], Joachim Behr[1,2,3]

[1]Department of Psychiatry, Psychotherapy and Psychosomatic Medicine, Brandenburg Medical School, 16816 Neuruppin, Germany

[2]Institute of Neurophysiology, Charité - Universitätsmedizin Berlin, 14195 Berlin, Germany

[3]Department of Psychiatry and Psychotherapy, Charité - Universitätsmedizin Berlin, 10117 Berlin, Germany

[4]Present address: Institute of Physiology I, Westfälische Wilhelms-Universität Münster, 48149 Münster, Germany

Hippocampal synaptic plasticity is susceptible to stress. Within the hippocampal formation, the subiculum plays an important role as the major output node. The ventral subiculum is associated with coordinating the stress response, due to its feedback control of the hypothalamic-pituitary-adrenal axis and its dense norepinephrine innervation. To date, the impact of stress on synaptic plasticity in the ventral subiculum has been poorly addressed. Thus, we determined the effect of acute stress (active avoidance conditioning) on the induction of long-term potentiation (LTP) at CA1 - subiculum synapses in ventral hippocampal slices from young adult male rats. Our results demonstrate that acute stress enhances LTP one day after stressor exposure and lowers the induction threshold for late-onset LTP at excitatory CA1 to subicular burst-spiking neuron synapses. This late-onset LTP is β-adrenergic and glutamatergic N-methyl-D-aspartate receptor dependent. We thereby present a metaplastic cellular mechanism that might contribute to behavioral adaptation after confrontation with emotionally charged challenging situations.

Sox11 - a novel activity-dependent gene with dentate gyrus-specific expression

Julia von Wittgenstein[1], Fang Zheng[3], Marie-Theres Wittmann[4], Elli-Anna Balta[2], Fulvia Ferrazzi[4], Maria J. Valero-Aracama[3], Arif B. Ekici[4], Andrè Reis[4], Christian Alzheimer[3], D. Chichung Lie[2]

[1]Friedrich-Alexander-Universität Erlangen, Department of Biology, Division of Animal Physiology, Staudtstraße 5, 91058 Erlangen, Germany

[2]Friedrich-Alexander-Universität Erlangen, Institute of Biochemistry, Emil Fischer Center, Fahrstraße 17, 91054 Erlangen, Germany

[3]Friedrich-Alexander-Universität Erlangen, Institute of Physiology and Pathophysiology, Universitätsstraße 17, 91054 Erlangen, Germany

[4]University Hospital Erlangen, Institute of Human Genetics, Schwabachanlage 10, 91054 Erlangen, Germany

Neuronal plasticity is an important prerequisite for the nervous system to adapt to a constantly changing environment. Throughout the brain, plasticity displays distinct characteristics and is well adjusted to the functions of different brain regions and neuron subtypes. Activity-dependent changes in gene transcription are an important mechanism to shape long-term neuronal plasticity. Surprisingly, most known activity-dependent genes are broadly expressed in all brain regions and neuron subtypes. Thus, how regional and neuronal subtype-specific plasticity are established on the transcriptional level remains poorly understood. Here, we present evidence that the developmental transcription factor *Sox11* is a region specific activity-dependent gene.

We studied the expression pattern of Sox11 in the hippocampus of adult C57Bl/6 mice after exposure to electroconvulsive stimulation (ECS) or exploration of a novel enriched environment (EE). Upon both treatments we observed a transient upregulation of SOX11 in DG granule cell neurons. Immunofluorescence co-labeling with the immediate early gene c-FOS confirmed that the majority of SOX11-expressing neurons had been activated by a stimulus. Strikingly, activity-dependent expression of SOX11 was restricted to the DG of the hippocampus. By using AAV-mediated overexpression of SOX11 in C57Bl/6 mice and a conditional KO mouse line for *Sox11* and the closely related *Sox4*, we found that *Sox11* regulates the intrinsic excitability of DG granule cells. Transcriptome analysis identified a dysregulation of the K+ channel genes *Kcnh5* and *Kcnc2* in SOX11-OE and in *Sox11/4* cKO mice, suggesting a SOX11-dependent regulation of granule cell excitability via modification of K+ channel conductances.

Based on our findings we hypothesize that *Sox11* acts as a dentate gyrus (DG)-specific activity-dependent transcription factor and might play a role in fine tuning regional plasticity in the hippocampal circuit.

Long-term potentiation in an innexin-based electrical synapse

Georg Welzel[1], Stefan Schuster[1]

[1]University of Bayreuth, Department of Animal Physiology, Universitaetsstrasse 30, 95440 Bayreuth, Germany

Electrical synapses are formed by two unrelated gap junction protein families, the primordial innexins (invertebrates) or the connexins (vertebrates). Although molecularly different, innexin- and connexin-based electrical synapses are strikingly similar in their membrane topology. However, it remains unclear if this similarity extends also to more sophisticated functions such as long-term potentiation which is only known in connexin-based synapses. Here we show that this capacity is not unique to connexin-based synapses. Using a method that allows us to quantitatively measure gap-junction conductance we provide the first and unequivocal evidence of long-term potentiation in an innexin-based electrical synapse. Our findings suggest that long-term potentiation is a property that has likely existed already in ancestral gap junctions. They therefore could provide a highly potent system to dissect shared molecular mechanisms of electrical synapse plasticity.

Stress affects the dynamics of hippocampal CA1 synapses and CA1-dependent learning and memory

Ghabiba Weston[1,2,3], Tommaso Carlo Caudullo[1,2], Tim Phillip Castello-Waldow[1,2], Alon Chen[1,2,3,4], Alessio Attardo[1]

[1]Max Planck Institute of Psychiatry, Attardo Lab, Department of Stress Neurobiology and Neurogenetics, Kraepelinstr. 2-10, 80804 Munich, Germany

[2]Ludwig-Maximilians-Universität, Munich, Germany

[3]Graduate School of Systemic Neurosciences, Munich, Germany

[4]Weizmann Institute of Science, Rehovot, Israel

Chronic stress is associated with impairments in learning and memory, as well as changes in dendritic structure and synaptic connections of the hippocampus, a brain region key for spatial and episodic learning and memory. However, the cellular- and circuit- level mechanisms by which stress-induced structural synaptic changes impair learning and memory are not yet clear. Structural changes have historically been studied mostly by ex vivo preparations, due to the necessity to sacrifice the subjects to quantify structural changes. This approach however, is limited in its temporal resolution and lacks the ability to study the dynamic response to stress within the same subjects. This is important because individuals - both human and animal models – show high variability in their responses to stress and learning and memory are intrinsically dynamic processes.

In order to overcome these drawbacks, we employed deep brain 2-photon *in vivo* time lapse optical imaging to longitudinally study the hippocampal dorsal CA1 region in live mice. We used a transgenic mouse model where a green fluorescent protein targeted to the cytoplasm sparsely labels a subset of excitatory neurons (Thy1-GFPm line). This enabled us to visualize the structure and the spines – protrusions on the dendrites where most of the excitatory synapses of excitatory neurons occur – of CA1 pyramidal neuron's basal dendrites, further allowing us to track structural synaptic changes upon multi-modal stress (MMS). MMS involves exposure of the mice to multiple simultaneous stressors.

In this work we first characterized the effect of MMS on the numbers of both excitatory and inhibitory synapses in the dorsal hippocampal CA1 *ex vivo*. To this aim we quantified dendritic spines and inhibitory synapses upon acute and repeated MMS in Thy1-GFPm mice. We correlated each individual's excitatory and inhibitory synaptic densities to the respective stress response by measuring the levels of circulating corticosterone.

Second, we investigated the extent to which acute and repeated MMS impair CA1 dependent learning and recall by using the Morris Water Maze spatial learning paradigm.

Finally, we tracked synaptic dynamics over two weeks during baseline and repeated MMS by deep brain 2-photon *in vivo* time lapse optical imaging of groups of Thy1-GFPm mice. This enabled us to investigate – for the first time *in vivo* – stress-induced changes in synaptic dynamics.

With this approach we were able to determine that MMS has a negative impact on spatial learning and on synaptic density. In addition we found that MMS alters dynamics of a specific population of CA1 synapses.

Understanding how stress impacts the dynamics of synapses is important to elucidate the basic mechanisms by which stress impairs learning and memory and it will be important for the development of therapeutic interventions both in terms of time scales and targets.

The anesthetic state of the hippocampus and its effect on spine dynamics

Wei Yang[1], J.Simon Wiegert[1]

[1]Research Group Synaptic Wiring and Information Processing, Center for Molecular Neurobiology Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

The hippocampus is a brain structure, which plays an important role in spatial navigation and episodic memory. CA1 pyramidal neurons constitute the major output elements of the hippocampal trisynaptic circuit. They are thought to be essential for the comparison of internal and external representations of the environment by integrating excitatory synaptic input from CA3 and the entorhinal cortex. Axons from these two brain areas connect to spatially segregated spine synapses at distinct dendritic locations. Thus, the formation and elimination of dendritic spines at these dendrites may reflect wiring changes and alteration of information flow within the hippocampal network. Recent in vivo imaging studies on hippocampal structural plasticity report high spine turnover rates at CA1 pyramidal cells suggesting frequent rewiring of the circuit. However, all these data derive from anesthetized mice and it is unknown whether anesthetics have effects on structural plasticity of hippocampal synapses in vivo. In humans and mice, the risk for transient amnesia is increased after isoflurane-mediated anesthesia, which may be related to alterations in hippocampal activity and/or connectivity. We therefore assessed both transient and long term effects of anesthetics on hippocampal functional and structural plasticity. We systematically determined structural spine plasticity at basal, oblique and tuft dendrites along CA1 pyramidal cells in vivo under different anesthetics compared to wakefulness. To investigate spine turnover, we used repetitive two-photon microscopy in Thy1-GFP-M mice implanted with a chronic hippocampal window to visualize dendritic spines throughout all CA1 hippocampal layers. The same animals were sequentially imaged over 3 months under isoflurane-, ketamine- and fentanyl-mediated anesthesia in a pseudo-randomized order, followed by imaging during wakefulness. As a control, we also imaged a group of animals only under awake conditions without prior exposure to anesthesia.Using repeated calcium imaging in mice expressing GCaMP6f in CA1, we further analyzed CA1 cell network activity in dorsal hippocampus to test acute effects of the three different anesthesia conditions compared to wakefulness. Visiting the same field of view across different anesthetic conditions and in awake animals enabled us to directly compare dynamics of identified neurons over time.

Homeostatic regulation of mossy fiber LTP by TGF-β family member activin

Fang Zheng[1], Christian Alzheimer[1]

[1] University of Erlangen-Nurnberg, Institute of Physiology and Pathophysiology, Universitatsstrasse 17, 91054 Erlangen, Germany

Activin, a neurotrophic and neuroprotective member of the TGF-β family, is now also recognized as a modulator of neuronal excitability and synaptic transmission in the adult brain, with an impact on cognitive functions and affective behavior. Importantly, physiological and, more so, pathophysiological stimuli can strongly enhance activin signaling. Thus, in rodents, activin levels are markedly increased after brief trains of LTP-inducing electrical stimuli or after exploration of an enriched environment (EE), and even higher levels are observed after electroconvulsive seizure (ECS), a rodent analog to electroconvulsive therapy in antidepressant treatment. Since activin levels in the dentate gyrus are particularly responsive to such stimuli, we asked how an increase in activin signaling would affect the firing properties of dentate gyrus granule cells (DGGCs) as well as their synaptic output onto CA3 pyramidal cells. Hippocampal slices were prepared from adult mice under control conditions and after 12 h housing in EE or 12 h post ECS. When slices from control mice not subjected to EE or ECS were incubated with recombinant activin, we observed always an increase in the intrinsic excitability of DGGCs. By contrast, recombinant activin exerted a bi-directional effect on mossy fiber LTP as its levels rose, facilitating plasticity at lower and dampening it at higher concentrations. The functional significance of these findings became evident when we examined mossy fiber LTP after preceding EE or ECS. Whereas slices from mice exposed to EE, which engenders a moderate increase in endogenous activin, exhibited augmented LTP, slices from mice exposed to ECS, which causes a strong rise in activin, displayed attenuated LTP compared to control slices. Establishing a causal link to activin signaling, slices from transgenic mice with a forebrain-specific disruption of activin signaling failed to show the augmenting effects of EE. We propose that activin signaling controls the extent of mossy fiber LTP in a bi-directional fashion that is inversely coupled to the strength of the preceding behavioral or electrical stimulation.

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AMPA-mediated calcium signalling in olfactory ensheathing cells

Antonia Beiersdorfer[1], Christian Lohr [1]

[1]University of Hamburg , Neurophysiology , Martin-Luther-King Platz 3, 20146 Hamburg , Germany

Olfactory ensheathing cells (OECs) is a specialized population of glial cells, enwraping fascicles of axons of olfactory sensory neurons (OSN) in the superficial olfactory nerve layer (ONL) and thus support axonal growth and guidance into the olfactory bulb. Extrasynaptic release of glutamate and ATP by OSN axons initiates Gq-mediated calcium release from internal stores in OECs via mGluR1 and P2Y1 receptors. However, confocal calcium imaging experiments in the mouse olfactory bulb provide evidence, that inhibition of ionotropic AMPA/Kainate receptors additionally reduces calcium signalling in OECs, induced by axonal stimulation.
We investigated the question wheather AMPA/Kainate receptor stimulation initates calcium signalling and membrane currents in OECs. We performed confocal calcium imaging and whole cell patch clamp recordings analyzing calcium signalling and membrane currents in response to kainate application. Kainate evoked calcium signalling in OECs, that persisted in the presence of TTX and carbenoxolone (CBX, gap junction inhibitor), thus being direct responses. However, kainate induced calcium responses were suppressed by the AMPA/Kainate receptor antagonist, NBQX.
Whole cell patch clamp recordings showed that kainate induced a robust inward current in OECs, which was inhibited by the AMPA receptor specific antagonist GYKI53655, indicating that kainate induced responses are attributed to AMPA receptors exclusivly. Additionally kainat-induced inward currents were reduced by 60% in the presence of NASPM, which inhibits the GluA2-lacking Ca2+ permeable AMPA receptor. Studying the expression of AMPA receptor subunits using immunohistochemistry, we showed that, GluA1, GluA2 and GluA4 are all present on OECs. Our results indicate that OECs express functional AMPA receptors, initiating calcium signalling in response to kainate application or neuronal activity.

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Critical contribution of astrocytes to motor learning *in vivo*

Chloe Delepine[1], Keji Li[1], Mriganka Sur[1]

[1]Massachusetts Institute of Technology, Brain and Cognitive Science Department, 43 Vassar Street, 02139 Cambridge, MA, USA

Astrocytes, long thought to operate only as a support network for neurons, are now emerging as key players in the modulation of brain information processing. Astrocytes influence synaptic transmission via glutamate transporters, and respond to, as well as modulate, neuronal activity with calcium signaling. However, many questions remain in understanding the contribution of astrocytes in vivo to complex behaviors and cognition. During motor learning, primary motor cortex (M1) is functionally and structurally reorganized. The learning of a new movement is associated with changes in neuronal activity and dendritic spine turnover. We hypothesize that astrocytes are modulators of learning-associated neuronal network reorganization by influencing synaptic strength through glutamate clearance and calcium signaling. Here we investigate the role and plasticity of cortical astrocytes in a motor learning, lever-push task in vivo. Using the engineered human muscarinic G protein-coupled receptor DREADD-hM3Dq activated by low doses of clozapine-N-oxide (CNO), we find that modulation of astrocyte calcium activity perturbs performance of the animal in the lever-push task (causing decreased lever push responses to a cue sound). Moreover, we use a transgenic mouse line in which the expression of the glutamate transporter GLT1 can be inhibited locally in M1 and show that decreasing astrocyte glutamate clearance prevents learning of smooth motor trajectory. Using genetically encoded calcium indicators and high-resolution two-photon imaging, we then show that perturbation of astrocyte calcium activity and GLT1 knockout modulate the correlation structure of neuronal population activity and the movement trajectory encoding. This project utilizes cutting-edge imaging techniques, and novel technologies for manipulating astrocyte activity, to unravel astrocyte function during a physiologically relevant task involving motor cortex plasticity.

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Activity-dependent alteration of anisotropic glial coupling in the auditory brainstem

Sara Eitelmann[1], Tatjana TX Schmitt[2], Jan J Hirtz[2], Jonathan Stephan[1]

[1]University of Kaiserslautern, Animal Physiology Group, Department of Biology, Erwin Schrödinger-Straße 13, 67663 Kaiserslautern, Germany

[2]University of Kaiserslautern, Physiology of Neuronal Networks Group, Department of Biology, Erwin Schrödinger-Straße 13, 67663 Kaiserslautern, Germany

One hallmark of the auditory system is the tonotopic organization of neuronal circuitry that is preserved from the cochlea, throughout auditory brainstem nuclei up to the auditory cortex. The formation of proper circuitry requires spontaneous neuronal activity in the pre-hearing animal. However, in $Ca_V1.3$ KO mice, where auditory brainstem nuclei are deprived of cochlea-driven neuronal activity, this precise organization is impaired in the lateral superior olive (LSO), a conspicuous auditory brainstem center (Hirtz JJ, Braun N, Griesemer D, Hannes C, Janz K, Lohrke S, Muller B, Friauf E. 2012. Synaptic refinement of an inhibitory topographic map in the auditory brainstem requires functional $Ca_V1.3$ calcium channels. J Neurosci 32(42):14602-16). It was reported previously that in the LSO astrocytes and oligendrocytes form panglial, anisotropic networks that are preferentially oriented orthogonal to the tonotopic axis (Augustin V, Bold C, Wadle SL, Langer J, Jabs R, Philippot C, Weingarten DJ, Rose CR, Steinhauser C, Stephan J. 2016. Functional anisotropic panglial networks in the lateral superior olive. Glia 64(11):1892-911). However, it remained unknown whether an impaired neuronal arrangement is followed by an altered glial network topography. We used acute brainstem slices of wildtype and $Ca_V1.3$ KO mice at postnatal days 10-12 and whole-cell patch-clamp to load single sulforhodamine 101-labeled LSO astrocytes with the gap junction-permeable tracer neurobiotin. After visualization of the tracer with avidin conjugated with alexa fluor 488, tracer-coupled networks were documented at a confocal microscope. Subsequently, we analyzed the shape and orientation of tracer-coupled networks using a newly developed vector-based approach in combination with an intensity-based cell detection method. The basic electrophysiological properties of LSO astrocytes, i.e. membrane potential, membrane resistance, and IV-relationship, were not altered in $Ca_V1.3$ KO mice. In wildtype mice, tracer-coupled LSO networks were preferentially oval shaped and primarily oriented orthogonal to the tonotopic axis, as seen before. In contrast, networks in the LSO of $Ca_V1.3$ KO mice exhibited a variable topography without any preferential orientation. Furthermore, the density of tracer-coupled cells was decreased compared to wildtype.

Taken together, our results show that the loss of $Ca_V1.3$ alters gap junctional coupling in the LSO, as demonstrated by disturbed network topography and reduced coupling of glial cells. Thus, our data indicate that gap-junctional coupling in the LSO is influenced by $Ca_V1.3$-mediated maturation of neuronal circuitry.

Development of microglia in fetal cortex of European wild boar, Sus scrofa

Laura Ernst[1], Eric Sobierajski[1], Christa Beemelmans[2], Christoph Beemelmans[2], Gundela Meyer[3], Petra Wahle[1]

[1]Ruhr University, Developmental Neurobiology, Universitatsstraße 150, 44801 Bochum, Germany
[2]Regionalverband Ruhr Grün, Forsthof Üfter Mark, Forsthausweg 306, 46514 Schermbeck, Germany
[3]University of La Laguna, Faculty of Medicine, 38200 Tenerife, Spain

Knowledge on cortical development is based mainly on small rodents besides primates and carnivores, all being altricial nestlings. Ungulates are precocial and born with nearly mature sensory and motor systems. Almost no information is available on ungulate brain development. We recently decribed the maturation of the Neuropeptide Y neuron system in wild boar fetuses derived from the forests of the Üfter Mark, Germany, reporting that the cell types resemble those seen in other mammals, however, the timing differs such that the cell types typical for the adult cortex already mature during prenatal life. Here we stained sections from E45 to P30 for the microglial marker Iba-1. At E45, a majority of labeled cells with a rather macrophage-like morphology reside in the meninges (pia mater). A few cells are already present in VZ and SVZ, but none has been detected in dorsoparietal cortex IZ, CP and MZ. At E60, VZ and SVZ display the highest density of microglia cells, followed by IZ/WM; CP and MZ display only a few labeled cells. Most cells at this age are intensely labeled, have large somata, and are poorly arborized resembling ameboid, macrophage-like microglia. At E70, all laminar compartments display microglia cells at a moderate-to-low density, at a slightly higher density at E85, followed by a massive increase in density at E100 (birth at E114) which is roughly similar to densities present at P5 and P30. Thus, the density increases substantially with the developmental increase in cortical volume and the emergence of the final lamination. From E70 onwards, more and more cells with smaller somata and ramified processes are present in MZ down to WM whereas more cells with macrophage-like morphology persist in VZ/SVZ. These results suggest that microglial invasion and development proceeds fast during the third fetal month reaching near-adult status around birth.

Analysis of exosome release from NG2-glial cells.

Dmitry Fedorov[1], Jochen Walter[2], Volkmar Gieselmann[3], Christian Steinhäuser[1], Konstantin Glebov[2], Ronald Jabs[1]

[1]Institute of Cellular Neurosciences, Univ. of Bonn; Sigmund-Freud-Straße 25, 53105 Bonn, Germany

[2]Deptartment of Neurology, Univ. of Bonn; Sigmund-Freud-Straße 25, 53105 Bonn, Germany

[3]Institute of Biochemistry and Molecular Biology, Univ. of Bonn; Nussallee 11. D- 53115 Bonn, Germany

NG2-glia is an abundant and in the CNS widely distributed subtype of macroglial cells. One of their outstanding properties is the ability to receive synaptic input from neurons. Several studies confirmed a fast information flow from neurons to NG2-glia through synaptic structures. NG2-glial cells are not able to fire action potentials and do not have pre-synaptic membrane specializations. Therefore, NG2-glia cannot directly signal to post-synaptic cells through classical exocytosis of transmitters. Although, there is implicit evidence for the existence of feedback from NG2-glia to neurons. This raises the question how NG2-glial cells signal to their neighborhood and what might be the relevance of synaptic input in this feedback pathway. It has been suggested that in white matter, neuronal synaptic input stimulates NG2-glial differentiation into myelinating oligodendrocytes, regulated by the requirements of the neural circuit. In contrast, in the sparsely myelinated grey matter NG2-glia persist throughout adulthood, with function and feedback mechanisms of these cells remaining largely unknown yet. Our preliminary results demonstrate exosome release from NG2-glia that might be a promising candidate for a NG2-glia to neuron signaling pathway.

In the presented study we analyzed Ca^{2+}-dependent mechanisms of exosome release from NG2-glia. We used membrane capacitance recordings in the Femtofarad range to detect fusion events of intracellular vesicular structures with the plasma membrane of NG2-glia. Additionally we proved the existence of triggered exosome release from NG2-glia by differential centrifugation and staining against exosome specific antigens. Furthermore, we identified non-polymerized GFAP monomers and NG2 protein as components of the exosomal cargo of NG2-glia.

Together, this work sheds new light on the enigmatic functional relationship between NG2-glia and neurons, gives clues about their physiological role in grey matter, and gauges the importance of fast synaptic input onto glial cells.

Spontaneous Na^+ Signalling in the Neonatal Hippocampus

Lisa Felix[1], Christine Rose[1]

[1]Heinrich Heine Universität, Institut für Neurobiologie, Universitaetsstraße 1, 40225 Dusseldorf, Germany

In the rodent hippocampus, drastic changes occur during the first weeks after birth as the neuronal network develops. In neonates (P2-4), the pyramidal cell population undergoes bursts of electrical activity termed giant depolarising potentials (GDPs) which are mediated by GABAergic and glutamatergic transmission (Ben-Ari et al. 1989). These are concomitant with early network calcium oscillations (ENOs) (Garaschuk et al. 1998). While GABA operates as an excitatory neurotransmitter in neonates, glutamatergic excitation takes over at the end of the first postnatal week, and major synaptogenesis and synapse maturation start to surge in the second week. Astrocyte differentiation and maturation parallel and promote this development and regulate transmission throughout (Ullian et al. 2004). Because excitatory electrical activity is largely based on the flux of sodium ions (Na^+) across neuronal membranes, neonatal electrical activity might also be accompanied by oscillations in intracellular Na^+. As Na^+ provides the driving force for a variety of cellular processes, such Na^+ oscillations might play an important role in early network formation.

To test this hypothesis, we used SBFI-AM, a ratiometric Na^+ indicator to monitor somatic Na^+ changes in astrocytes and neurons in acutely isolated tissue slices of the neonatal mouse hippocampus (p2-4). We found that 20% of neurons and 35% of astrocytes exhibit spontaneous Na^+ fluctuations that are restricted to the first postnatal week and not seen in animals at p14-18. Spontaneous Na^+ signals (like ENOs) are highly variable, however, other properties have little in common with their calcium counterparts. Na^+ signals are exceptionally long, lasting on average around 8 minutes, with a mean peak amplitude of roughly 2mM. Neuronal Na^+ fluctuations are repressed through the blocking of voltage-dependent sodium channels by TTX or by blocking GABAergic signalling components, while these manipulations have no impact on the astrocytic Na^+ signals. The blocking of other pathways including different receptors and transporters for GABA or glutamate, as well as the Na-Ca exchanger, Na-K-Cl cotransporter, stretch-activated receptors, endothelial Na^+ channels and the Na-H exchanger do not alter the occurrence of the astrocytic signals. Additionally, while the chelation of intra- and extracellular calcium eradicated Ca^{2+} ENOs in both cells types, it did not reduce the number of astrocytic Na^+ fluctuations. Intriguingly, removal of extracellular Ca^{2+} also produced rhythmic, synchronised Na^+ activity in neurons, possibly due to surface charge or competitive occupation of voltage gated Na^+ channels.

In summary, these results demonstrate for the first time that neonatal astrocytes and neurons display spontaneous Na^+ activity. These signals are prevalent during the first few postnatal days but are significantly reduced by the end of the first week after birth. Like with ENOs, neuronal Na^+ signals seem to depend on GABA release and receptor activation. However, astrocytic Na^+ signals, although having properties similar to their neuronal counter parts, appear to have a separate trigger and mechanism, the source of which remains unclear at present.

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Dopamine induces calcium signals in olfactory bulb astrocytes

Timo Fischer[1], Christian Lohr[1]

[1]University of Hamburg, Division of Neurophysiology, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

Whereas it is well established that astrocytes in culture respond to dopamine (DA) with cytosolic Ca^{2+} rises, less is known about the dopamine sensitivity of astroglia in situ. It has been shown, e.g., that application of DA acts on astroglia of the stratum radiatum in a dose dependent manner, however, the effect of DA on olfactory bulb astrocytes remains unknown. To elucidate this, we aimed to establish whether astrocytes in situ respond to DA with an intracellular Ca^{2+} rise. To exclude neuronal influence, we did all experiments in the presence of Na+-channel blocker TTX, glutamate and GABA receptor blockers (AP-5, NBQX, MPEP, gabazine). Our results show that DA leads to cytosolic Ca2+ rises in Fluo-8 AM bulk-loaded astroglia with constant signal amplitude over multiple applications of DA in the same experiment, indicating that no significant rundown occurs. DA-induced Ca^{2+} signalling was completely blocked by the mixture of the D1/5 receptor blocker SCH23390 and D2/3 receptor blocker sulpiride. Application of the IP3 receptor blocker 2-APB completely abolished the DA-evoked Ca2+ response in astrocytes. Furthermore, we tested the effect of Ca2+ store depletion by the Ca^{2+} pump inhibitor cyclopiazonic acid (CPA). CPA induced a prominent Ca2+ elevation by itself, reflecting store depletion, and suppressed Ca^{2+} transients by DA. Our data show that DA evokes Ca2+ release from internal stores by activation of both D1/5 and D2/3 receptors in olfactory bulb astrocytes. Supported by the DFG (LO779/11).

Electrophysiological properties of proliferating astrocytes after traumatic brain injury

Stefanie Götz[1], Benedikt Grothe[1], Lars Kunz[1]

[1]LMU Munich, Graduate School of Systemic Neurosciences & Dept. Biologie II, Div. of Neurobiology, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

Injuries to the brain have severe consequences due to the low healing potential of brain tissue. However, in recent years, a rising number of regenerative processes in the brain have been identified. Astrocytes are major players in this process since they become reactive under these conditions. Especially juxtavascular astrocytes with their somata directly adjacent to blood vessels proliferate after traumatic brain injury (TBI; Bardehle et al. 2013, Nat. Neurosci. 16(5):580). With respect to the differences between juxtavascular and non-juxtavascular astrocytes we focussed on ion channels and electrophysiological properties since it is well-known from other cell types – especially tumour cells - that ion channels have a role in cell cycle regulation and, thereby, also in proliferation. We addressed this question in the BAC Aldh1l1 eGFP transgenic mouse strain with astrocytes expressing eGFP for identification. Performing whole-cell patch-clamp recordings as well as immunohistochemical stainings on brain slices of the somatosensory cortex, we asked two major questions: (1) Are juxtavascular astrocytes per se different from non-juxtavascular ones, which might provide the basis for proliferation upon TBI? (2) Does TBI induces different development of juxtavascular and non-juxtavascular astrocytes resulting in manifestation of electrophysiological differences? We did not find any electrophysiological differences between juxtavascular and non-juxtavascular astrocytes in healthy control animals. Regarding resting membrane potential (V_r) and membrane conductance at V_r, somatosensory astrocytes exhibited a high level of heterogeneity, but no significant differences between juxtavascular and non-juxtavascular ones. Moreover, immunohistochemistry revealed no differences in expression of $K_{ir}4.1$, the K^+ channels giving rise to the major astrocytic current, and of $K_{ir}6.2$, the pore-forming alpha subunit of metabolic K^+ channels (K_{ATP}). To answer the second question we introduced a stab wound lesion in the somatosensory cortex and performed electrophysiological and immunohistochemical experiments 5 days after the TBI. We found two major electrophysiological alterations in astrocytes. First, the ratio between passive and non-passive astrocytes changed significantly after the lesion from predominantly passive (70-80%) to equal (non-juxtavascular) or even predominantly non-passive ones (65%, juxtavascular). Second, we found a pronounced downregulation of $K_{ir}4.1$ in proliferating astrocytes - identified by Ki67 expression - due to TBI. In conclusion, juxtavascular and non-juxtavascular astrocytes in the somatosensory cortex did not show any obvious electrophysiological differences that could explain proliferation of juxtavascular ones upon TBI. Nevertheless, the cortical lesion induced pronounced alterations in electrophysiological properties of astrocytes that might well be related to their proliferative potential and should therefore be further studied in the context of brain tissue regeneration.

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Serotonin regulation of astrocyte cell number and morphology in raphe nuclei

Melin Hasan[1], Mary Newland[1], Natalia Alenina[2], Valentina Mosienko[1]

[1]University of Exeter Medical School, United Kingdom
[2]Max-Delbrück Center for Molecular Medicine Berlin-Buch, Germany

Depression is the most common mental health illness and the second leading cause of disability worldwide. The most prevalent theory of depression is monoaminergic. According to this theory disbalance in brain monoaminergic neurotransmitters such as serotonin results in depressed mood. Depression is also characterised by the changes in non-neuronal cells in the brain, glial cells. For example, post-mortem analysis of human brain tissue obtained from depressed individuals reveals reduction in glia cell density and changes in astrocyte cell morphology and decreased expression of genes involved in astrocyte functions. However, in animal models of depression antidepressants which elevate serotonin level in the brain are shown to either induce or reverse “depression-like” phenotype of astrocytes and there is no solid understanding on serotonin modulation of astrocyte cell number and morphology. Here, we propose to characterise astrocytes in the brain of a unique animal model lacking central serotonin. This was achieved by knocking out central isoform of serotonin synthesising enzyme, Tryptophan hydroxylase 2 (TPH2). Using Tph2KO mice we propose to study a direct effect of serotonin reduction on astrocyte cell density and morphology in the raphe nuclei, a main hub of serotonergic neurons.

We will employ immunohistochemistry and label astrocytes with antibodies against two proteins exclusively expressed in astrocytes, glial fibrillary acidic protein (GFAP) and calcium binding protein B (S100B). Following the staining, we will acquire images and process them using ImageJ software and quantify astrocyte nuclei size (diameter and perimeter), area occupied by an astrocyte, astrocyte cell density (cells/mm3) and number.

This study will lay the foundation for further studies investigating the modulatory effect of serotonin on glial cells' functions.

Gap junction uncoupling and cytoskeletal changes in astrocytes occur independent of neuroinflammation during early epileptogenesis

Lukas Henning[1], Julia Müller[1], Zhou Wu[1], M. T. Heneka[2], Peter Bedner[1], Christian Steinhäuser[1]

[1]Institute of Cellular Neurosciences, University of Bonn

[2]Department of Neurodegenerative Disease and Gerontopsychiatry,University of Bonn / German Center for Neurodegenerative Diseases

Astrocytes build networks via gap junction channel (GJC) proteins, which in the hippocampal CA1 region mainly comprise connexin Cx43 and Cx301. These networks allow for dissipation of extracellular ions and other molecules, including K+, and prevent neuronal hyperactivity2.
Temporal lobe epilepsy (TLE) is often accompanied by hippocampal sclerosis (HS), and in sclerotic tissue astrocytic coupling is completely lost3. We have previously shown in a mouse model of TLE with HS, that (i) loss of astrocyte coupling precedes neuronal death and seizure generation and (ii) that uncoupling is caused by cytokine-induced phosphorylation at the C-terminus of Cx434. The precise time at which astrocyte uncoupling is initiated following kainate (KA) injection remains unknown. As production and release of proinflammatory cytokines occurs rapidly after the induction of epilepsy, it can be assumed that GJ uncoupling already takes place within the first hour after KA injection. Here, we examined astrocyte coupling 1 h post KA injection by quantifying the intercellular spread of biocytin injected into individual astrocytes during whole-cell patch clamp recordings in acute hippocampal brain slices. Additionally, we conducted ELISA and immunohistochemistry to measure the concentration of IL-1ß and TNF-α; and the nucleus-to-cytoplasm transfer of the early proinflammatory factor HMGB-1 in the brain 1 h following KA injection. Our results demonstrate impaired GJ coupling on the injected (ipsilateral) compared to the non-injected (contralateral) hemisphere. However, in contrast to the 4 h post KA time-point, neither an increase in IL-1ß or TNF-α, nor any indication for a nucleus-to-cytoplasm transfer of HMGB-1 could be detected 1 h post KA injection. The morphology of microglia was also not altered at that time, further substantiating the absence of neuroinflammation. Importantly, however, we observed significant cytoskeletal changes of GFAP+ astrocytes on the ipsi- vs. contralateral side. Western blotting is currently under way to investigate the phosphorylation status of Cx43 at the 1 h time. Our data suggest that the interplay of rapid functional and morphological changes in astrocytes is responsible for the initiation of epileptogenesis.

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Exosomal release of astroglial vimentin: implications on the neuronal growth-promoting properties of clostridial C3 transferase?

Markus Höltje[1], Andrej Adolf[1], Rohrbeck Astrid[2], Agnieszka Münster-Wandowski[1], Malin Johansson[3], Hans-Georg Kuhn[3], Marcel Alexander Kopp[4], Benedikt Brommer[4], Jan Markus Schwab[5], Gudrun Ahnert-Hilger[1]

[1]Institute of Integrative Neuroanatomy, Charité – Universitätsmedizin Berlin, Germany

[2]Institute of Toxicology, Hannover Medical School (MHH), Germany

[3]Department of Clinical Neuroscience at Institute of Neuroscience and Physiology, University of Gothenburg, Sweden

[4]Department of Neurology and Experimental Neurology, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-
Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany

[5]Paraplegiology (Spinal Cord Injury Division), Belford Spinal Cord Injury Center, Departments of Neurology, Neuroscience and Physical Medicine and Rehabilitation, The Neurological Institute, The Ohio State University, Wexner Medical Center, Columbus, USA.

Clostridium botulinum C3 transferase (C3bot) ADP-ribosylates rho proteins to change cellular functions in a variety of cell types including astrocytes and neurons. The intermediate filament protein vimentin as well as transmembrane integrins are involved in internalization of C3bot into cells. The exact contribution, however, of these proteins to binding of C3bot to the cell surface and subsequent cellular uptake remains to be unraveled. By comparing primary astrocyte cultures derived from wild type with $Vim^{-/-}$ mice we demonstrate that astrocytes lacking vimentin exhibited a delayed ADP-ribosylation of RhoA concurrent with a blunted morphological response. This functional impairment was rescued by the extracellular excess of recombinant vimentin. Binding assays using C3bot harboring a mutated integrin-binding RGD motif (C3bot-G89I) revealed the involvement of integrins in astrocyte binding of C3bot. Axonotrophic effects of C3bot are vimentin dependent and postulate an underlying mechanism entertaining a molecular cross talk between astrocytes and neurons. We present functional evidence for astrocytic release of vimentin by exosomes using an in vitro scratch wound model. Exosomal vimentin+ particles released from wild type astrocytes promote the interaction of C3bot with neuronal membranes. This effect vanished when culturing $Vim^{-/-}$ astrocytes. Specificity of these findings was confirmed by recombinant vimentin propagating enhanced binding of C3bot to synaptosomes from rat spinal cord and mouse brain. We hypothesize that vimentin+ exosomes released by reactive astrocytes provide a novel molecular mechanism constituting axonotrophic (neuroprotective) and plasticity augmenting effects of C3bot after spinal cord injury.

HIF-1α is stable and active in astrocytes under physioxia

Sherif Idriss[1], Silje Zimek[1], Katia Monsorno[1], Olaf Joehren[1]

[1]University of Luebeck, Center of Brain, Behaviour and Metabolism, Marie Curie Strasse, 23562 Luebeck, Germany[1]

The physiological levels of oxygen in the body (physioxia; 3-5% oxygen in brain) are significantly lower than normal oxygen levels (21%) in the air (normoxia) due to the dramatic decrease in blood oxygen levels across the lung and to organs throughout the body. Oxygen levels below the physiological range (hypoxia; 1% Oxygen) can result in disturbed functions of organs, tissues and cells, leading to injury. In response to hypoxia, Hypoxia Inducible Factors (HIFs) are activated, stabilised and HIF-1/2α enter the nucleus, where they heterodimerise with HIF-1β and bind to a conserved DNA sequence known as the hypoxia responsive element (HRE), leading to an increase of transcription of HIF target genes such as glucose transporter 1 (GLUT1), hexokinase (HK), phosphofructokinase (PFK) and lactate dehydrogenase A (LDHA), monocarboxylate transporter 4 (MCT4).
Aims: We investigated whether HIF-1α is stabilised in primary astrocytes by NO under physioxia as we have shown before for normoxia. We investigated whether that HIF-1α is active under physioxia without further stimulation by NO. We also address whether further studies of HIF-1α are best conducted under physioxia.
Methods: Cortical astrocytes obtained from 1 day old pups in primary culture are treated with nitric oxide donor DetaNONOate (Deta) and HIF-1α was knocked down by siRNA independently under normoxia, physioxia and hypoxia. HIF-1α and its target gene expression was analysed at mRNA and protein levels by RT-qPCRs and Western blots respectively.
Results: HIF-1α is not only active under normoxia but also at physioxia by DETA treatment. Following HIF-1α knockdown, MCT4 is selectively downregulated but not GLUT1 or HK.
Conclusions: Under physioxia HIF-1α is stable and active and differentially regulates its target genes. We also conclude that further HIF-1α investigations in astrocytes should be done in physioxia.

Neuroimmunological function of osteopontin in activation of astrocytes in stab wound mouse brain and LPS stimulated primary culture

Hiroko Ikeshima-Kataoka[1], Motoko Furukawa[2], Sayaka Inui[2], Yutaka Matsui[3], Toshimitsu Uede[4], Masato Yasui[2]

Faculty of Science and Engineering, Waseda University, Tokyo, Japan[1]
Department of Pharmacology and Neuroscience, Keio University School of Medicine, Tokyo, Japan[2]
Department of Cardiovascular Medicine, Tonan Hospital, Sapporo, Hokkaido, Japan[3]
Department of Matrix Medicine, Institute of Genetic Medicine, Hokkaido University, Sapporo, Hokkaido, Japan[4]

Osteopontin (OPN) is an inflammatory cytokine inducer involved in cell proliferation and migration in inflammatory diseases, injuries or tumors. To clarify the functional role of OPN in reactivation of astrocytes during brain injury, we compared OPN-deficient (OPN/KO) with wild type (WT) mouse brains after stab wound injury on the cerebral cortex as a brain traumatic injury model. Furthermore, primary culture of astrocytes and microglial cells from either genotype of postnatal mouse brains was prepared, and treated with lipopolysaccharide (LPS) to induce inflammation in the cells. By the immunofluorescent analysis on the injured brain sections, either astrocytes or microglial cell activation was attenuated in OPN/KO mice compared with WT mice confirmed with bromo-deoxy urine incorporation as a cell proliferation marker. Activation efficiency of astrocytes in primary culture was accessed using Western blotting analysis by examining the protein expression levels of glial fibrillary acidic protein (GFAP) and tenascin-C (TN-C), which are the markers for reactive astrocytes. The expression levels of both GFAP and TN-C were downregulated in the primary culture of astrocytes from OPN/KO mice compared to that from WT mice. Additionally, primary culture of astrocytes prepared from OPN/KO mice showed only 25% of normal shaped astrocytes in a flask were produced compared to that from WT mice. These data suggest that OPN is essential for proper astrocytic generation in vitro culture prepared from mouse cerebral cortex. Moreover, OPN is indispensable for astrocyte activation in the mouse brain injury model and in LPS stimulated primary culture.

Synaptic protein turnover inside and outside the adult *Drosophila* photoreceptors

Eugene (Jennifer) Jin[1], Eugenia Patsouri[1], Sarah Green[1], Friederike Elisabeth Kohrs[1], Peter Robin Hiesinger[1]

[1]Freie University Berlin, Neurobiology, Koenigin Luise Strasse 1-3, 14195 Berlin, Germany

The longevity and morphological complexity of neurons create a formidable challenge to maintain neurons healthy and functional. Defects in protein degradation mechanisms cause neuronal degeneration, yet little is known about synaptic protein sorting and degradation during their functional turnover. We have recently reported direct observation of constitutive local sorting and degradation of both synaptic vesicle (SV) and plasma membrane (PM) proteins at axon terminals of developing *Drosophila* photoreceptors. To investigate where, when and how synaptic proteins are sorted and degraded in adult neurons, we used our established live imaging method of acidification-sensing probes in adult *Drosophila* photoreceptor synaptic terminals, axons and cell bodies. We have characterized degradation of the following cargo proteins: (1) general myristoylation-tag (myr) as a plasma membrane protein, (2) functional and dysfunctional variants of the SV protein Synaptotagmin (Syt1), (3) the synaptic vesicle SNARE protein neuronal Synaptobrevin (n-Syb), and (4) an active zone protein Bruchpilot (Brp). We found that all cargo probes are sorted and degraded in adult photoreceptor synaptic terminals, with increased degradation in aged adult photoreceptors. Interestingly, degradation in adult photoreceptors occurs predominantly in the cell bodies and in glia: both functional and dysfunctional Syt1 are degraded predominantly in the cell bodies, Brp is degraded similarly in both cell bodies and at synaptic terminals, and both myr and n-Syb are degraded predominantly in glia. To understand how the cargo probes are released from the photoreceptors and taken up by glia, we are currently systematically characterizing the requirement of all *Drosophila* Rab GTPases for either the release and/ or the uptake events. Rab GTPases are master regulators of intracellular membrane trafficking in all eukaryotic cells. Identification and characterization of Rab proteins required for degradation of cargo probes in glia would further our understanding of how neurons stay healthy and functional for the entire lifetime of an organism.

Essential contribution of NBCe1 in modulation of astrocytic metabolism by neuronal signals

Susanne Köhler[1], Ulrike Winkler[1], Marit Sicker[1], Johannes Hirrlinger[1,2]

[1]Carl-Ludwig-Insitute for Physiology, University Leipzig, Medical Faculty, Liebigstraße, 27 A, D-04103 Leipzig, Germany

[2]Department of Neurogenetics, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Strasse 3, D-37075 Göttingen, Germany

Brain function is dependent on an appropriate supply of energy and astrocytes crucially contribute to brain energy metabolism. Astrocytes extend numerous processes towards neighboring neurons but they also enwrap blood vessels with their endfeet. Due to this strategical location, astrocytes are prone to take up nutrients from capillaries and provide neighboring cells with energy metabolites. This process of metabolic coupling takes a key role in the relationship of astrocytes and neurons. However, the detailed mechanisms and the regulation of these processes are still a matter of intense debate. To address this issue we investigated the regulation of astrocytic metabolism by neuronal signals. As read out for astrocytic metabolism, the NADH/NAD^+ redox state constituting a central integrating node between metabolism and signaling was assessed. To characterize the dynamics of cytosolic NADH/NAD^+, Peredox-mCherry, a genetically encoded fluorescent biosensor, was expressed in primary cultured cortical astrocytes. Upon increasing the extracellular potassium concentration ($[K^+]_e$) as well as application of the neurotransmitters ATP and glutamate the intracellular NADH/NAD^+ redox ratio in astrocytes increased, implying a higher catabolism in astrocytes triggered by neuronal signals. Mechanistically, a prime candidate for regulation of astrocytic metabolism is the sodium-dependent bicarbonate cotransporter NBCe1, which is known to stimulate glycolysis in astrocytes via activation of sAC and cAMP production. Indeed, functional and pharmacological inhibition of NBCe1 abrogated the observed modulation of the astrocytic NADH/NAD^+ redox state by K^+, ATP and glutamate. These results suggest that the NADH/NAD^+ redox state in astrocytes is a metabolic node regulated by neuronal signals reflecting physiological activity via a NBCe1 dependent pathway, most likely contributing to adjust astrocytic metabolism to energy demands of the brain.

Dendritic ATP release evokes calcium signaling in olfactory bulb astrocytes

Kristina Losse[1], Damian Droste [1], Janina Popp[1], Antonia Beiersdorfer[1], Christian Lohr [1]

[1]University of Hamburg, Neurophysiology , Martin-Luther-King Platz 3, 20146 Hamburg , Germany

Astrocytes respond to a large range of neurotransmitters, neuromodulators and growth factors with increases in the cytosolic calcium concentration, which can trigger neurovascular coupling and release of gliotransmitters such as D-serin, glutamate and GABA. Astrocytes in the glomerular layer of mouse olfactory bulbs have been shown to respond to ATP co-released from nerve terminals of olfactory sensory neurons via activation of P2Y1 receptors and, after degradation of ATP to adenosine, via A2A adenosine receptors. However, axons of sensory neurons are not found in deeper layers of the olfactory bulb, which raises the questions whether ATP is also released in these layers, which cells release ATP and whether astrocytes respond to released ATP. We used GCaMP6s, expressed under control of GLAST-CreERT2 in astrocytes, to visualize calcium changes in acute olfactory bulb slices. Retrograd electrical stimulation of mitral and tufted cells (M/T cells) resulted in calcium transients in astrocytes of the external plexiform layer, innervated by dendrites of M/T cells. Blocking ionotropic and metabotropic glutamate receptors, GABA receptors and GAT3 GABA transporters, which have been demonstrated to evoke calcium signaling in olfactory bulb astrocytes, only weakly reduced calcium signaling elicited by retrograde stimulation. The olfactory bulb is innervated by centrifugal fibres releasing noradrenalin, acetylcholine, serotonin and dopamine, however, additional blockage of receptors for these neurotransmitters did not affect stimulation-evoked responses in astrocytes. A combination of antagonists for P2Y1 and A2A receptors (MRS 2179 and ZM 241385), however, almost completely suppressed stimulation-induced calcium transients in astrocytes. The results demonstrate that retrograde stimulation of M/T cells evokes ATP release without major contribution of GABAergic granule cells or centrifugal fibres, therefore suggesting ATP release from dendrites of the stimulated M/T cells.
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Astrocytes and oligodendrocytes in the thalamus jointly maintain synaptic activity by supplying metabolites

Camille Philippot[1], Stephanie Griemsmann[1], Ronald Jabs[1], Helmut Kettenmann[2], Christian Steinhäuser[1]

[1]Institute of Cellular Neurosciences, Medical Faculty, University of Bonn

[2]Cellular Neuroscience, Max-Delbrück-Center for Molecular Medicine, Berlin

Over the past decades increasing evidence has revealed the importance of glia for proper brain function. Astrocytes are the most abundant subtype of glial cells, fulfilling a variety of functions, e.g. metabolic support, ion homeostasis and modulation of synaptic transmission. During development astrocytes establish gap junction channels with each other and with oligodendrocytes to form a panglial network. The thalamus is known as the “gate to consciousness” and plays an important role in relay and modulation of information to the cortex. Most studies focused on its neuronal functions while little is known about glial cells. Here, we investigated properties of thalamic glia and their role in energy metabolism and neuron-glia signaling.

Using the tracer biocytin, we found more abundant panglial coupling in the thalamus as compared to the hippocampus. Indeed, filling an individual astrocyte, around 55% of the biocytin-labeled cells were oligodendrocytes in thalamic networks, in contrast to 13% in the hippocampus (Griemsmann et al., Cereb Cortex 2015). The functional impact of astrocyte-oligodendrocyte coupling in grey matter is still unclear. It has been shown that white matter oligodendrocytes support axonal function by transport of metabolites (Lee et al., Nature 2012; Meyer et al., Cell Reports 2018). Since oligodendrocytes do not directly contact blood vessels, the panglial network is a possible route of transport for energy metabolites from blood vessels to the oligodendrocytes. We assessed whether oligodendrocytes in the thalamus contribute to the maintenance of synaptic activity by providing metabolites through the coupled network. Stimulating the cortico-thalamic pathway we found that extracellular glucose deprivation (EGD) suppresses thalamic post synaptic field potentials, which could be rescued in part by extracellular lactate or pyruvate. Importantly, the loss of field potentials during EGD was fully prevented when filling an individual astrocyte with glucose or lactate, a process that required glucose and monocarboxylate transporter activity. Interestingly, filling individual oligodendrocyte with glucose also rescued thalamic post synaptic field potentials during EGD. Thus, in the thalamus astrocytes and oligodendrocytes are jointly engaged in maintaining synaptic activity by delivery of metabolites through the panglial coupling network. Whether oligodendrocytes exert their effect by supporting axonal/presynaptic function and/or by assisting astrocyte metabolite transport to the synapse remains to be investigated.

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Immunohistochemical and functional analysis of $P2X_7$ receptors in microglia of the mouse olfactory bulb

Natalie Clara-Maria Rotermund[1], Judith Seliger[1], Niklas Hormanns[1], Annette Nicke[2], Christian Lohr[1]

[1]University of Hamburg, Neurophysiology, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

[2]Ludwig-Maximilian University Munich, Walther-Straub-Institute of Pharmacolgy and Toxicology, Nußbaumstr. 26, 80336 München

Microglia are an important part of the immune system in the central nervous system and further involved in the regulation of brain-homeostasis as well as establishment and refinement of neuronal networks and synaptic transmission. Their importance in all aspects of brain function has only recently been fully recognised. Therefore, there is great interest in a better understanding of microglia physiology. It has been shown that the purinergic transmitter system is an important part of microglial activation and involved in different aspects of their cell-to-cell communication. The ionotropic $P2X_7$ receptor, for example, is widely expressed in microglia and involved in triggering their proliferation and activation.

We analysed the expression pattern of $P2X_7$ receptors in the olfactory bulb by using a P2X7R-reporter mouse in combination with immunohistochemical stainings and found a co-localisation of $P2X_7$ positive cells with IBA1, a microglia marker. Additionally, we generated a mouse line, which expresses the calcium indicator GCaMP6s exclusively in microglia and confirmed the microglia-specific expression of GCaMP6s by co-staining with anti-IBA1. Using this mouse-line, we investigated calcium signalling in microglia and analysed the physiological properties of $P2X_7$ receptors in microglia of the main olfactory bulb. Our results demonstrate that olfactory bulb microglial cells express $P2X_7$ receptors that mediate calcium signalling.

NG2 glia-specific gene knockout as a tool to understand the impact of neuron-glia synaptic signaling

Aline Timmermann[1], Anne Boehlen[1], Magdalena Skubal[1], Andras Bilkei-Gorzo[2], Andreas Zimmer[2], Ronald Jabs[1], Frank Kirchhoff[3], Gerald Seifert[1], Christian Steinhäuser[1]

[1]Institute of Cellular Neurosciences, Medical Faculty, University of Bonn, Bonn, Germany
[2]Institute of Molecular Psychiatry, Medical Faculty, University of Bonn, Bonn, Germany
[3]Molecular Physiology, Center for Integrative Physiology and Molecular Medicine (CIPMM), University of Saarland, Homburg, Germany

NG2 glia represents the 4th type of CNS glia cells. In white matter, most of them differentiate into oligodendrocytes but in grey matter a majority retains their NG2 phenotype throughout life. NG2 glia receives direct synaptic input from glutamatergic and GABAergic neurons. However, the functional consequence of this input is not yet understood. During development, NG2 glia upregulate Kir4.1 channels, leading to low membrane resistance and a resting potential close to the K+ equilibrium potential. To test if Kir currents impact the efficiency of synaptic activation of NG2 glia and assess whether/how these cells influence brain function, we generated NG2-CreERT2 knock-in mice where conditional knockout of the Kir4.1 gene upon tamoxifen administration was induced.
In tamoxifen-treated mice, semi-quantitative RT-PCR of FAC sorted NG2 glial cells proved a downregulation of Kir4.1 mRNA by 91% in the hippocampus. NG2 glia devoid of Kir currents displayed more positive resting potentials and a significantly increased membrane resistance. This change in passive membrane properties affected the glial response upon quantal transmitter release at the neuron-NG2 glia-synapse as miniature EPSP amplitudes were increased and prolonged in Kir-deficient NG2 glia. Short-term plasticity at the neuron-NG2 glia-synapse was changed as indicated by an increased release probability of neurotransmitters from the presynapse in Kir4.1 flox mice. To investigate the impact of Kir4.1 deletion in NG2 glia on neuronal signaling, field potentials were recorded in the hippocampus after stimulation of Schaffer collaterals. Long term potentiation, induced by theta-burst stimulation, was significantly impaired in the hippocampal CA1 region of mice with NG2 glia-targeted Kir4.1-deficiency whereas basal neuronal excitability was not affected. Interestingly Kir4.1-deficient mice showed increased novelty preference in the object location recognition memory task and improved partner recognition in the partner recogniton paradigm. These findings assume an improved declarative and social memory of mice lacking Kir4.1 in NG2 glia cells. NG2 glia-targeted deletion of the Kir4.1 gene further entailed an increase of MBP and MAG mRNA in recombined cells and an upregulation of MBP protein, a main component of myelin sheaths, 8 weeks after tamoxifen injection. These findings show that Kir4.1 channels in NG2 glial cells set their excitability, influence myelination and are important for proper hippocampal synaptic plasticity.
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Investigating Glia-Neuron Protein Interactions In Purified Neuronal Cultures using BONCAT and SILAC metabolic labelling

Paul Turko[1], Juliane Schiweck[2], Keenan Groberman[1], Cristina Kroon[2], Britta Eickholt[2], Imre Vida[1]

[1]Charite University Medicine, Integrative Neuroanatomy, Charite, 10117 Berlin, Germany

[2]Charite University Medicine, Institute of Biochemistry, Charite, 10117 Berlin, Germany

Glial cells continually secrete proteins into the extracellular space which alone or in synergy may mediate many biologically relevant effects. Recent experiments from our lab reveal an important difference in the way glial-secreted signals influence the development of GABAergic and glutamatergic networks (Turko et al. 2018). Our results suggest that glial-secreted proteins are required for the establishment of glutamatergic synaptic transmission, but not GABAergic synaptic transmission. This important difference between the 2 dominant neuronal classes has major implications for our understanding of how neuronal networks develop. To study this further, we have established metabolic labelling techniques, such as bioorthogonal non-canonical amino acid tagging (BONCAT) and stable isotope labelling with amino acids in cell culture (SILAC), to label glial-secreted proteins and to study their interaction with specific neuron types. Our goal is to identify novel glial-secreted proteins which differentially influence of the development and function of GABAergic and glutamatergic neurons. Through a combination of mass spectroscopy, gene analysis, electrophysiology and imaging techniques we will study in detail how glial-secreted proteins regulate neuron development. Overall, these experiments should provide better insights in the ever growing role of glial cells in the development of neuronal networks.

Mapping Glutamate Receptors On NG2 Cells With 2P Glutamate Uncaging

Natascha Vana[1], Wenjing Sun[1], Shane McMahon[1], Susanne Schoch[2], Dirk Dietrich[1]

[1]Department of Neurosurgery, University Clinic Bonn, 53105 Bonn, Germany
[2]Institute of Neuropathology, University Clinic Bonn , 53105 Bonn, Germany

Glutamatergic input to NG2 cells is mainly mediated by AMPARs and universal to all brain areas. Ultrastructural studies confirmed the existence of a similar synaptic junction between neurons and NG2 cells when compared to neuron-neuron contacts.
Glutamate signaling between NG2 cells and neurons has been implicated in a variety of functions such as proliferation, migration and differentiation into oligodendrocytes as well as myelination. However, it is not known how NG2 cells and neurons first establish synaptic contacts, whether this needs to be pre- or postsynaptically induced and whether glia-neuron synapses can be initialized at any site along the NG2 cell surface.
One characteristic of conventional neuron-neuron synapses is the accumulation of glutamatergic receptors at excitatory synapses but until now it has remained enigmatic whether NG2 cells exhibit similar AMPAR clusters at synaptic sites. Taking the neuron-neuron synapse as a reference we want to compare the local glutamate responsivity of NG2 cells to that of neuronal spines.
Using 2-photon glutamate uncaging paired with the whole cell patch-clamp technique in CA1 stratum radiatum of hippocampal brain slices of NG2 DsRed mice between P7 and P15 we map functional AMPARs on the NG2 cell processes. Using bath application, MNI-caged glutamate (5mM) was uncaged for 0.6 ms at consecutive sites along NG2 cell processes. To keep experimental conditions comparable across all cells and cell types we only uncaged between 20 and 30 µm below the surface of the slice using a wavelegth of 720 nm and a laser power of 25 mW.
MNI glutamate was able to elicit inward currents in NG2 cells at all distances measured even at the very distal ends of their processes. The mean response elicited at one uncaging spot was -2.89 pA (± 0.15 pA, n = 332 uncaging spots on 6 cells) being largest next to the soma and decreasing with distance from the soma. Normalizing the uncaging response size to the process diameter showed that the decline in response size with distance is due to a thinning of the NG2 cells processes. Hierarchical clustering of both, the amplitude and the current density divided all uncaging runs (n = 56 sets of 6 sequential uncaging spots) in basically two groups, one with a low baseline responsivity and the other with consistently bigger responses. A first analysis of synthetic data to validate our finding shows that this distinct separation of the uncaging runs is unlikely to happen by chance. Our data indicate that NG2 cells express functional glutamate receptors along their processes at all distances tested and experience also sites of elevated glutamate responsivity. This suggests NG2 cells might be prepared to establish glutamatergic synapses anywhere on their surface.

Metabolic heterogeneity of astrocytes: insights from nanosensor imaging in the brain

Ulrike Winkler[1], Susanne Köhler[1], Johannes Hirrlinger[1,2]

[1]Carl-Ludwig-Institute for Physiology, Faculty of Medicine, University of Leipzig, Liebigstr. 27, 04103 Leipzig, Germany
[2]Department of Neurogenetics, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Str. 3, D-37075 Göttingen, Germany

Astrocytes are a glial cell type participating in all essential brain functions, including energy metabolism, ion homeostasis and neurotransmission. Based on histological studies it has been known for a long time, that CNS astrocytes have no uniform morphology. Protoplasmic astrocytes in the grey matter are complex “star-shaped”- cells with numerous fine processes, while fibrous astrocytes in the white matter are less complex with fewer branching processes. Astrocytes in grey matter mainly contact synapses, blood vessels and other brain cells, while white matter astrocytes mainly contact axons, oligodendrocytes and their myelin. Functionally, the major task for neurons within grey matter is transmission and computation of information at synapses; white matter tracts are specialized to allow reliable axon potential propagation along axons for long distances. Astrocytes display molecular and structural properties that are perfectly matched to the function of neighboring neurons. We hypothesized that astrocytes in grey and white matter differ in their basal energy metabolism as well as in the main regulatory mechanisms affecting astrocytic energy metabolism, but also providing feedback from metabolism to signaling events. We aim at unraveling the discriminative metabolic events in astrocytes of grey and white matter, the underlying regulatory principles as well as their physiological relevance for brain function. To address these questions we took advantage of genetically encoded, fluorescent biosensors for metabolites and studied in acutely isolated brain slices the dynamics of key metabolites including ATP (“ATeam”-sensor) and the NAD+/NADH-redox state (“Peredox”-sensor) as a key integrating node between metabolism and signaling. We identified distinct differences in astrocytic energy metabolism as well as in the main regulatory mechanisms between astrocytes located in the mouse cortex and corpus callosum, respectively. These results support the hypothesis that metabolism of astrocytes is subject to cellular heterogeneity between different brain regions and contribute to a deeper understanding of how brain energy metabolism is embedded in brain physiology.

Characterization of astrocytic calcium signals from intensity based fluorescence indicators

Andre Zeug[1], Volodymyr Cherkas[1], Gebhard Stopper[2], Franziska E. Müller[1], Evgeni Ponimaskin[1]

[1]Hannover Medical School, Cellular Neurophysiology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2]Saarland University, Department of Physiology, CIPMM Building 48, 66421 Homburg

Astrocytes, an abundant type of glial cells in the mammalian brain and spinal cord, play an important role in regulation of neuronal network functions. Because of their unique morphology, astrocytes can modulate the functional properties of thousands of synapses over defined anatomical regions. It has become evident that astrocytes can also be directly involved in modulation of synaptic signalling and synaptic plasticity, and furthermore that these functions are related to their intracellular Ca^{2+} dynamics. These Ca^{2+} signals in astrocytes can occur spontaneously and mainly rely on Ca^{2+} release from intracellular stores.

Continuous development of methods for quantitative detection of $[Ca^{2+}]_i$ opens up new horizons for scientists to study such physiological activities at a subcellular levels. The unique characteristic of astrocytic Ca^{2+} activity, however, still require the development of event detection algorithms, which correctly describe its versatile characteristics. Here we describe a novel approach for the analysis and quantification of astrocytic Ca2+ activity, obtained by intensity based genetically encoded Ca^{2+} indicators (GCaMPs).

Since the Ca^{2+} indicator signal not only scales with $[Ca^{2+}]_i$, but also with indicator concentration, we show that it is mandatory for quantification to correctly estimate fluorescence intensity at basal $[Ca^{2+}]_i$ (F_0). For that we developed a robust pixel based algorithm to estimate F0 on the basis of fluorescence fluctuation analysis, which runs in a fully automatic manner. Furthermore, on the basis of $\Delta F/F_0$ we developed a novel Ca^{2+} event detection algorithm, which handles multiple threshold levels and allows to identify and characterize dynamic and overlapping patterns of activity. Due to the versatile and flexible functionality of the algorithm we can show that the characteristics of astrocytic Ca^{2+} activity in primary hippocampal culture is highly dependent on incubation temperature. At room temperature we observe long lasting Ca^{2+} events with strong Ca^{2+} release and large regions of activity, while at 37°C much shorter events at limited regions and significat lower Ca^{2+} release were detected. On the basis of our event detection algorithm we are now able to provide a possible model for the found temperature dependence. We can furthermore identify the neuronal impact on the astrocytic Ca^{2+} activity.

Our strategy is robust and applicable for many kinds of microscopy, including its usage in two-photon excitation, confocal and epi-fluorescent microscopy.

Mechanisms and consequences of sodium signals in astrocytes of the mouse neocortex

Daniel Ziemens[1], Franziska Oschmann[2], Niklas J. Gerkau[1], Christine R. Rose[1]

[1]Heinrich Heine University Düsseldorf, Institute of Neurobiology, Universitätsstrasse 1, 40223 Düsseldorf, Germany
[2]Technical University Berlin, Neural Information Processing Group & Bernstein Center for Computational Neuroscience, 10587 Berlin, Germany

Activity-related sodium signaling resulting from activation of glutamate uptake represents a new form of astrocyte excitability. Astrocytes of the neocortex, as opposed to the hippocampus proper, also express ionotropic glutamate receptors, which might provide additional sodium influx pathways. We compared the mechanisms of sodium signaling in astrocytes and neurons in tissue slices of the neocortex and hippocampus of mice of both sexes, employing wide field and multiphoton imaging. Stimulation of glutamatergic afferents or glutamate application induced sodium transients that were twice as large in astrocytes of the neocortex than in the hippocampal CA1 area, despite similar neuronal responses. Astrocyte sodium signals were reduced by ~50% upon blocking of NMDA receptors in the neocortex, but not the hippocampus. Neocortical, but not hippocampal, astrocytes exhibited marked sodium increases in response to NMDA. Moreover, NMDA evoked local calcium transients in processes of neocortical astrocytes, which were dampened upon blocking sodium/calcium exchange (NCX) with KBR7943. Mathematical computation based on our data predict that NMDA-induced sodium increases drive the NCX into reverse mode, resulting in calcium influx. Taken together, our study reveals a considerable regional heterogeneity in astrocyte sodium signaling. Neocortical astrocytes respond with much larger sodium transients to glutamatergic activity than hippocampal astrocytes. Moreover, they experience NMDA-receptor-mediated sodium influx, which hippocampal astrocytes lack. NMDA-mediated sodium increases promote import of calcium through reverse NCX in astrocyte processes, adding to the calcium influx through NMDA receptors. This pathway thereby represents a new mechanism for the generation of local astrocyte calcium signaling in the neocortex.

Poster Topic

T10: Aging and Developmental Disorders

T10-1A Creatine transporter disorder: new insights into epileptic phenotype and diagnostic biomarkers
Laura Baroncelli, Francesco Cacciante, Leonardo Lupori, Raffaele Mazziotti, Elena Putignano, Tommaso Pizzorusso

T10-2A Excessive generation of NMDA receptor-dependent early network oscillations in a human stem cell-derived model of autism
Katharina Behr, Philippe Valmaggia, Ravi Jagasia, Josef Bischofberger

T10-3A Modulation of the Hyaluronan-Based Extracellular Matrix in Mouse Models of Epilepsy
Armand Blondiaux, Shaobo Jia, Renato Frischknecht, Alexander Dityatev, Eckart D. Gundelfinger, Constanze I. Seidenbecher

T10-4A Intranasal oxytocin enhances perceptual mechanisms for voice-identity recognition.
Kamila Borowiak, Katharina von Kriegstein

T10-1B Impact of caloric restriction on the *in vivo* cortical function in aged mice
Nicole Fröhlich, Nithi Asavapanumas, Chommanad Lerdkrai, Elizabeta Zirdum, Olga Garaschuk

T10-2B Novel mutations in the asparagine synthetase gene (ASNS) associated with microcephaly
Johannes Hirrlinger, Dorit Schleinitz, Ruth Stassart, Katrin Hoffmann, Peter Kovacs

T10-3B Long-term studies in mice assessing delayed effects of low and moderate radiation doses
Sabine M. Hölter, Marie-Claire Ung, Lillian Garrett, Claudia Dalke, Sarah Kunze, Daniel Pawliczek, Elizabeth A. Ainsbury, Jochen Graw

T10-4B The role of the metabotropic glutamate receptor 5 in the pathophysiology and therapy of generalized dystonia in the dtsz model
Stefanie Perl, Franziska Richter, Angelika Richter

T10-5B The role of cholinergic interneurons in dystonia: optogenetic stimulations in DYT1 knock-in mice reveal an endophenotype but no overt dystonic symptoms
Franziska Richter, Anne Bauer, Stefanie Perl, Anja Schulz, Angelika Richter

T10-1C The role of CBP in neurodevelopment and mental retardation
Melanie Schoof, Michael Launspach, Dörthe Holdhof, Malte Hellwig, Lynhda Nguyen, Beat Lutz, Ulrich Schüller

T10-2C Genetic variation of IL-6 expression modulates age-related memory decline
Björn Hendrik Schott, Lea Knopf, Hartmut Schütze, Constanze I. Seidenbecher, Emrah Düzel,

Anni Richter

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Creatine transporter disorder: new insights into epileptic phenotype and diagnostic biomarkers

Laura Baroncelli[1], Francesco Cacciante[2], Leonardo Lupori[2], Raffaele Mazziotti[3], Elena Putignano[1], Tommaso Pizzorusso[1,3]

[1]Neuroscience Institute, CNR, via Moruzzi 1, I-56124 Pisa, Italy

[2]Scuola Normale Superiore, piazza Cavalieri 7, I-56126 Pisa Italy

[3]Department of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA, University of Florence, Area San Salvi – Pad. 26, I-50135 Florence, Italy

Creatine (Cr) transporter (CrT) deficiency is an orphan disorder (CTD, OMIM #300352) characterized by intellectual disability, epilepsy and autistic-like behavior. Epilepsy is one of the symptoms with the greatest impact on everyday life of patients and families. Animal models are crucial tools to analyze disease mechanisms and to develop new therapeutic strategies. Four murine models of CTD are available so far. However, they have been analyzed only at the behavioral, neurochemical and anatomical level. To expand our knowledge about the face validity of the murine model, we monitored brain excitability and seizure susceptibility in the CrT knockout mice using video-EEG recording sessions. Our data show that CrT loss-of-function results in higher susceptibility to kainic acid (KA)-induced seizures, as assessed both at behavioral and electrophysiological level. Accordingly, we detected a prominent reduction of parvalbuminergic synapses in the cerebral cortex. This activity allowed us to fill a substantial gap in the current literature and to provide a more comprehensive set of normative data for the evaluation of potential therapeutic approaches. In addition, since CTD patients show impaired activity of cerebral cortex, we monitored visual responses in CrT ko mice throughout the disorder progression using longitudinal transcranial intrinsic optical signal (IOS) imaging and visual evoked potential (VEP) recordings. A peculiar increase of IOS and VEP response was detected in CrT ko mice, indicating that integrated visual assessment could be used as a classifying biomarker for CTD diagnosis and treatment assessment with high reliability. Importantly, VEP recordings and IOS imaging can be readily applied to humans, increasing the translational value of the visual biomarker.

Excessive generation of NMDA receptor-dependent early network oscillations in a human stem cell-derived model of autism

Katharina Behr[1], Philippe Valmaggia[1], Ravi Jagasia[2], Josef Bischofberger[1]

[1]Institute of Physiology, Group Bischofberger, Pestalozzistr. 20, 4056 Basel, Switzerland

[2]Pharma Research and Early Development: Discovery Neuroscience Department, F. Hoffmann-La Roche Ltd- Basel, Basel, Switzerland

Valproic acid (VPA) is an antiepileptic drug with histone deacetylase (HDAC) inhibition activity. VPA intake during pregnancy is associated with an increased risk of the unborn child to develop autism spectrum disorder (ASD). In rodent models, in utero VPA exposure results in an ASD-related behavioral phenotype probably mediated by changes in glutamatergic and GABAergic transmission as well as enhanced NMDA receptor-dependent plasticity. However, nothing is known about the effect of VPA on human neuronal development.

We used hESC-derived neural stem cells that can be differentiated into synaptic networks. Human neurons co-cultured with primary mouse astrocytes were treated with VPA throughout the differentiation process. Patch-clamp recordings were used to study the maturation of neuronal properties.

At 6 weeks of differentiation, we observed an increased frequency and amplitude of mEPSCs in VPA-treated neurons, indicating enhanced formation of glutamatergic synapses. 83% percent of VPA-treated cultures showed repetitive burst activity, which could not be detected in control neurons at the same developmental stage. After 10 weeks, also untreated cultures showed rhythmic oscillations, although at lower frequencies. The burst oscillations were critically dependent on NR2B NMDA-receptors, thereby resembling cortical early network oscillations (cENOs) during early brain development. The increased burst frequency in VPA-treated neurons was accompanied by larger burst-evoked NMDA currents.

Our results indicate that early VPA exposure of developing human neurons promotes premature onset and enhanced frequency of early network oscillations (ENOs) that can be rescued by NR2B NMDA receptor blockade. This suggests that modulation of NR2B NMDA receptors might ameliorate network deficits in ASD.

Modulation of the Hyaluronan-Based Extracellular Matrix in Mouse Models of Epilepsy

Armand Blondiaux[1], Shaobo Jia[2], Renato Frischknecht[1,3,4], Alexander Dityatev[2,3], Eckart D. Gundelfinger[1,3], Constanze I. Seidenbecher[1,3]

[1]Leibniz Institute for Neurobiology, Brenneckestraße 6, 39118 Magdeburg, Germany
[2]German Center for Neurodegenerative Diseases, Leipziger straße 44, 39120 Magdeburg, Germany
[3]Center for Behavioural Brain Science (CBBS), Magdeburg, Germany
[4]Current address: University of Erlangen-Nuremberg, Dept. Biology, Erlangen, Germany

Epilepsy is a frequent brain disorder which affects over 50 million people worldwide. Its hallmarks are bursts of synchronised activity in different brain regions called seizures. These seizures can happen at any time and have behavioural consequences; thus, they severely affect daily life and can lead to changes in the brain parenchyma and in synaptic connectivity.
Our project, set within the Marie Curie network ECMED, focuses on the hypothesis that the epileptic phenotype can be related to the composition and integrity of the hyaluronan-based (HA) extracellular matrix (ECM). This ECM is mostly composed of proteoglycans such as brevican or aggrecan, which bind HA with the help of link proteins such as HAPLN1, and glycoproteins like tenascin-R and -C. Moreover, network activity and synaptic changes have an impact on ECM gene expression and ECM proteolytic processing, including in cases of epilepsy.
In order to investigate the correlation between seizures and the state of the ECM, we used a new epilepsy model: three different mouse lines, mutant for the presynaptic scaffolding protein Bassoon (*Bsn* mutants). We recorded brain activity with EEG and then extracted the brains of these same animals. After separation of sub-cellular fractions, we quantify the level of HA-based ECM in different fractions of the recorded animals and correlate it to seizure parameters. To validate our findings, we also compare the *Bsn* mutant models to the widely used kainic acid (KA) model of epilepsy.
Our findings clearly show that a lack of functional Bsn leads to frequent seizures in adult mice. These seizures present with two phases, a first one with high frequency and low amplitude that evolves into the second stage with high amplitude coupled to a low frequency. A survival study suggests that the epilepsy could start in early life in these mutants, as the death rate is much higher for *Bsn* mutants during the first 20 weeks of life.
Regarding the ECM composition in the brain, there are some differences between Wild-Type (WT) and *Bsn* mutant animals. The most prominent changes affect tenascin-C, clearly more abundant in animals with high seizure numbers; brevican expression is also affected in *Bsn* mutants. Interestingly, we observe some differences in the ECM regulation when comparing with the KA model. This may be due to the different molecular causes of the epileptic phenotype (e.g. a pre-synaptic origin against a post-synaptic one; however, the site and the developmental timing of onset may also play an important role).
To check for the cause of epilepsy in *Bsn* mutants, we will investigate conditional KO of Bsn in inhibitory and excitatory neurons to see whether the epileptic phenotype involves more one of these networks over the other.

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Intranasal oxytocin enhances perceptual mechanisms for voice-identity recognition.

Kamila Borowiak[1,2,3], Katharina von Kriegstein[1,2]

[1]Technical University of Dresden, Chair of Cognitive and Clinical Neuroscience, Bamberger Straße 7, 01187 Dresden, Germany

[2]Max Planck Institute for Human Cognitive and Brain Sciences, Stephanstraße 1, 04103 Leipzig, Germany

[3]Berlin School of Mind and Brain, Humboldt University of Berlin, 10117 Berlin, Germany

Background: Individuals with autism spectrum disorder (ASD) have difficulties recognizing other people by the voice – a deficit associated with reduced responses in the right posterior superior temporal sulcus/ gyrus (pSTS/STG) (Schelinski et al., 2016, SCAN; Schelinski et al., 2017, Autism Res). The right pSTS/STG has been implicated in the perceptual analysis of voice-identity information (Maguiness et al., 2018, Neuropsychologia). Intranasal oxytocin can promote face recognition and modulate face-sensitive brain responses in ASD (Domes et al., 2013, Biol Psychiatry; Guastella et al., 2010, Biol Psychiatry). Here, we aimed to investigate whether intranasal oxytocin can enhance the impaired voice-identity recognition and increase the right pSTS/STG responses in ASD.

Methods: Nineteen adults with high-functioning ASD (ASD group) and nineteen typically developed (TD) controls (control group) participated in a randomized, double-blind, placebo-controlled, cross-over design study. The groups were pairwise matched on age, gender, handedness and intelligence quotient (IQ). All participants had normal hearing and were free of psychopharmacological medication. Participants with ASD had already received a formal clinical diagnosis of ASD (Asperger Syndrome/ childhood autism (1 male; verbal IQ = 109)) according to the diagnostic criteria of the International Classification of Diseases (ICD 10; World Health Organization, 2004). Within the study, they underwent additional clinical assessment using Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000, J Autism Dev Disord) conducted by researches with formal training.
Participants completed two sessions of a functional magnetic resonance imaging (fMRI) experiment on voice-identity recognition: after oxytocin and after placebo administration. The experiment included two conditions: voice-identity task and speech task. In both conditions, participants listened to blocks of neutral two-word sentences spoken by four male speakers. In the voice-identity task, participants matched the identity of each speaker to a target speaker. In the speech task, participants matched the content of each sentence to a target sentence. Targets were presented at the beginning of each block.
We calculated a three-way ANOVA: Substance (oxytocin, placebo) x Task (voice-identity, speech) x Group (ASD, control). Region of interest (ROI) was the right pSTS/STG, because it is known to show decreased BOLD response to voice-identity vs. speech recognition in ASD compared to TD controls (Schelinski et al., 2016, SCAN).

Results: Oxytocin did not have any effect on the behavioral performance in the voice-identity recognition experiment. For the ROI, we found a significant three-way interaction Substance x Task x Group ($p < .05$ FWE corrected). Post hoc t-tests revealed that oxytocin increased right pSTS/STG responses to voice-identity vs. speech in the control group, but not in the ASD group.

Conclusions: Our results suggest that oxytocin can enhance perceptual analysis of voice-identity features in the right pSTS/STG in TD individuals. This was not the case in ASD, at least not to the same extent as in TD population, which supports previous evidence that perception of voice identity is atypical in ASD.

Impact of caloric restriction on the *in vivo* cortical function in aged mice

Nicole Fröhlich[1], Nithi Asavapanumas[1], Chommanad Lerdkrai[2], Elizabeta Zirdum[1], Olga Garaschuk[1]

[1]University Tübingen, Institute of Physiology, Department of Neurophysiology, Keplerstraße 15, 72074 Tübingen, Germany

[2]Kasetsart University Bangkok, Faculty of Veterinary Medicine, Bangkok 10900, Thailand

Caloric restriction diet is known to extend the lifespan and to improve the cognitive function in several species. However, the underlying cellular mechanisms still remain inconclusive. Here we employed the *in vivo* two-photon Ca^{2+} imaging in wild type C57BL/6N mice to study effects of ageing and caloric restriction on the spontaneous as well as sensory-evoked Ca^{2+} signals. Layer 2/3 neurons in the frontal/motor and the primary visual cortex showed a robust ageing-related increase in the frequency of spontaneous neuronal Ca^{2+} transients in both cortical areas. Interestingly, ageing-related hyperactivity in the frontal/motor cortex developed already in 10-14 months old mice, whereas significant neuronal hyperactivity in the primary visual cortex was seen at the age of 18 months only. In addition, when compared to 3-month-old mice, neurons in the primary visual cortex of 18-month-old mice showed a significant decrease in orientation selectivity. Caloric restriction significantly reduced neuronal hyperactivity in both cortical areas. Moreover, 18-month-old mice with a 12-month-long caloric restriction diet showed a significant improvement of the orientation tuning in the primary visual cortex compared to age-match *ad libitum* fed mice. Furthermore, the caloric restriction regimen used significantly improved both pattern detection and pattern discrimination abilities of aged mice to the levels observed in 3-month-old controls.

Taken together, our data reveals a robust ageing-related neuronal hyperactivity in both the frontal/motor and the primary visual cortex, with frontal/motor cortex ageing earlier than the visual cortex. In the primary visual cortex, ageing-related neuronal hyperactivity is accompanied by an impairment of the orientation tuning. Caloric restriction reduces ageing-related neuronal hyperactivity in both cortical areas and prevents ageing-related impairment of sensory processing in aged mice.

Novel mutations in the asparagine synthetase gene (ASNS) associated with microcephaly

Johannes Hirrlinger[1,2], Dorit Schleinitz[3], Ruth Stassart[4], Katrin Hoffmann[5], Peter Kovacs[3]

[1]Carl-Ludwig-Institute for Physiology, University of Leipzig, Liebigstrasse 27, D-04103 Leipzig, Germany
[2]Department of Neurogenetics, Max-Planck-Institute for Experimental Medicine, Hermann-Rein-Str. 3, D-37075 Göttingen, Germany
[3]IFB AdiposityDiseases, Leipzig University Medical Center, University of Leipzig, Liebigstr. 19, D-04103 Leipzig, Germany
[4]Division of Neuropathology, Leipzig University Medical Center, Liebigstr. 26, D-04103 Leipzig, Germany
[5]Institute of Human Genetics, Martin-Luther-University Halle-Wittenberg, Magdeburger Str. 2, 06112 Halle (Saale), Germany

Microcephaly is a devastating condition defined by a small head and small brain compared to the age- and sex-matched population. Mutations in genes involved the synthesis of several non-essential amino acids have been described which cause a severe neurological and/or neurodevelopmental phenotype. This includes the gene encoding the enzyme asparagine synthetase (ASNS). Mutations in the ASNS gene were recently identified as causal mutations for rare forms of microcephaly and several different mutations in ASNS have been described worldwide. In a family with two affected girls, we identified novel compound heterozygous variants in ASNS (c.1165G>C, p.E389Q and c.601delA, p.M201Wfs*28). The first mutation (E389Q) is a missense mutation resulting in the replacement of a glutamate residue evolutionary conserved from Escherichia coli to Homo sapiens by glutamine. Protein modeling based on the known crystal structure of ASNS of E. coli predicted a destabilization of the protein by E389Q. The second mutation (p.M201Wfs*28) results in a premature stop codon after amino acid 227, thereby truncating more than half of the protein. While, the pathophysiological mechanisms linking mutations in the ASNS gene to the development of microcephaly remain enigmatic so far, these novel variants expand the growing list of microcephaly causing mutations in ASNS.

Long-term studies in mice assessing delayed effects of low and moderate radiation doses

Sabine M. Hölter[1], Marie-Claire Ung[1], Lillian Garrett[1], Claudia Dalke[1], Sarah Kunze[1], Daniel Pawliczek[1], Elizabeth A. Ainsbury[2], Jochen Graw[1]

[1]Helmholtz Zentrum München GmbH, Institute of Developmental Genetics, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany [2]Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Oxford OX11 0RQ, UK; for LDLensRad Consortium

In Western societies exposure to low dose ionizing radiation is pervasive, and the average annual exposure per person has doubled in the last two decades, mainly because of advances in the medical field. However, because in the nuclear age radiation research and protection were focused on high doses, the biological consequences of low dose exposure have hardly been investigated, particularly potential delayed long-term effects.

The INSTRA and LDLensRad projects use mice irradiated with low and moderate doses of γ-rays (^{60}Co) to study long-term radiation effects over the lifetime in a mammalian animal model. Of particular interest are radiation-sensitive tissues like the eye lens and the brain, and behavioural changes as potential early indicators of radiation effects.

Young adult mice of different genetic constitution (wild-type C57BL/6 x C3HeB/FeJ hybrids and *Ercc2*$^{+/-}$) were acutely whole body irradiated at the age of 10 weeks (0, 0.063, 0.125 and 0.5 Gy at 0.063 Gy/min; 0, 0.5, 1 and 2 Gy at 0.3 Gy/min). The *Ercc2* gene codes for the XPD protein that is involved in DNA repair, and heterozygous *Ercc2*$^{+/-}$ mice were expected to be more sensitive to radiation-induced DNA damage since their peripheral lymphocytes had been shown to be when irradiated *in vitro*. During the lifespan of the mice lens opacification was analysed monthly and alterations in behaviour were repeatedly assessed starting 4, 12 and 18 months post irradiation (p.i.). We used the open field test to assess motor and exploratory activity and anxiety, acoustic startle/prepulse inhibition to assess sensorimotor recruitment and gating, a social discrimination test for social memory and spontaneous alternation in the Y-maze for working memory assessment. Organs were collected at different time points p.i. (4 and 24 hours, 6, 12, 18 and 24 months) for histology and immunohistochemistry.

In the INSTRA study with irradiation doses up to 0.5 Gy over the lifetime a significantly altered survival rate and a dose-dependent increase for several types of tumours were found (Dalke et al. 2018, Radiat Environ Biophys 57:99-113). Behaviourally, long-term dose-dependent sensorimotor recruitment alterations were detectable as early as 4 months p.i., and decreases in motor and exploratory activity were found 12 and 18 months p.i.. The effect size of alterations induced by 0.5 Gy radiation was about a third of the effect size of age-related reductions in affected parameters. Lens opacification showed a subtle but statistically significant dose-dependent increase (approx. 1%) over the follow-up period of 24 month p.i., while the age-dependent increase of lens density was about 2%. At present it is unclear if the radiation-induced effects on the eye resulted in functional vision impairments that might have contributed to the reductions in motor behaviour. So far heterozygous *Ercc2*$^{+/-}$ mice did not prove to be more sensitive to radiation effects *in vivo*; at 18 months p.i. *Ercc2* heterozygosity rather seemed to be protective against age-related reductions in motor and exploratory activity independent of radiation.

In conclusion, at least in mice, a single low-dose ionizing irradiation event can have dose-dependent delayed long-term effects that are detectable in specialized tests. In further steps, we assess morphological and cellular changes in eye and brain p.i.. Upon completion of the INSTRA and LDLensRad projects there will also be more information about potential effects of the radiation dose rate.

The role of the metabotropic glutamate receptor 5 in the pathophysiology and therapy of generalized dystonia in the dtsz model

Stefanie Perl[1], Franziska Richter[2], Angelika Richter[3]

[1]University of Leipzig, VMF, Institute of Pharmacology, Pharmacy and Toxicology, An den Tierkliniken 15, 04103 Leipzig, Germany

Dystonia is a common movement disorder characterized by involuntary muscle contractions leading to abnormal postures and movements. The metabotropic glutamate receptor 5 (mGluR5) belongs to the family of G-protein coupled receptors and has a widespread expression within the striatum – a region with crucial importance in the pathophysiology of dystonia. It could be a promising pharmacological target in this disease, because inhibition of mGluR5 has been shown to improve dystonic symptoms in animal models of levodopa-induced dyskinesia.

Examinations of mGluR5 were carried out in the dtsz mutant hamster, a well-characterized model of paroxysmal dystonia with an age-dependent phenotype. The mGluR5 negative allosteric modulator fenobam (20, 30, 50 mg/kg) and the positive allosteric modulator CDBBP (10, 20 mg/kg) were injected intraperitoneally and the severity of the dystonic attacks was rated using an established score system.

The expression of mGluR5 was examined by immunohistochemistry (IHC) in striatal and cortical areas of mutant hamsters at the age of maximum severity of dystonic symptoms and after age-dependent spontaneous remission in comparison with controls. To examine the differences of the protein expression on the mRNA level, a qPCR was performed in dystonic hamsters at the age of high severity scores and age-matched non-dystonic animals.

Both investigated mGluR5 modulators did not exert significant effects on the severity of dystonia at all tested doses. However, positive modulation of mGluR5 by CDPPB transiently caused signs of general dyskinesia in dystonic dtsz hamsters. In contrast, no dyskinesia could be provoked by the injection of CDPPB in non-dystonic control hamsters.

By IHC we found a 35 % higher expression of mGluR5 in the striatum and cortex of mutant hamsters at an age of high dystonia severity scores compared to controls, notably not after remission of dystonia. As shown by qPCR there were no differences in the expression of striatal und cortical mGluR5 mRNA between dystonic and non-dystonic hamsters, suggesting altered post-transcriptional mechanisms.

The lack of acute effects of mGluR5 modulators on the severity of dystonia indicates that antagonists like fenobam do not provide potential for the treatment of inherited dystonia.
However, the induction of dyskinetic effects by CDPPB only in mutant hamsters as well as the age-dependent difference in mGluR5 expression indicate a role of mGluR5 in maladaptive plasticity as previously shown by increased corticostriatal long-term potentiation in several models of dystonia.
Therefore, ongoing studies will be done in an etiologic mouse model of dystonia and examine if deep brain stimulation, known to be effective in human dystonia, influence the expression of mGluR5.

The role of cholinergic interneurons in dystonia: optogenetic stimulations in DYT1 knock-in mice reveal an endophenotype but no overt dystonic symptoms

Franziska Richter[1], Anne Bauer[1], Stefanie Perl[1], Anja Schulz[1], Angelika Richter[1]

[1]Leipzig University, VMF Pharmacology, An den Tierkliniken 15, 04103 Leipzig, Germany

Dystonias are movement disorders defined by sustained or intermittent muscle contractions causing twisting movements and postures. The most prevalent inherited form of dystonia is caused by a mutation in the gene for torsin A (DYT1, deltaGAG) with incomplete penetrance. While the pathophysiology of DYT1 dystonia is largely unknown, ex vivo electrophysiological recordings in brain slices of animal models without dystonic phenotype suggested a role of striatal cholinergic interneurons. Here we aim to dissect the role of this alteration for motor dysfunctions in vivo by using optogenetic activation of striatal cholinergic interneurons in freely behaving mice.

For this purpose we crossed DYT1 deltaGAG heterozygous knock-in mice (DYT1) with mice expressing a light-dependent cation channel (Channelrhodopsin2, ChR2), essential for optogenetic examinations, specifically in cholinergic interneurons (Chat promotor). ChR2 is opened by blue light (wavelength 470 nm), leading to depolarization and acetylcholine release. The light (LED) is conveyed by optical cannulae, which were chronically implanted by stereotaxic surgery into the striatum of DYT1/ChR2 mice and wildtype (WT)/ChR2 littermates. Mice (males, n=6-11/group) at 3 and 6 months of age were stimulated with optical light pulses of varied durations and behavioral effects and neuronal activity were recorded.

Six months old DYT1 KI mice but not wildtype controls responded with hyperactivity to blue light specifically at 25 ms pulse duration, 10 Hz frequency. Neuronal activity (c-Fos) in cholinergic interneurons was increased immediately after light stimulation and persisted only in DYT1 KI over 15 min. Substance P was increased specifically in striosome compartments in naïve DYT1 KI mice compared to wildtype. Under optogenetic stimulation substance P increased in wildtype to match levels in Dyt1 KI. At 3 months of age neither DYT1 KI nor wildtype controls responded with hyperactivity or persistent neuronal activity to optogenetic stimulations. No signs of dystonic movements were observed under stimulation of up to one hour in both genotypes and age groups, and the sensorimotor deficit previously observed in 6 months old Dyt1 KI mice persisted under stimulation.

Increased cholinergic neuronal activity upon optogenetic stimulation and higher expression of substance P are likely involved in observed hyperactivity in 6 months old DYT1 KI mice. This disturbed regulation of the striatal cholinergic system was, however, not sufficient to exert dystonic symptoms. Furthermore, the sensorimotor phenotype previously observed in this model of dystonia was not altered. Thus alterations in cholinergic interneurons may not be the crucial culprit in development of overt dystonia in mutation carriers.

The role of CBP in neurodevelopment and mental retardation

Melanie Schoof[1,2], Michael Launspach[1,2], Dörthe Holdhof[1,2], Malte Hellwig[1,2], Lynhda Nguyen[1,2], Beat Lutz[4], Ulrich Schüller[1,2,3]

[1]Research Institute Children's Cancer Center Hamburg, Martinistraße 52, 20251 Hamburg, Germany
2 Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Germany
3 Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Germany
4 Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany

Mutations in the cAMP-response element binding protein (CREB)-binding protein (CBP) are causing the Rubinstein-Taybi-Syndrome (RSTS), a neurodevelopmental disease leading to numerous different symptoms including distinct facial features, physical anomalies as well as intellectual disability (ID).
Here, we investigate the molecular mechanisms of CBP function in brain development and adult neurogenesis in order to understand morphological and functional reasons for ID in RSTS patients.
Due to the embryonic lethality of conventional CBP knockout mice, we employed a tissue specific knockout model (hGFAP-cre::$CBP^{Fl/Fl}$) to achieve a homozygous deletion of CBP in cells of the central nervous system (CNS). The knockout mice are analyzed by behavioral, immunohistochemical, as well as in vitro methods to uncover mechanisms and abnormalities.
Our results reveal a phenotype resembling multiple aspects of RSTS like microcephaly and behavioral anomalies. Prominent histological findings include a hippocampus that is significantly smaller in size and has fewer stem cells residing in the subgranular zone of the dentate gyrus. In the SVZ, we observe large cell aggregations at the beginning of the rostral migratory stream due to a migration deficit caused by impaired attraction from the CBP-deficient olfactory bulb.
The cerebral cortex of mutant mice is characterized by a shorter dendrite length, a diminished spine number and a relatively increased number of immature spines in pyramidal neurons.
In summary, we provide evidence that CBP is shaping neuronal morphology and is involved in neuronal cell migration. These findings may help to understand the molecular basis of mental retardation in RSTS patients and may be employed to establish treatment options to improve patients' quality of life.

Genetic variation of IL-6 expression modulates age-related memory decline

Björn Hendrik Schott[1,2], Lea Knopf[1,3], Hartmut Schütze[3,4], Constanze I. Seidenbecher[1], Emrah Düzel[3,4], Anni Richter[1]

[1]Leibniz Institute for Neurobiology, Department of Behavioral Neurology, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]University Medicine Göttingen, Department of Psychiatry and Psychotherapy, Göttingen, Germany
[3]Otto von Guericke University, Magdeburg, Germany
[4]Helmholtz Center for Neurodegenerative Diseases (DZNE), Magdeburg, Germany

BACKGROUND: Cognitive performance is subject to age-related decline, with explicit memory being particularly affected by aging, even in otherwise healthy individuals. Subclinical inflammatory processes have been implicated in age-related cognitive impairment, and we hypothesized that genetic variations affecting the expression of pro-inflammatory cytokines might thus influence memory performance in the elderly. We focused on the interleukin 6 (IL-6) -174G>C polymorphism (rs1800795), which has previously been associated with IL-6 expression levels in vitro and with cognitive deficits in humans (Napolioni & MacMurray, 2016)

METHODS: We investigated explicit memory performance and memory-related hippocampal activation in healthy participants who were genotyped for the Il- 6 -174G>C polymorphism (rs1800795). Explicit memory performance was assessed using the Verbal Learning and Memory Test (VLMT; Helmstaedter et al., 2001) in a cohort of young (18-35 yrs), middle aged (50-65 yrs), and elderly (66-80 yrs) participants (N = 195). Furthermore, functional MRI was acquired in a subsample of the cohort (N = 129, including 54 young and 75 elderly participants), while participants performed a previously described visual memory-encoding task (Düzel et al., 2011).

RESULTS: The G allele, which was previously linked to higher IL-6 expression in vitro, was associated with significantly lower VLMT performance. When performing separate analyses for each age group, we found that this effect was largely restricted to elderly participants.
During successful encoding of novel scene stimuli, we further observed a genotype by age interaction in the hippocampus, with elderly G homozygotes showing diminished encoding-related hippocampal activation.

CONCLUSION: Our findings indicate that IL-6 rs1800795 is associated with hippocampus-dependent memory in healthy humans, with G homozygotes showing relatively lower encoding performance and reduced hippocampal activation. Notably, this genotype-dependent difference was age-dependent, suggesting that genetically mediated differences in the expression of pro-inflammatory IL-6 modulate memory decline in elderly humans.

retracted

NEUROPATHOLOGICAL FINDINGS IN CATTLE WITH HISTOLOGICALLY SUSPECTED BOVINE SPONGIFORM ENCEPHALOPATHY IN NIGERIA

James Clinton Shawulu[1], Joseph Olajide Hambolu[2], Samuel Adeniyi Ojo[2], James Olukayode Olopade[3], Anne Balkema-Buschmann[4], Reiner Ulrich[5], Martin Hermann Groschup[4]

[1]University of Abuja, Veterinary Anatomy, PMB 117, Garki, 900001 Gwagwalada-Abuja, Nigeria

[2]Ahmadu Bello University Zaria, Veterinary Anatomy,PMB 2555 Samaru Zaria, Nigeria.

[3]University of Ibadan, Veterinary Anatomy, Ibadan Nigeria.

[4]Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Sudufer 10/17493 Greifswald, Germany.

[5]Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeffler-Institut,Sudufer 10/17394 Greifswald, Germany.

Introduction: The aim of the study was to diagnose encephalitis of unclear origin and neurodegerative diseases in cattle breeds in Nigeria.
Materials and methods: Formalin-fixed paraffin-embedded (FFPE) brain tissues from 246 cattle of both sexes sacrificed for human consumptions and obtained from abattoir were investigated for age-related features, rift valley fever virus, Aujeszky's disease and rabies RNA virus by Immunohistochemistry (IHC). GFAP and Iba1 assessments of microgliosis and astrogliosis activation. The use of mab 12F10 immunoreactivity in the assessment of PrPBSE was also carried out in older animals. RNA isolation was carried out and PCR was done for rabies viruses, rift valley fever viruses and Aujeskys disease virus.
Results: Major changes observed were intracellular accumulation of substances involving neuronal vacuolations and lipofuscin accumulations (61.4%), neuronal and or axonal degenerations and loss involving axonal spheroids (51.6%), Inflammatory changes mostly perivascular cuffings (50.0%), extracellular accumulation of substances involving brain sands and vascular calcium mineralization (29.7%), hypercellularity (19.1%) and spongy state (0.4%) in 233 cattle. Histopathological changes, some of which were age related, were identified in the brains of 233 cattle involving all the four breeds. These changes started early in life (2-4 years) in these animals. Intracellular vacuolations were all observed in neuronal cells and this depicts the vulnerability of neurons to injury or trauma in the nervous system. Immunohistochemistry for the lesions in two suspected cattle for BSE was positive.
Conclusions: The histological findings were highly suspicious for an infectious etiology. However, frozen samples were not available to run western blot assay on these animals. The isolation of RNA from FFPE brains did not yield products more than 200bp. Alternative PCRs have to be established to detect potential emergent and re-emergent infections in Nigeria using FFPE materials.

Modeling channelopathies: From ion currents to firing behavior

Lukas Sonnenberg[1,2], Yuanyuan Liu[2], Holger Lerche[2], Jan Benda[1]

[1]Institute for Neurobiology, University of Tübingen, Germany

[2]Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tuebingen, Germany

Mutations in Na-channels can lead to a variety of neurological diseases like epilepsy or autism. In order to characterize the effects of the mutations on their biophysical properties, the mutated ion channels are usually expressed in cell lines without endogenous ion channels, and the resulting ionic currents are measured in voltage-clamp experiments with standardized protocols. An still open question is, how the altered biophysical properties of an ion channel measured in an expression system translate into modifications of firing properties in a real neuron, measured, for example, in a brain slice.

We developed a self-consistent procedure to retrieve the biophysical properties of the ionic currents from the voltage-clamp experiments, based on fitting a Hodgkin-Huxley type model of the ionic current to the data. Using this parameterization of the sodium current mutant we predicted its effect on the cellular level by plugging it into a conductance-based model. We validate the prediction experimentally by blocking sodium currents in a slice preparation and replacing them by the measured sodium channel via dynamic clamp.

This allowed us to construct and test quantitative models and to asses the predictive power of characterizations of ionic currents in expression systems. This is a first step towards personalized medicine for patients with channelopathies.

Decreased Auditory Nerve Population Activity in Aged Gerbils

Friederike Steenken[1], Rainer Beutelmann[1], Henning Oetjen[1], Georg M. Klump[1], Christine Köppl[1]

[1]Cluster of Excellence "Hearing4all", Dept. of Neuroscience, Carl von Ossietzky University Oldenburg, Germany

Introduction
Gerbils are an attractive presbyacusis model due to their good low-frequency hearing and short life expectancy (Cheal, 1986, Exp Aging Res 12(1): 3-21). The origin of age-related hearing loss is still debated (Gleich et al., 2016, Exp Gerontol 84: 61-70). Here, mass potentials (or neural noise) recorded at the round window (RW) of quiet-aged Mongolian gerbils (*Meriones unguiculatus*) were used to measure the neural index of the auditory nerve in response to broadband noise. This has been shown to be a sensitive metric for the number of active auditory nerve fibers, including low-spontaneous-rate fibers (Batrel et al., 2017, PLoS One 12(1):e0169890).

Methods
An electrode was placed in the RW niche of Mongolian gerbils under ketamine/xylazine anesthesia. Third-octave band noise (300 ms) with center frequencies of 1.6-16 kHz was presented monaurally at 60 dB SPL in stimulus pairs of opposite polarities. Only the steady-state response (last 200 ms) was analyzed. The mean of the responses to both polarities was calculated, eliminating the cochlear microphonic, and the power spectral density (PSD) computed. A neural gain was obtained by dividing point-by-point the PSD of the unstimulated by the stimulated condition. Finally, a neural index for each frequency band was derived by integrating the area under the neural gain curve, resulting in an auditory-nerve excitation pattern across frequency.

Results
In a total of 9 aged (23-39 months) and 38 young adult (4-8 months) ears, the neural index of young gerbils peaked broadly at two frequencies, as previously shown. The overall auditory-nerve excitation of aged gerbils was significantly reduced compared to that of young adults (mixed-model ANOVA, $p<0.001$). Post-hoc tests for each frequency band showed significant reductions between 1.6 and 3.3 kHz and at 16 kHz, with similar trends at all other frequencies. On average, the decrease of the neural index indicated a loss of about 28% of auditory nerve fibers, and up to 45% at 2.5 kHz.

Conclusions
Gleich et al. (2016) showed a numerical decrease in ribbon synapses in aged gerbils, with the largest loss, near 40%, in apical regions. Consistent with that, the neural index suggests losses of active auditory nerve fibers of a similar magnitude. Importantly, both metrics show the clearest loss in low-frequency cochlear regions. This was unexpected, since synaptopathy after noise exposure preferentially affects low-spontaneous-rate fibers that, in the gerbil, predominate at high frequencies.

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Effects of Amphetamine and Ecstasy in Mice Lacking the Postsynaptic Scaffolding Protein SHANK1: Link to Catecholamine and Indolamine Systems?

Özge Sungur[1,2], Tobias M. Redecker[1], Elena Andres[3], Wiebke Dürichen[1], Rainer K.W. Schwarting[1,2], Adriana del Rey[3], Markus Wöhr[1,2]

[1]Philipps-University of Marburg, Behavioral Neuroscience, Gutenbergstr. 18, 35032 Marburg, Germany

[2]Center for Mind, Brain, and Behavior (CMBB), Philipps-University of Marburg, Hans-Meerwein-Str. 6, 35032 Marburg, Germany

[3]Research Group Immunophysiology, Division of Neurophysiology, Institute of Physiology and Pathophysiology, Philipps-University of Marburg, Deutschhaus-Str. 2, 35032 Marburg, Germany

SHANK proteins, with three known members SHANK1, SHANK2, and SHANK3, are the master scaffolding proteins of the postsynaptic density, connecting neurotransmitter receptors and other membrane proteins with downstream signaling cascade and actin cytoskeleton. Shankopathies are implicated in various neuropsychiatric disorders, including autism spectrum disorder (ASD), schizophrenia (SCZ), and bipolar disorder (BPD). Psychostimulant-induced hyperactivity is a commonly applied paradigm to assess behavioral phenotypes related to BPD and can help elicit mania-like elevated drive in mouse models. Therefore in the present study we tested (i) whether *Shank1* plays a role in the behavioral effects of psychostimulants d-amphetamine (AMPH) and 3,4-methylenedioxymethamphetamine (MDMA, commonly known as ecstasy), and (ii) whether this is associated with neurochemical alterations. Male and female null mutant *Shank1-/-* mice were treated with AMPH (2.5 mg/kg) and MDMA (20 mg/kg), and psychostimulant-induced hyperactivity was compared to heterozygous *Shank1+/-* and wildtype *Shank1+/+* littermate controls. *Shank1-/-* mice displayed reduced psychostimulant-induced hyperactivity, although psychostimulants robustly stimulated locomotor activity in littermate controls. While AMPH-induced hyperactivity was reduced but still detectable in *Shank1-/-* mice, MDMA-induced hyperactivity was completely absent in *Shank1-/-* mice. Although there was an overall increase in circling behavior following MDMA treatment, the increase occurred irrespective of genotype and can thus not explain the lack of MDMA-induced hyperactivity in *Shank1-/-* mice. Reduced efficacy of psychostimulants to stimulate hyperactivity in *Shank1-/-* mice might be associated with alterations in the concentrations of catecholaminergic and indolaminergic neurotransmitters, metabolites, and precursors in prefrontal cortex, nucleus accumbens, and hypothalamus.

Cellular and molecular causes of normal motor aging in *Drosophila*

Jessica Thiem[1], Jean-Yves Roignant[2], Susanne Gerber[3], Christos Consoulas[4], Carsten Duch[1]

[1]Johannes Gutenberg-Universität, Institute of Developmental Biology and Neurobiology, Hanns-Dieter-Hüsch-Weg 15, 55128 Mainz, Germany

[2]Institute of Molecular Biology gGmbH, Ackermannweg 4, 55128 Mainz, Germany

[3]Johannes Gutenberg-Universität, Institute of Developmental Biology and Neurobiology, Staudingerweg 9, 55128 Mainz, Germany

[4]National and Kapodistrian University of Athens Medical School, Division of Experimental Physiology, Athens, Greece

With the dramatically increasing life expectancy in modern human societies, a mechanistic understanding of the biological principles of age-related functional nervous system decline becomes increasingly relevant for developing strategies toward healthy aging. We utilize experimental advantages and the relatively short life span of the genetic model, *Drosophila melanogaster*, to study cellular and molecular causes of motor system decline during aging.

With respect to identifying age-related changes in gene expression, numerous studies have utilized *Drosophila* to acquire nervous system, and more recently, even cell type specific transcriptome at different chronological ages. However, it is often difficult to distinguish whether transcriptional changes are cause or consequence of functional decline. We have therefore established a motor behavioral paradigm to separate middle aged flies with low from flies with high life expectancy, before these individuals show obvious motor deficits. We now employ differential RNA Seq of these two groups to identify transcriptional causes for low versus high life expectancy. Preliminary data reveal known molecular players of brain aging, like insulin and immune signaling factors, thus providing proof of principle. In addition, this approach starts identifying previously unknown players, which are currently being validated.

In an effort to map the cellular causes of functional motor decline, we focus on the visually induced escape response of *Drosophila*, because it is an essential behavior for survival and the underlying neural circuit is well mapped and genetically accessible. We found that flies lose this escape response at high ages. Electrophysiological and neuroanatomical data indicate that the motor part of the circuit as well as sensory neurons remain fully functional even when the behavior is lost. Similarly, axonal action potential conduction, and synaptic output from the giant fiber interneuron remain normal. This now leaves a small number of identified interneurons and synapses to pinpoint the cellular cause of age-dependent motor behavior decline. In addition to offering a chance to map age related loss of a motor behavior precisely into a known circuit, the data already demonstrate differential vulnerability within a defined neural circuit.

Neural and Heart Rate Variability and their Relation to Cognitive Performance in Young and Old Adults

Diana Toscano Tejeida[1,5], Kristina Miloserdov[1,2,3], Iris Steinmann[1], Carsten Schmidt-Samoa[1], Annekathrin Schacht[4], Melanie Wilke[1,2,3]

[1]Universitätsmedizin Göttingen, Institut für Kognitive Neurologie, Robert-Koch-Straße 40, 37075 Göttingen, Germany

[2]German Primate Center, Leibniz Institute for Primate Research, Kellnerweg 4, Göttingen, 37077, Germany

[3]DFG Center for Nanoscale Microscopy & Molecular Physiology of the Brain (CNMPB).

[4]Affective Neuroscience and Psychophysiology, Georg-Elias-Müller-Institute of Psychology, Goßlerstraße 14, Göttingen, 37073, Germany

[5]International Max-Planck Research School Neuroscience Program, European Neuroscience Institute, Grisebachstraße 5 ,Göttingen, 37077, Germany

Introduction: As life-expectancy increases worldwide, so does the need for early markers of age-related cognitive decline. While high behavioral intraindividual variability (IIV) has been associated with poor cognition, it is not clear whether behavioral and neural IIV represent the same phenomenon, and if there is an association between neural IIV and cognitive performance. Additionally, it is still not known how both neural and behavioral IIV are related to Heart Rate Variability (HRV), whose reduction has been correlated to cognitive decline. Variability quenching is a measure of neural IIV and refers to the reduction in trial-by-trial variability observed after the presentation of a stimulus. In this study, we assessed variability quenching and HRV in young and elderly healthy subjects during an image recognition task to determine how neural IIV and HRV relate to behavioral IIV and cognitive performance.

Methods: Variability quenching and HRV were measured using EEG and EKG recordings while participants (young: n = 8, mean age: 25 years ± 4.7 SD; elderly: n = 8, mean age 62 years ± 7.6 SD) performed an image recognition task. Several neuropsychological tests were used to measure the cognitive performance and behavioral IIV of the subjects.

Results: Young participants exhibited significantly higher HRV compared to elderly subjects. High variability quenching levels (i.e. reduced neural IIV) were significantly correlated with better scores and lower behavioral IIV in neuropsychological tests measuring executive functions, while higher HRV was significantly correlated to better cognitive performance. No association was found between HRV and neural or behavioral IIV.

Conclusions: Our results provide evidence for the relationship between neural IIV and executive cognitive functions, contributing to a better understanding of the neural correlates of behavioral IIV. Additionally, our results support an association of HRV and variability quenching to cognitive performance, making them candidates for early markers of age-related cognitive decline. The lack of association between HRV and neural variability quenching suggests that they may be reflecting two distinct mechanisms underlying the cognitive decline that comes with aging.

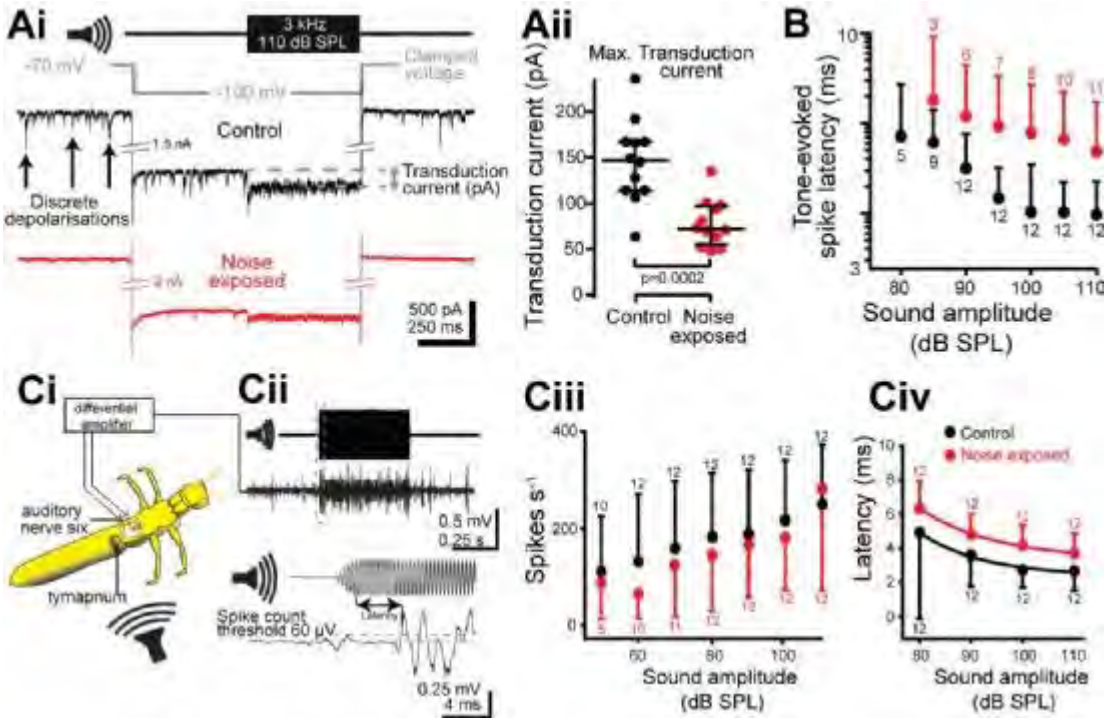
Understanding noise-induced hearing loss using the ear of the desert locust.

Ben Warren[1]

[1]University of Leicester, Department of Neuroscience, Psychology and Behaviour, University Road, LE1 7RH Leicester, United Kingdom

If you listen to loud music for long enough you will suffer noise-induced hearing loss (NIHL). The louder you listen the more rapidly you lose your hearing. At the level of the auditory neurons the loudness of the music is positively correlated to the open state of the transduction channels that convert sound energy into electrical potentials. Thus, when enough cations have entered the auditory neurons a tipping point is reached and the pathologies of NIHL begin. My work uses the tympanal ears of the desert locust to understand how auditory neurons cope with exposure to loud sound.

Using whole-cell patch-clamp from individual auditory neurons we found that the sound-evoked transduction current, and the spikes that this current triggers, are reduced in auditory neurons exposed to excessive loud noise (3kHz tone, 24 hrs at 126 dB SPL), compared to their silent counterparts (Figure 1A). The latency of the transduction current and resulting spikes are also reduced (Figure 1B) - a hallmark of NIHL in humans and in a wide range of animal models. These ex vivo findings were complemented by in vivo extracellular recordings from the auditory nerve in an intact auditory system (Figure 1C). The sound-transducing ion channels reside at the ciliated distal end of the locust's auditory neurons. The transduction current then triggers small dendritic spikes that travel along the 100 µm-long dendrite to the soma. At the axon hillock larger axonal spikes are generated by the sound-evoked dendritic spikes. We injected current into the soma and found that axonal spikes are unaffected by noise exposure. We, thus, hypothesise that the transduction channels are reduced upon noise-exposure and this helps protect downstream spike-generating machinery within the auditory neurons.



The use of biosensors in studying ageing

Alexander Wirth[1], Andre Zeug[1], Franziska Müller[1], Evgeni Ponimaskin[1]

[1]Medical School Hannover, Cellular Neurophysiology, Carl Neuberg Straße 1, 30625 Hannover, Germany

Age-induced memory impairment (AMI) can be a result of physiological ageing. Reducing nutrient-signalling (caloric restriction) and upregulation of autophagy has been described as major contributors to increased healthy life expectancy. While the level of polyamines such as spermidine declines with ageing, restoring polyamine levels as well as the induction of autophagy by nutrient-signalling is thought to play a key role in healthy ageing.

Here we aimed to analyse cellular and molecular mechanisms underlying physiological ageing, a prerequisite for ageing-induced cognitive deficits. By stereotactic injection of adeno-associated viruses (AAVs) into the hippocampus of young as well as aged mice, we transduced biosensors to investigate changes in different signalling cascades during ageing in space and time. Using Förster resonance energy transfer (FRET)-based biosensors and two-photon excitation we investigated mTOR kinase signalling, a metabolic key kinase in nutrition sensing. Furthermore, we looked at the induction of autophagy in aged mice, using ATG-homolog cleavage sensors. We also characterised calcium waves in astrocytes of aged mice. To elaborate the cellular and molecular changes occurring in those pathways, we compared young (i.e., five month old mice) with aged 24 month old mice. To address if the restoration of spermidine levels slows down pathological changes in signalling during ageing, we also investigated late-in-life spermidine fed aged mice using the above mentioned biosensors.

Poster Topic

T11: Alzheimer's, Parkinson's and other Neurodegenerative Diseases

T11-1A miRNA-expression profiling in midbrains of Parkinson's Disease patients
Lucas A. Caldi Gomes, Anna-Elisa Roser, Gaurav Jain, André Fischer, Paul Lingor

T11-2A Quantitative imaging of spreading-depression associated ROS production
Marc Ackermann, Katharina Dietrich, Michael Müller

T11-3A Intracerebroventricular administration of histidine reduces kainic acid induced convulsive seizures in mice.
Serdar Alpdogan, Felix Neumaier, Jürgen Hescheler, Toni Schneider

T11-4A Abnormal Amyloid beta accumulation leads to neuronal loss and alterations in the process of adult hippocampal neurogenesis
Sanila Amber, Fatima Javed Mirza, Deeba Hassan, Saadia Zahid

T11-5A Pharmacological and genetic inhibition of ADAM10 reduce brain damage after experimental traumatic brain injury
Dominik Appel, Regina Hummel, Larissa Dangel, Maryam Treiber, Johanna Merz, Martin Weidemeier, Christina Gölz, Mirko H.H. Schmidt, Kristina Endres, Michael K.E. Schäfer

T11-6A Complexome profiling of the mitochondrial respiratory chain – Mechanistic insights into the regulation of energy metabolism in neurodegenerative diseases
Susanne Arnold

T11-7A Copper, zinc and HSPGs influence trans-dimerization of the amyloid precursor protein family members
Alexander August, Nadine Schmidt, Johannes Klingler, Frederik Baumkötter, Jessica Klement, Carolyn Vargas, Klemens Wild, Sandro Keller, Stefan Kins

T11-8A Fe65 and Fe65L1 have distinct functions in synaptic plasticity
Vanessa Augustin, Paul Strecker, Susann Ludewig, Elisa Kraechan, Marcel Daas, Martin Korte, Jonathan Stephan, Stefan Kins

T11-9A AP-2 prevents amyloidogenic processing of APP via endocytosis-independent regulation of BACE1 trafficking in neurons
Sujoy Bera, Elena Calleja Barca, Albert Negrete, Julia Racho, Christoph Wittich, Nina Ellrich, Soraia Martins, James Adjaye, Natalia L. Kononenko

T11-10A Nanoscale analysis of cytoskeletal alterations during acute axonal degeneration in primary neuron cultures

Arndt Lucas Biller, Elisabeth Barski, Elisa D´Este, Mathias Bähr, Paul Lingor, Jan- Christoph Koch

T11-11A In vivo Imaging and Transcriptome Analysis of Astrocytes in an Alzheimer's Disease Mouse Model
Nelli Blank, Lech Kaczmarczyk, Stefanie Herresthal, Walker S. Jackson, Joachim L. Schultze, Gabor C. Petzold

T11-12A Tau blocks amyloid-β dependent neuronal hyperactivity in vivo
Marc Aurel Busche, Susanne Wegmann, Simon Dujardin, Caitlin Commins, Julia Schiantarelli, Tarun V. Kamath, Naomi Klickstein, George Carlson, Israel Nelken, Bradley T. Hyman

T11-13A The NMDA antagonist memantine attenuates the okadaic acid induced short-term spatial memory impairment and hippocampal cell lossin rats
Mariam Chighladze, Manana Dashniani, Khatuna Rusadze

T11-14A Neddylation-dependent protein degradation as a nexus between neuronal insulin signaling, amyloidosis and metabolic syndrome
Alessandro Dario Confettura, Guilherme Monteiro Gomes, PingAn Yuanxiang, Andreas Hentschel, Robert Ahrends, Michael Kreutz

T11-15A Identification of signaling mechanisms regulating mitochondria-endoplasmic reticulum contact sites
Renata Couto, Ira Milosevic, Nuno Raimundo

T11-16A Small conductance calcium-activated (SK3) potassium channel overexpression in nigrostriatal system of mice
Elaine Del Bel, Sabine Martin, Marcio Lazzarini, Miso Mitkovski, Luis Pardo, Walter Stuhmer

T11-1B Microstructural analysis of endo- and perineurial cells in human neuroma
Patrick Dömer, Bettina Kewitz, Christian Heinen, Thomas Kretschmer, Ulrike Janssen-Bienhold

T11-2B Trimethyltin-induced neurodegeneration is associated with gradual up-regulation of ecto-5^{I} nucleotidase on activated microglia
Milorad Dragic, Nataša Mitrovic, Nadežda Nedeljkovic, Ivana Grkovic

T11-3B Arbutin promotes functional recovery following lysolecithin-induced demyelination in rat optic chiasm
Forough Ebrahimtabar, Fatemeh Ebrahimtabar, Atena Nazari, Mahdi Pouramir, Manuchehr Ashrafpour, Fereshteh Pourabdolhossein

T11-4B APP gene family members as synaptic adhesion molecules
Simone Eggert, Sandra Schilling, Jonathan Stephan, Mathieu Meleux, Marius Zimmermann, Alexander August, Martin Korte, Edward H. Koo, Ulrike C. Müller, Stefan Kins

T11-5B Impact of voluntary running and environmental enrichment on learning and memory performance in APP/PS1 mice
Thomas Endres, Monique Klausch, Georgia-Ioanna Kartalou, Elke Edelmann, Kurt Gottmann,

Volkmar Lessmann

T11-6B Stimulation of mGluR1/5 improves the defective internalization of AMPA receptors in NPC1 mutant mouse
Xiao Feng

T11-7B Redox homeostasis modulates axonal microtubule dynamics: Effects on microtubule-dependent transport and tau-microtubule interaction
Chrsitian Gach, Benedikt Niewidok, Nancy Monteiro Abreu , Daniel Villar Romero , Lena Schünemann, Jacqueline Becker, Maike Schober, Anna-Sophie Schwarze, Roland Brandt

T11-8B Nanobody-based Sensor for Detecting α-Synuclein-Transmission in Parkinson's Disease Models
Christoph Gerdes, Natalia Waal, Hannes Verbarg, Nora Wender, Buket Basmanav, Stefan Becker, Silvio Rizzoli, Sebastian Kügler, Felipe Opazo

T11-9B Hereditary spastic paraplegia in *Danio rerio*
Bart Geurten, Gudrun Kracht, Wiebke Möbius, Torben Ruhwedel, Hauke Werner, Ralf Heinrich, Roland Dosch

T11-10B Combination of different approaches for the characterization of a Rodent Alzheimer's Disease Model
Barbara Hinteregger, Tobias Madl, Joerg Neddens, Birgit Hutter-Paier, Robert Wronski

T11-11B Deep brain stimulation in the inferior colliculus induces anxiolytic effect and improves haloperidol-induced catalepsy in rats
Hannah Ihme, Rainer K. W. Schwarting, Liana Melo-Thomas

T11-12B The role of 5-HT_7-receptor signalling in neurodegenerative diseases
Kathrin Jahreis, Josephine Labus, Evgeni Ponimaskin

T11-13B Modification of the spreading of α-synuclein pathology in vivo
Karina Joppe, Lars Tatenhorst, Anna-Elisa Roser, Stefan Becker, Mathias Bähr, Paul Lingor

T11-14B Rescue of dendritic spine pathology in the hippocampus of APP/PS1 mice
Georgia-Ioanna Kartalou, Thomas Endres, Volkmar Lessmann, Kurt Gottmann

T11-15B Early changes in hippocampal network oscillations and parvalbumin protein expression in a mouse model of Alzheimer's disease
Jochen Kuhse, Jan-Oliver Hollnagel, Oliver Kann, Joachim Kirsch, Eva Kiss

T11-16B Disruption in the hippocampal network function in inducible *FMR1* premutation mice
Ufuk Emre Kul, Guersel Caliskan, Renate Hukema, Rob Willemsen, Monica Santos Santos, Oliver Stork

T11-1C Cortical and subcortical volumetry in patients with Parkinson's disease and cognitive impairment
Martin Kunz, Martin Gorges, Hans-Jürgen Huppertz, Inga Liepelt-Scarfone, Alexander Storch, Richard Dodel, Rüdiger Hilker-Roggendorf, Daniela Berg, Elke Kalbe, Hans-Peter Müller, Simon Baudrexel, Jan Kassubek

T11-2C Impaired organelle transport in a neuronal cell model derived from Niemann-Pick type C1 patient-specific induced pluripotent stem cells
Maik Liedtke, Franziska Peter, Christin Völkner, Michael Rabenstein, Moritz J. Frech

T11-3C The NMDA receptor antagonist ketamine transiently reduces thalamocortical spindle and slow oscillations in a rodent model of non-REM sleep
Ali Mahdavi, Didier Pinault, Stefan Rotter, Yi Qin, Marine Bertschy, Damaris Cornec

T11-4C Misperceptions and performance fluctuations and their relation to resting state functional connectivity in Parkinson patients
Kristina Miloserdov, Carsten Schmidt-Samoa, Kathleen Williams, Christiane Anne Weinrich, Kathrin Bürk, Claudia Trenkwalder, Mathias Bähr, Melanie Wilke

T11-5C Optogenetic stimulation inhibits seizure generation in a mouse model of mesial temporal lobe epilepsy
Enya Paschen, Philipp Janz, Katharina Heining, Diego Vieira, Dr. Ute Häussler, Antje Kilias, Prof. Dr. Ulrich Egert, Prof. Dr. Carola Haas

T11-6C Apolipoprotein D-mediated regulation of lysosomal membrane integrity preserve lysosomal function and promotes cell survival in Niemann-Pick Type A disease.
Raquel Pascua-Maestro, María D. Ganfornina, María D. Ledesma, Diego Sanchez

T11-7C Replicative reprogramming in the context of physiological CNS aging and age-related neurodegeneration
Diane Penndorf, Alessandro Ori, Ivonne Heinze, Joanna Kirkpatrick, Otto W Witte, Alexandra Kretz

T11-8C scRNAseq analysis of brain organoids to study molecular mechanism of Leigh syndrome
Tancredi Massimo Pentimalli, Agnieszka Rybak-Wolf, Nikos Karaiskos, Gizem Inak, Alessandro Prigione, Nikolaus Rajewsky

T11-9C Mitochondrial and lysosomal dysfunction have opposite effects on lipid biosynthesis
Leonardo Gabriel Pereyra, Nuno Raimundo

T11-10C Axonal changes upon toxin-induced myelin remodeling
Friederike Pfeiffer, Petra Fallier-Becker

T11-11C GABAergic Synaptic Input to Cerebellar Purkinje Cells is Affected in a Niemann-Pick Type C1 Mouse Model
Michael Rabenstein, Christin Völkner, Maik Liedtke, Arndt Rolfs, Moritz J. Frech

T11-12C Specific Mutations in Presenilin 1 have a Differential Role on Mitochondrial Phenotype and Function
Liliana Rojas-Charry, Laura Heikaus, Harmut Schlueter, Christian Hagel, Markus Glatzel, Diego Sepulveda-Falla

T11-13C Combination of sesamin and alpha-mangostin attenuates hydrogen peroxide-induced neurodegeneration
Waralee Ruankham, Wilasinee Suwanjang, Virapong Prachayasittikul, Supaluk

Prachayasittikul, Kamonrat Phopin

T11-14C Alterations of neurogenesis in dentate gyrus precede development of Alzheimer's disease-like pathology in OXYS rats
Ekaterina Rudnitskaya, Tatiana Kozlova, Alena Burnyasheva, Nataliya Kolosova, Natalya Stefanova

T11-15C Epigenetic profiling in experimental models entailing mitochondrial and non-mitochondrial toxins - implications for movement disorders
Ranganayaki Sathyanarayanan, Srinivas Bharath MM

T11-16C Astrocyte – T-lymphocyte communication under neuroinflammatory conditions
Samantha Schmaul, Julian Loeffel, Dirk Luchtman, Stefan Bittner

T11-1D Linking cognition to amyloid-β burden in the brain of a non-human primate (*Microcebus murinus*)
Daniel Schmidtke, Elke Zimmermann, Stéphanie G. Trouche, Pascaline Fontès, Jean- Michel Verdier, Nadine Mestre-Francés

T11-2D Effect of novel acetylcholinesterase inhibitors 3-nitro-6-amino-substituted imidazo[1,2-b] pyridazine derivative compounds on mitochondrial physiology
Rakesh Kumar Sharma, Manisha Singh, Sridhar Pulavarthi, Sabbasani Rajasekhara, Ravi Shankar Akundi

T11-3D Baseline neuronal-activity dependence of Aβ-induced dysfunction
Manuel Simon, Benedikt Zott, Arthur Konnerth

T11-4D Increase of neuronal activity by 4-Aminopyridine in vivo, improves sensory-motor dysfunction in a mouse model of SMA
Christian Marc Simon, John G. Pagiazitis , Emily V. Fletcher, Beatriz Blanco-Redondo, George Z. Mentis

T11-5D Aberrant nitric oxide and redox signalling induces glycation activity resulting in enhanced neuronal dysfunction in neurodegeneration
Joern Steinert, Julie Bourgognon , Jereme Spiers

T11-6D The neurovascular unit in Alzheimer's disease
Stephanie Lauren Taylor, Gabor Petzold

T11-7D Two-photon glutamate imaging suggests mechanism for amyloid-β-dependent neuronal dysfunction
Felix Gabriel Unger, Benedikt Zott, Hsing-Jung Chen, Arthur Konnerth

T11-8D Evaluation of potential pharmacological chaperones in a neuronal cell model derived from Niemann-Pick type C1 patient-specific induced pluripotent stem cells
Christin Völkner, Franziska Peter, Maik Liedtke, Michael Rabenstein, Moritz J. Frech

T11-9D Cytoskeletal alterations contributing to synapto-axonal dysfunction in Parkinson´s disease.
Carmina Carelia Warth Perez Arias, Lucas Caldi Gomes, Kim-Ann Saal, Christof Lenz, Henning Urlaub, Silvio Rizzoli, Paul Lingor

T11-10D Effects of cannabidiol on diabetes outcomes and chronic cerebral hypoperfusion comorbidities in middle-aged rats
Rúbia Maria Weffort de Oliveira, Amanda Santiago, Marco Aurelio Mori, Humberto Milani, Francisco Silveira Guimarães

T11-11D Endocytic defects impair lysosomal and mitochondrial function
Katarzyna Wieciorek, King Faisal Yambire, Lorena Fernández Mosquera, Ángela Sánchez Guerrero, Ira Milosevic, Nuno Raimundo

T11-12D Role of the subthalamic nucleus in impulse control
Zifeng Xia, Franziska Richter, Frank W Ohl, Kentaroh Takagaki

T11-13D Lysosomal and mitochondrial crosstalk: a case for neurodegeneration in LSDs?
King Faisal Yambire, Ira Milosevic, Nuno Raimundo

T11-14D Vulnerability of highly active brain regions in Alzheimer's disease
Benedikt Zott, Arthur Konnerth

T11-15D Neuroinflammation in a mouse model of amyotrophic lateral sclerosis with FUS gene mutation and effects of standard and new therapies.
Diana Ivanovna Babaevskaia, Johannes de Munter, Alexander Trofimov, Joao Costa-Nunes, Dmitry Pavlov, Ekaterina Veniaminova, Margarita Oplatchikova, Anna Gorlova, Klaus-Peter Lesch, Erik Wolters, Daniel Anthony, Tatyana Strekalova

T11-16D a-Synuclein Aggregation Mechanisms and the Role of Lysosomal Cathepsins in Parkinson's Disease
Josina Bunk, Alice Drobny, Susy Prieto Huarcaya, Friederike Zunke

miRNA-expression profiling in midbrains of Parkinson's Disease patients

Lucas A. Caldi Gomes[1,2,3], Anna-Elisa Roser[1,3,4], Gaurav Jain[5], André Fischer[5], Paul Lingor[1,3,4]

[1]University Medical Center Göttingen, Neurology Dept., Göttingen, Germany
[2]International Max Planck Research School for Neurosciences, Göttingen, Germany
[3]Center for Biostructural Imaging of Neurodegeneration (BIN), Göttingen, Germany
[4]DFG Research Center Molecular Physiology of the Brain (CNMPB), University Medicine Göttingen, Göttingen, Germany
[5]German Center for Neurodegenerative Diseases (DZNE), site Göttingen, Germany

Parkinson's Disease (PD) is a highly prevalent age-related neurodegenerative disorder, affecting up to 2% of individuals aged over 60 years. PD patients present a pronounced dopaminergic cell loss in the substantia nigra and a consequent dopamine depletion in the striatum, triggering severe deficits of motor control. Recently, a number of studies showed that dysfunctions in the regulation of gene expression are critical to the development of brain diseases. microRNAs (miRs), a class of small non-coding RNAs, act as post-transcriptional regulatory factors of gene expression and seem to play a key role in the pathophysiology of neurodegenerative diseases. They target mRNAs by complementarity, which can result in either translational repression or mRNA degradation. Profiling the miRNA expression patterns in PD midbrains is a promising way to explore its pathophysiological mechanisms. Therefore, the aim of this study was to assess the expression profiles of miRs in midbrains of PD patients and aged-matched controls (AMCs).

Fresh-frozen human post-mortem midbrain samples were provided by the UK PD Brain Bank, accounting to 19 PD samples and 15 AMCs samples. Total RNA was isolated from the samples and small RNA libraries were prepared (Illumina TruSeq small RNA library prep Kit; TruSeq SR cluster Kit v3), followed by massive parallel small RNA deep-sequencing (Illumina HiSeq4000). miRs were by far the most abundant small RNA species found in the sequenced samples, accounting to 89.80 % and 90.38 % of total small RNA reads in the PD and AMC condition, respectively.

Differential expression analyses between PD and controls revealed 28 miRs significantly regulated among all differentially expressed small RNA sequences (considering FDR<0.1, log2fc> ±0.5 and minimum of 20 reads in each condition), the majority (21/28) being up-regulated in PD patients. Several of the de-regulated miRs have been already reported in the context of neurodegenerative diseases, brain injury and aging, and their functions have been attributed to regulation of neuronal apoptosis, cell survival, neuronal development and differentiation, inflammation, immune response and α-synuclein pathology. Pathway enrichment analyses (conducted in DAVID Bioinformatics Resources 6.8) with the predicted targets of the deregulated miRs reveal important biological processes and pathways that might be altered. Those include apoptotic process (GO:0006915; p=2.87E-02), nervous system development (GO:0007399; p= 5.57E-08); ubiquitin-dependent protein catabolic process (GO:0045893; p= 1.29E-02), positive regulation of apoptotic process (GO:0043065; p= 1.35E-02), dopaminergic synapse (KEGG:hsa04728; p= 1.93E-03) and PI3K-Akt signaling pathway (KEGG:hsa0415; p= 2.09E-02), for example. Up- and down-regulated miRs were analyzed separately.

Regulated miRs, target genes and related pathways will be further studied and validated by real-time PCR and Western Blotting. Our findings are likely to reveal molecular networks involved in PD pathogenesis as well as drugable targets for the development of novel therapeutic alternatives.

Quantitative imaging of spreading-depression associated ROS production

Marc Ackermann[1], Katharina Dietrich[1], Michael Müller[1]

[1]Universitätsmedizin Göttingen, Inst. Neuro- und Sinnesphysiologie, Humboldtallee 23, 37073 Göttingen, Germany

Spreading depression (SD) is a neurological phenomenon characterized by a massive neuronal and glial depolarization, which slowly propagates in brain tissue at velocities of a few millimeters per minute. Due to the near-complete depolarization and the associated massive disturbance of ionic homeostasis, proper neuronal network function is severely disturbed or may even complete collapse in the invaded brain areas. The occurrence of SD is closely associated with pathophysiological conditions such as migraine, cerebral hemorrhage, stroke, trauma, and edema. In view of the massive metabolic burden that is associated with the occurrence of an SD episode, it can be assumed that also marked amounts of reactive oxygen species (ROS) are generated. Yet so far, proper optical tools were not available to address this aspect with spatiotemporal detail. We now took advantage or our recently generated transgenic redox-indicator mice, which express in excitatory projection neurons the genetically-encoded redox-sensor roGFP1 (reduction oxidation sensitive green fluorescent protein 1). RoGFP1 is ratiometric by excitation (395/470 nm), responds reversibly to reduction and oxidation, and it reports in quantitative manner subcellular thiol redox-conditions. With this advanced model, we monitored SD-related ROS production in acute hippocampal tissue slices of adult male mice, focusing on stratum pyramidale of the CA1 subfield. SD was induced by local pressure-injection of high K^+, O_2 withdrawal or mitochondrial uncoupling by FCCP. In the submerged slices (35-36°C), the negative DC potential deflection was accompanied by a clear oxidation of roGFP1, indicating marked ROS release during each type of SD. In the case of hypoxia- and FCCP-induced SD an initial, gradually increasing oxidation was already evident before HSD onset, which then turned into a steep increase as the DC potential deflection occurred. Inducing SD by high K^+ did not give rise to an initial roGFP1-oxidation. Upon the K^+-induced SD, the roGFP1 oxidation slowly recovered over the course of 10-15 min. With the other stimuli, a recovery of the SD-related ROS production was limited as FCCP or the hypoxic-solution (95%N_2 - 5%CO_2 plus 2 mM Na_2SO_3) washed-out only slowly from the tissue. Removing extracellular Ca^{2+} largely dampened the SD-related oxidation of roGFP1 and markedly improved its reversibility, pointing out to a key-role of cellular Ca^{2+} load in the SD-related ROS-production. Neither mitochondrial uncoupling, nor pharmacological inhibition of NADPH-oxidase or xanthine oxidase abolished the SD-related oxidation of roGFP1. Therefore, involvement of both mitochondrial as well as extra-mitochondrial ROS sources has to be assumed. In conclusion, our transgenic redox-indicator mice provide a novel technical basis to decipher the very subcellular mechanisms of SD-related ROS generation. This will foster the understanding of the physiological and pathophysiological consequences that may arise from SD episodes in various parts of the brain.

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Intracerebroventricular administration of histidine reduces kainic acid induced convulsive seizures in mice.

Serdar Alpdogan[1], Felix Neumaier[1], Jürgen Hescheler[1], Toni Schneider[1]

[1]University of Cologne,Institute for Neurophysiology, Robert-Koch Str. 39, 50931 Cologne, Germany

Kainic acid (KA)-induced seizures and other experimental models of epilepsy have proven instrumental in identifying novel targets that could be responsible for human icto- and epileptogenesis. We have previously shown that the ablation of pharmacoresistant voltage-gated Ca2+ channels with Cav2.3 as central ion conducting pore (R-type Ca2+ channel) reduces the sensitivity towards KA-induced epilepsy in mice (Weiergräber et al. 2007;Dibue-Adjei et al. 2017). In vivo, Cav2.3 channels are thought to be under tight allosteric control by endogenous loosely-bound trace metal cations (Zn2+ and Cu2+) that suppress channel gating via a high-affinity trace metal binding site (Shcheglovitov et al. 2012; Neumaier et al. 2015; Neumaier et al. 2018). Metal dyshomeostasis in the brain, which is a common feature of (KA-induced) seizures, could therefore alter the normal function of Cav2.3 channels and may shift hippocampal and neocortical signaling towards hyperexcitation.

In order to investigate the role of loosely-bound metal ions for KA-induced hyperexcitation in vivo, we examined the effects of manipulating brain trace metal homeostasis in mice. To this end, we developed a murine system for intracerebroventricular administration of trace metal ions and / or histidine (His), which can bind Zn2+ and Cu2+ and is involved in their transendothelial transport at the blood-brain-barrier. Unexpectedly, our preliminary findings indicate that application of His alone but not in the presence of Zn2+ has substantial beneficial effects on the outcome of KA-induced epilepsy in mice. As such, our results emphasize previous findings on the complex, two-sided role of loosely-bound metal ions with regard to neuronal excitation and –degeneration under pathophysiological conditions.

Abnormal Amyloid beta accumulation leads to neuronal loss and alterations in the process of adult hippocampal neurogenesis

Sanila Amber[1], Fatima Javed Mirza[1], Deeba Hassan[1], Saadia Zahid[1]

[1]National University of Sciences and Technology, Atta ur Rahman School of Applied Biosciences, Kashmir Highway, Islamabad, 44000 Islamabad, Pakistan

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by deposition of Amyloid-beta (Aβ) plaques and neurofibrillary tangles in sub-cortical brain regions that eventually lead to cognitive impairment. Progressive Aβ accumulation leads synaptic loss, behavioral anomalies, sustained neuronal damage and alterations in the process of adult hippocampal neurogenesis. In the present study, Aβ (1-42) (3µg/3µl) induced mouse model (BALB/c) of AD was developed to underscore the effects of abnormal Aβ accumulation on neurodegeneration, subsequent cognitive loss and hippocampal neurogenesis. Cognitive abilities were assessed using a battery of behavioral tests including Morris water maze, elevated plus maze and novel object recognition tasks. Morris water maze task revealed significant decline in spatial memory of Aβ (1-42) treated animals as compared to control group. Impaired social interactive abilities and novelty preference was evident in Aβ (1-42) treated group as indicated by novel object test. Aβ (1-42) treated animals spent less time and exhibited less entries in open arms during elevated plus maze task that is an indication of anxiety like behavior which is accredited to anxiogenic properties of Aβ (1-42). Histopathological assessment revealed accumulation of Aβ plaques and marked neurodegeneration in Aβ (1-42) treated group as compared to control group. To assess the effects of Aβ (1-42) on the process of adult hippocampal neurogenesis, qRT-PCR and immunohistochemistry were carried out using stage specific neuronal markers i.e. Ki67, DCX and NeuN. Increased expression of neuronal makers was revealed in the hippocampus of Aβ (1-42) treated mice via qRT-PCR whereas marked reduction in Ki67 and NeuN immunoreactivity was observed in the dentate gyrus of Aβ (1-42) treated animals. These findings support the notion that Aβ accumulation mediates memory impairment and alterations in the process of adult hippocampal neurogenesis. However, the observed discrepancy between gene and protein expression analysis may be attributed to the protein loss mediated by Aβ induced oxidative stress.

Pharmacological and genetic inhibition of ADAM10 reduce brain damage after experimental traumatic brain injury

Dominik Appel[1], Regina Hummel[1], Larissa Dangel[1], Maryam Treiber[1], Johanna Merz[1], Martin Weidemeier[1], Christina Gölz[1], Mirko H.H. Schmidt[2], Kristina Endres[3], Michael K.E. Schäfer[1]

[1]Universitätsmedizin Mainz, Klinik für Anästhesiologie, Abteilung Neuroprotektion, Langenbeckstraße 1, 55131 Mainz, Germany

[2]Universitätsmedizin Mainz, Institut für Mikroanatomie und Neurobiologie, Langenbeckstraße 1, 55131 Mainz, Germany

[3]Universitätsmedizin Mainz, Klinik für Psychiatrie und Psychotherapie, Untere Zahlbacher Str. 8, 55131 Mainz, Germany

The alpha-secretase A disintegrin and metalloprotease 10 (ADAM10), known for its role in non-amyloid cleavage of Amyloid Precursor Protein, has been suggested to play a beneficial role in central nervous system (CNS) injury. However, up-regulation of ADAM10 in cortical injury has been also associated with impaired functional recovery. The objective of this study was to investigate whether inhibition of ADAM10 affects the neurological and histopathological outcome after experimental traumatic brain injury (TBI). Adult C57Bl/6 mice were subjected to the controlled cortical impact (CCI) model of TBI and received the ADAM10 inhibitor GI254023X by intraperitoneal administration (2.5 mg, 30 min and 24 h after CCI). Pharmacological inhibition of ADAM10 had no effect on neurological deficits but resulted in smaller brain lesions compared to vehicle-treated animals at 7 days after CCI. Additionally, GI254023X treated mice exhibited less axonal injury as revealed by SMI-32 antibody immunoreactivity specific for dephosphorylated neurofilament. Moreover, pharmacological ADAM10 inhibition attenuated the generation of spectrin breakdown products (SBDPs) suggesting improved maintenance of Ca2+ homeostasis. To study the role of neuronal ADAM10, transgenic mice heterozygous for dominant-negative ADAM10 (ADAM10 DN/WT) under control of the neuron-specific Thy-1 promoter were subjected to CCI. Similar to pharmacological inhibition of ADAM10, ADAM10 DN/WT mice exhibited significantly smaller brain lesions compared to WT littermates. Our results demonstrate that both, pharmacological and genetic inhibition of ADAM10 reduce the histopathological brain damage in a clinically relevant model of TBI.

Complexome profiling of the mitochondrial respiratory chain – Mechanistic insights into the regulation of energy metabolism in neurodegenerative diseases

Susanne Arnold[1]

[1]Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Geert Grooteplein-Zuid 10, route 774, 6525 GA Nijmegen, Netherlands

Complexome profiling exploits the powerful combination of blue-native electrophoresis and state-of-the-art mass spectrometry and is ideally suited to characterize size and composition of multi-protein complexes and multiple assembly intermediates comprehensively in a single experiment. This recently developed approach allowed us to decipher the complete assembly pathway of mitochondrial respiratory chain complex I in great detail (Guerrero-Castillo et al., 2017).
Here, we have applied complexome profiling to study the subunit assembly and composition of human and rodent respiratory chain complexes. We present evidence that the assembly of respiratory chain complexes from mitochondrial and nuclear encoded subunits follows a precisely coordinated, module-based pathway. Fully assembled complexes I, III and IV then associate into supercomplexes.
We have identified new proteins that comply with the characteristics of protein subunits or assembly factors and defined their role under different physiological conditions and in neurodegenerative disease. This not only deepens our understanding of the structure and function of these respiratory chain complexes, but also sheds further light onto their involvement in regulatory and disease mechanisms, as already reported recently for MR-1S, a newly identified assembly factor of complex IV (Vidoni et al., 2017).

Guerrero-Castillo S., Baertling F., Kownatzki D., Wessels H.J., Arnold S., Brandt U., Nijtmans L. (2017) The assembly pathway of mitochondrial respiratory chain complex I. Cell Metab. 25, 128-139.
Vidoni S., Harbour M.E., Guerrero-Castillo S., Signes A., Ding S., Fearnley I.M., Taylor R.W., Tiranti V., Arnold S., Fernandez-Vizarra E., Zeviani M. (2017) MR-1S interacts with PET100 and PET117 in module-based assembly of human cytochrome c oxidase. Cell Rep. 18, 1727-1738.

Copper, zinc and HSPGs influence trans-dimerization of the amyloid precursor protein family members

Alexander August[1], Nadine Schmidt[1], Johannes Klingler[2], Frederik Baumkötter[1], Jessica Klement[2], Carolyn Vargas[2], Klemens Wild[3], Sandro Keller[2], Stefan Kins[1]

[1]Technische Universität Kaiserslautern, Human Biology and Human Genetics, Kaiserslautern, Germany
[2]Technische Universität Kaiserslautern, Department of Molecular Biophysics, Kaiserslautern, Germany
[3]University of Heidelberg, Heidelberg University Biochemistry Center (BZH), Heidelberg, Germany

The amyloid precursor protein (APP) and its homologues APLP1 and APLP2 are type I transmembrane proteins with a large extracellular part. The ectodomain is further subdivided into the E1 and E2 domain, able to bind copper, zinc and heparan sulfate proteoglycans (HSPGs). APP/APLPs were reported to play an essential role in synapse formation and synaptic plasticity likely mediated by *trans*-cellular dimers. We reported a novel copper binding site in the GFLD within the E1 domain, essential for *trans*-dimerization of APP. Further, zinc was reported to affect *trans*-dimerization of APLP1. This suggests that APP and APLP1 synaptogenic activity is preferentially regulated by copper and zinc, respectively.
Using ITC analyses, we found a novel zinc-binding site in the E1 domain of APP, likely localized in the interface of the GFLD and CuBD. Notably, copper binds with higher affinity as zinc. However, pre-incubation with zinc suppressed copper-binding, but not vice versa. This argues for two independent binding sites. To investigate *trans*-dimerization, we used a bead aggregation assay. We observed an increased *trans*-dimerization of APP only at low zinc concentrations, whereas high zinc levels inhibited copper-mediated *trans*-dimerization of APP. This suggests a zinc-mediated structural change of APP and APLP1 that abolishes copper binding and copper-induced dimerization. Interestingly, we observed that also proteinaceous components secreted by different non-neuronal cells, including astrocytes, modulate APP/APLPs *trans*-dimerization. This activity was blocked by heparinase treatment, indicating involvement of HSPGs in APP/APLPs *trans*-cellular dimerization.
Taken together, our results show that extracellular matrix components, including HSPGs, as well as copper and zinc modulate APP/APLPs dimerization. As these trace metals are released upon synaptic activity, changes in copper/zinc homeostasis likely contribute to modulation of APP/APLPs synaptogenic activity.

Fe65 and Fe65L1 have distinct functions in synaptic plasticity

Vanessa Augustin[1], Paul Strecker[1], Susann Ludewig[2], Elisa Kraechan[3], Marcel Daas[1], Martin Korte[2], Jonathan Stephan[3], Stefan Kins[1]

[1]Technical University of Kaiserslautern, Human Biology and Human Genetics, Erwin-Schrödinger-Str. 13, 67663 Kaiserslautern, Germany

[2]Technical University of Braunschweig, Cellular Neurobiology, Spielmannstr.7, 38106 Braunschweig, Germany

[3]Technical University of Kaiserslautern, Animal Physiology, Erwin-Schrödinger-Str. 13, 67663 Kaiserslautern, Germany

The Fe65 protein family, consisting in mammals of Fe65, Fe65L1, and Fe65L2, are multidomain adaptor proteins interacting with the carboxyl terminus of the amyloid precursor protein (APP), implicated in the pathogenesis of Alzheimer's disease. Our recent characterization of mice lacking Fe65 (Fe65 KO) and/or Fe65L1 (Fe65L1 KO, Fe65/Fe65L1 DKO) showed severe impairments in spatial learning and LTP. Interestingly, these analyses revealed besides overlapping also some distinct functions of Fe65 and Fe65L1. Field potential recordings in hippocampal CA1 pyramidal neurons exhibited no change in the input-output strength between the individual genotypes, but showed increased paired-pulse facilitation in Fe65L1 KO mice. In line with this, whole-cell patch-clamp recordings revealed only for Fe65L1 KO mice elevated mIPSC frequencies, suggesting different functions of Fe65 and Fe65L1 in short-term presynaptic plasticity. To get further insights in the putative underlying mechanisms, we extended the electrophysiological studies and performed detailed histological analyses of dendrites and spines of apical second order dendrites of hippocampal CA1 pyramidal neurons in the different genotypes. Fe65/Fe65L1 deficient mice showed reduced dendritic length and branching leading to a simplification of arborization and altered spine densities, presumably due to changes in Fe65/Fe65L1 interaction with the cytoskeleton. Together, our data demonstrate essential distinct functions of Fe65 and Fe65L1 in synaptic plasticity and learning, which also has important implications for understanding of APP pathophysiological function.

AP-2 prevents amyloidogenic processing of APP via endocytosis-independent regulation of BACE1 trafficking in neurons

Sujoy Bera[1], Elena Calleja Barca[1], Albert Negrete[1], Julia Racho[1], Christoph Wittich[1], Nina Ellrich[1], Soraia Martins[2], James Adjaye[2], Natalia L. Kononenko[1]

[1]CECAD Research Center, University of Cologne, Natalia L Kononenko Lab, Joseph-Stelzmann Strasse-26, 50931 Köln, Germany

[2]Institute for Stem Cell Research and Regenerative Medicine. University of Düsseldorf, Moorenstrasse 5, D-40225, Germany.

Cleavage of amyloid precursor protein (APP) by BACE-1 (β-site APP cleaving enzyme-1) is the rate-limiting step in amyloid-β (Aβ) production and a neuropathological hallmark of Alzheimer's disease (AD). Despite decades of research, molecular and cellular mechanisms of BACE1 trafficking and APP cleavage remain highly controversial. Here we show that in neurons amyloidogenic processing of APP is controlled by the adaptor protein complex-2 (AP-2). AP-2 prevents amyloidogenesis via endocytosis-independent regulation of BACE1 intracellular trafficking. AP-2 is decreased in IPSCs-derived neurons from sporadic AD patients, while conditional neuronal-confined AP-2 knock-out (KO) mice suffer from increased Aβ generation, resulting from intracellular accumulation of BACE1 within the late endosomes and autophagosomes. Deletion of BACE1 decreases amyloidogenic processing of APP and mitigates synapse loss in neurons lacking AP-2. Taken together, these data suggest a mechanism for BACE1 intracellular trafficking and degradation via an endocytosis-independent function of AP-2 and reveal a crucial role of endocytic proteins in the prevention of AD.

Nanoscale analysis of cytoskeletal alterations during acute axonal degeneration in primary neuron cultures

Arndt Lucas Biller[1], Elisabeth Barski[2], Elisa D´Este[3], Mathias Bähr[4], Paul Lingor[5], Jan-Christoph Koch[6]

[1]UMG Göttingen, Neurology, AG Lingor/Koch, Im Hassel 15, 37077 Göttingen, Germany

Axonal degeneration is a hallmark of many neurological diseases, not only traumatic (e.g. spinal cord lesion) but also neurodegenerative (e.g. Parkinson disease, Amyotrophic lateral sclerosis) and neuroinflammatory diseases (Multiple sclerosis). The degeneration often starts at the distal axon, from where it then propagates back to the soma. Neuroprotective treatments are still not available and the underlying pathophysiological mechanisms are not fully understood. Therefore, basic research is needed to create a better understanding of axonal degeneration for future therapeutic approaches.
Recently, optical nanoscopy techniques revealed that cytoskeletal proteins like actin and spectrin form highly periodic structures with precisely defined spacing. To further characterize the mechanisms of axonal degeneration, we studied the spatial and temporal changes of the axonal cytoskeleton in a lesion paradigm. To this, we plated cell cultures of primary rat neurons in microfluidic chambers and performed axotomy through vacuum aspiration to induce acute axonal degeneration of the proximal axon.
Using high resolution STED-nanoscopy and focusing on cytoskeletal proteins spectrin and tubulin, we show an early-onset degredation of spectrin ring-like periodicity, which progressed over the studied time period (up to 72h) and over the length of the lesioned axon. Furthermore, we show the development of axonal bulbs close to the axotomy lesion (10- 20 µm), which were both positive for spectrin and tubulin. Bulb formation starts as early as 5 min after lesion and bulbs continued to grow in diameter reaching a peak after 4h. More bulbs stained positive for tubulin than for spectrin.
Our results reveal the ultrastructural correlates of axonal dieback-degeneration showing an early and progressive degeneration of the actin/spectrin cytoskeleton, the stabilization of which could represent a promising therapeutic target.

In vivo Imaging and Transcriptome Analysis of Astrocytes in an Alzheimer's Disease Mouse Model

Nelli Blank[1], Lech Kaczmarczyk[1], Stefanie Herresthal[2], Walker S. Jackson[1], Joachim L. Schultze[1,2], Gabor C. Petzold[1,3]

[1]German Center for Neurodegenerative Diseases, Neurovascular Diseases, Sigmund-Freud-Str. 27, 53127 Bonn, Germany
[2]Department of Genomics and Immunoregulation, LIMES Institute, University of Bonn, 53115 Bonn, Germany
[3]Department of Neurology, University Hospital Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany

One of the most prominent hallmarks of Alzheimer's disease (AD) is the accumulation of neurotoxic amyloid-beta (Aβ) species, which have the tendency to form extracellular insoluble Aβ plaques and induce reactive astrogliosis in surrounding astrocytes. We have previously shown that peri-plaque reactive astrocytes become hyperactive in mouse models of AD, and that this hyperactivity is mediated by purinergic signaling. However, it has remained unclear how astrocytes and astrocytic hyperactivity contribute to the onset and progression of AD, and how astrocytic signaling is altered in awake mice in AD models. Therefore, we have started to perform in vivo imaging of calcium activity in astrocytes in awake behaving mice. By intravenous injection of a virus encoding for the green fluorescent calcium indicator GCaMP6f under control of the astrocyte-specific short GFAP promoter, we are able to longitudinally measure spontaneous calcium transients in the AD mouse model and their wildtype littermates, and to correlate these data to behavior and disease progression. In addition, we are analyzing the astrocytic transcriptome during aging in AD mice compared to wildtype mice using the RiboTag technique. Together, these approaches may lead to a better understanding of transcriptional alterations in astrocytes and their implications for functional changes, and may help to elucidate neurotoxic and neuroprotective elements of astrogliosis in AD.

Tau blocks amyloid-β dependent neuronal hyperactivity in vivo

Marc Aurel Busche[1], Susanne Wegmann[2], Simon Dujardin[3], Caitlin Commins[4], Julia Schiantarelli[5], Tarun V. Kamath[6], Naomi Klickstein[7], George Carlson[8], Israel Nelken[9], Bradley T. Hyman[10]

[1]University College London, UK Dementia Research Institute, London, United Kingdom
[2]German Center for Neurodegenerative Diseases, Berlin, Germany
[3]Massachusetts General Hospital, Charlestown, USA
[4]Massachusetts General Hospital, Charlestown, USA
[5]Massachusetts General Hospital, Charlestown, USA
[6]Massachusetts General Hospital, Charlestown, USA
[7]McLaughlin Research Institute, Great Falls, Montana, USA
[8]Department of Neurobiology, Silberman Institute of Life Sciences and Edmond and Lily Safra Center for Brain Sciences, Hebrew University of Jerusalem, Israel
[10]Massachusetts General Hospital, Charlestown, USA

Alzheimer´s disease is manifest neuropathologically by widespread amyloid-β (Aβ) plaques and tau neurofibrillary tangles (NFTs) in the brain. The relationship of these two lesions to one another is a central mystery of Alzheimer biology. Here we employed in vivo two-photon calcium-imaging of large populations of layer 2/3 cortical neurons in mice expressing both human Aβ and tau. We reveal a strong tau-dependent suppression of activity and silencing of a vast number of neurons, which dominates over Aβ-dependent neuronal hyperactivity. We show that NFTs are neither sufficient nor required for the silencing, which instead is dependent on soluble tau. We demonstrate that by repressing tau gene expression the silencing phenotype can be rapidly rescued in tau mice. Surprisingly, however, the same treatment was ineffective in mice harboring both Aβ and tau. Together, these new data may help explain the numerous failed clinical trials directed at reducing Aβ in the brains of patients, since the combination of Aβ and tau in the brain leads to a phenotype that is different than Aβ alone, and which is dominated by tau neural system silencing.

The NMDA antagonist memantine attenuates the okadaic acid induced short-term spatial memory impairment and hippocampal cell lossin rats

Mariam Chighladze[1], Manana Dashniani[1], Khatuna Rusadze[2]

[1]Ivane Beritashvili Center of Experimental Biomedicine, Laboratory of Behavior and Cognitive Functions, Gotua 14, 0160 Tbilisi, Georgia

[2] Akaki Tsereteli State University, Biology Department, Kutaisi, Georgia

Alzheimer`s disease (AD) is a neurodegenerative disease that causes progressive cognitive and behavior impairment in the elderly. It is widely believed that changes in the cerebral activity of protein phosphatases have been implicated in the pathogenesis of AD. Okadaic acid (OA) is a potent and selective inhibitor of protein phosphatases. Because of its property to inhibit phosphatase activity, OA is associated with protein phosphorylation and has been proved to be a powerful probe for studying the various regulatory mechanisms and neurotoxicity (intracerebral injection of OA, would provide a useful model of Alzheimer`s disease). OKA induced memory deficit and elevation of Ca2+ was found to be correlated with neurotoxicity and N-methyl-D-aspartate (NMDA) receptor emerged as a plausible link. The glutamatergic synapses are most likely site for initiation of neurodegenerative process. Activation of glutamate receptors is believed to play a major role in the neuronal cell death . The excitotoxicity induced by over activation of NMDA receptor is closely related to excess of intracellular Ca2+. OA increases Ca2+ in hippocampal neuronal cell culture through the ionotropic excitatory amino acid receptors resulting in neuronal degeneration.

In the present study, the possible beneficial effect of memantine on the Okadaic Acid (OA) induced spatial short-term memory impairment was examined in spatial alternation task, and the neuroprotective potential of memantine on OA-induced structural changes in the hippocampus was evaluated by Nissl staining. OA was dissolved in artificial cerebrospinal fluid (aCSF) and injected intracerebroventricularly (ICV) 200 ng in a volume of 10 µl bilaterally. Vehicle control received aCSF ICV bilaterally. Control and OA injected rats were divided into 2 subgroups injected i.p. with saline or memantine (5 mg/kg,) Memantine or saline were given daily for 13 days starting from the day of OA injection. Behavioral study showed that bilateral ICV microinjection of OA induced impairment in spatial short-term memory. Nissl staining in the present study showed that the ICV microinjection of OA significantly decreased the number of surviving pyramidal neurons in the CA1 region of the hippocampus. Chronic administration of memantine effectively attenuated OA induced spatial short-term memory impairment and the OA-induced neuropathological changes in the hippocampus. Therefore, ICV injection of OA can be used as an experimental model to study mechanisms of neurodegeneration and define novel therapeutics targets for AD pathology.

Neddylation-dependent protein degradation as a nexus between neuronal insulin signaling, amyloidosis and metabolic syndrome

Alessandro Dario Confettura[1], Guilherme Monteiro Gomes[1], PingAn Yuanxiang[1], Andreas Hentschel[2], Robert Ahrends[2], Michael Kreutz[1,3]

[1]Research Group Neuroplasticity, Leibniz-Institute for Neurobiology, Magdeburg, Germany
[2]Institute for Analytical Science ISAS, Dortmund, Germany
[3]Leibniz Group - Dendritic Organelles and Synaptic Function, Center for Molecular Neurobiology, Hamburg, Germany

The metabolic syndrome (MetS) is a civilization disease characterized by heterogeneous factors comprising overweight, high blood pressure, lipid metabolism's disorder and insulin resistance (IR). Neurons are insulin sensitive cells that can undergo to IR, which affects the activity of the Insulin Receptor Substrates (IRS), a protein family that organizes the cytosolic insulin signaling. The inactivation of IRS can occur via an abnormal phosphorylation or via protein degradation and the Interruption of the insulin signal has been linked to neurodegenerative diseases. It is well established that the IR and MetS have interplay with the amyloid-β; deposition in the brain: this is believed to be a major risk factor for sporadic Alzheimer's disease (AD).
MetS and AD seem to have a bidirectional interaction, in which the neuroinflammation may act as a key link: neuroinflammatory signal is induced by amyloidosis, which in turn promotes synaptic IR via alteration of IRS phosphorylation; prolonged exposure to insulin causes IRSs degradation; this results in an increase of pro-inflammatory cytokines (TNFα) release, thus driving amyloid-β; deposition.
Nedd8, a small molecule, analogous of Ubiquitin, which is conjugated to target proteins, leading these to proteasome-dependent protein degradation, may carry out a crucial role into IRS degradation. Little is known about its function in neurons about a possible role of neddylation into AD's onset, therefore we aim to find a nexus between IR, MetS and AD that can be explained by the action of Nedd8 on the insulin pathway.
Interestingly we found that a prolonged treatment with Insulin and TNFα; on primary dissociated hippocampal or cortical neurons induces IR and this associated to a down-regulation of IRS proteins, as wells as Insulin/IGF1 receptors.
To validate these results in-vivo, we developed an animal model for a high-risk population with the combination of genetically driven amyloid-β; deposition and high fat diet induced MetS. Our preliminary results showed that Nedd8 and the neddylation pathway have a role in the regulation of IRS level, thus regulating the insulin pathway in the synapses. Here we show that the neuroinflammatory signal, induced by amyloidosis promotes synaptic IR by driving neddylation of Insulin Receptor Substrates (IRS). Moreover we found that a pharmacological intervention that selectively blocks the neddylation pathway improves insulin sensitivity in-vitro and memory performances in-vivo.

Identification of signaling mechanisms regulating mitochondria-endoplasmic reticulum contact sites

Renata Couto[1,2], Ira Milosevic[3], Nuno Raimundo[1]

[1]Institute for Cellular Biochemistry, University Medical Center Göttingen (UMG), Göttingen, Germany
[2]Doctoral Program on Molecular Biology of the Cells, Göttingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences, University of Göttingen, Göttingen, Germany
[3]European Neuroscience Institute (ENI), Göttingen, Germany

Mitochondria and the endoplasmic reticulum (ER) form tight contact sites that are implicated in numerous cellular functions, and whose disruption has been associated with pathologies, particularly neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Despite these organelle contacts are one of the most studied and stable, the basic understanding of their regulation, disruption and its impact in cell faith, as well as the regulation of signaling pathways are topics that need further investigation.
Here, we explored the regulatory environment of the ER-mitochondria contact sites in various mammalian cells with mitochondrial malfunction. Specifically, we used human cells with knock-down (KD) of mitochondrial complex III subunit UQCRC1 and mouse fibroblasts without mitochondrial complex I subunit NDUFS4 (NDUFS4 KO). The mitochondrial function in these models was characterized by evaluating mitochondrial respiration, ROS production and mitochondrial membrane potential. Subsequent systematic electron microscopy (EM) and confocal microscopy analysis showed an elevated number of contact sites between mitochondria and ER in both models. Furthermore, we identified a key cellular kinase and a signaling pathway that regulate the number of ER-mitochondria contact sites.
Our findings reveal that ER-mitochondria contact sites respond to mitochondrial respiratory chain malfunction in a defined manner through a well-understood signaling pathway. They may also contribute to the better understanding on pathological conditions linked to ER-mitochondria contact sites.

Small conductance calcium-activated (SK3) potassium channel overexpression in nigrostriatal system of mice

Elaine Del Bel[4], Sabine Martin[1], Marcio Lazzarini[1], Miso Mitkovski[1], Luis Pardo[1], Walter Stuhmer[1,2,3]

[1]Department of Molecular Biology of Neuronal Signals, Max Planck Institute of Experimental Medicine, Göttingen, Germany
[2]Center Nanoscale Microscopy and Molecular Physiology of the Brain, Göttingen, Germany
[3]Bernstein Focus for Neurotechnology and Bernstein Center for Computational Neuroscience , Göttingen, Germany

Recent data showed that SK3 (small-conductance calcium-activated K+ channel subunit 3) modulates electrophysiological properties of dopaminergic cells of the substantia nigra compacta. Alterations of potassium channel expression and function in the basal ganglia have been linked to the pathogenesis of Parkinson's disease. Taking advantage of the conditional SK3 overexpressing mouse line (SK3-T/T) based on the delineated up-regulation of dopamine receptors DRD1A and DRD2 in the frontal cortex, dorsal striatum, and of DRD2 in the mesencephalon in SK3-T/T mice we hypothesized that SK3 channel overexpression would affect dopaminergic neurotransmission and associated behaviors. Our aim is to analyze how the SK3 channel overexpression impacts the dopaminergic neurons. We performed behavioral examination of haloperidol, a dopamine D2 receptor blocker induced catalepsy. We report here neurochemical differences in dopamine and acetylcholine in the striatum. We delineate unique molecular changes associated with the SK3-overexpression in the mouse striatum analyzing mRNA expression for dopamine/serotonin pathways. We carried out a detailed micro-anatomical study describing the distribution of SK3-channel with special attention to striatum-substantia nigra pathway in the brain of the SK3-T/T mice. The importance of the SK3 channel for the central nervous system is not only emphasized by these severe phenotype changes resulting from its overexpression, but these findings also indicate that ion channels may represent a new pathway to try to unveil the molecular mechanisms responsible for complex central nervous system diseases

Microstructural analysis of endo- and perineurial cells in human neuroma

Patrick Dömer[1,2], Bettina Kewitz[1], Christian Heinen[1], Thomas Kretschmer[3,5], Ulrike Janssen-Bienhold[2,4,5]

[1]Department of Neurosurgery, School of Medicine and Health Sciences, Faculty VI, University of Oldenburg, 26129 Oldenburg, Germany

[2]Research Center Neurosensory Science, University of Oldenburg, 26129 Oldenburg, Germany

[3]Department of Neurosurgery, Klinikum Klagenfurt am WS, 9020 Klagenfurt, Austria

[4]Department of Neuroscience and Neurobiology, School of Medicine and Health Sciences, Faculty VI, University of Oldenburg, 26129 Oldenburg, Germany

[5]the authors contributed equally to this work

Neuromas are pathologic nerve distensions caused by a nerve's response to trauma. Following traumatic peripheral nerve injury (PNI), sprouting axons attempt to cross the injury site as long as scar tissue, a gap or lacking axonal guidance do not counteract sprouting. If target-oriented sprouting is prevented, a neuroma will form. So far, rodent models have shown that regeneration associated genes play an important role in axonal regeneration. Furthermore, endoneurial cells are providing axonal guidance for successful regeneration, while perineurial cells form a diffusion-barrier via tight-junctions to provide a physiologically restricted intra-fascicular space. The cellular communication between those perineurial cells and the exchange of small molecules is mediated via connexins (Cx26 and Cx43). However, the precise cellular and molecular changes after PNI have not yet been resolved for human nerve. To provide further insight, the molecular and cellular alterations of endoneurial and perineurial cells after PNI were studied in human traumatic neuromas.

The endo- and perineurial cells were detected by immunohistochemistry and immuno-electron microscopy using antibodies directed against CD34 (endoneurial fibroblast like cells, EFLCs) and the Glucose-Transporter 1 (Glut1, perineurial cells). Furthermore, the formation of tight-junctions and the expression of connexins in endo- and perineurial cells was analyzed using antibodies directed against the tight junctional marker protein Claudin1 as well as Cx26 and Cx43 in six human traumatic neuromas. Image analysis and quantification was carried out using ImageJ and Graphpad Prism.

In intact peripheral nerves, immunohistological labeling revealed a sub-clustering of axons by endoneurial cells within the fascicle, while the perineurial cells formed a multi-layered sheath enclosing the fascicle. Immuno-ultrastructural analysis of perineurial cells showed a lamellar arrangement of overlapping perineurial cell processes, forming a diffusion barrier via tight-junctions intermingled with direct cell contacts via Cx26 and Cx43. Following PNI, axons as well as the endo- and perineurial structures were disrupted and mini-fascicles of regenerating axons were detectable in the neuromatous tissue. In the proximal neuroma, mini-fascicles were ensheathed by a single layer of CD34 expressing EFLCs and surrounded by a reduced number of Glut1 positive perineurial cells, compared to fascicles of an intact nerve. Those perineurial cells were expressing a highly reduced amount of Claudin1 and Cx26 and Cx43 protein. In the distal neuroma segment as well as in the degenerated end, located distal to the injury site, endo- and perineurial ensheathment of the regenerating axons was absent. The immunohistological labeling revealed scattered endo- and perineural cells in the distal and degenerated neuroma segments which showed no tight-junction formation and a highly decreased connexin expression.

Therefore, unsuccessful axonal regeneration might be related to the lack of endoneurial as well as perineurial structures in the distal neuroma and the degenerated end, which results in the disruption of the perineurial nerve barrier and the axonal guidance to the target organ.

Trimethyltin-induced neurodegeneration is associated with gradual up-regulation of ecto-5' nucleotidase on activated microglia

Milorad Dragic[1,2], Nataša Mitrovic[2], Nadežda Nedeljkovic[1], Ivana Grkovic[2]

[1]Department for General Physiology and Biophysics, Faculty of Biology, University of Belgrade, Studentski trg , 11000 Belgrade, Serbia

[2]Laboratory for Molecular Biology and Endocrinology, Institute of Nuclear Sciences Vinca, University of Belgrade, Mike Petrovica Alasa 12-14, 11000 Belgrade, Serbia

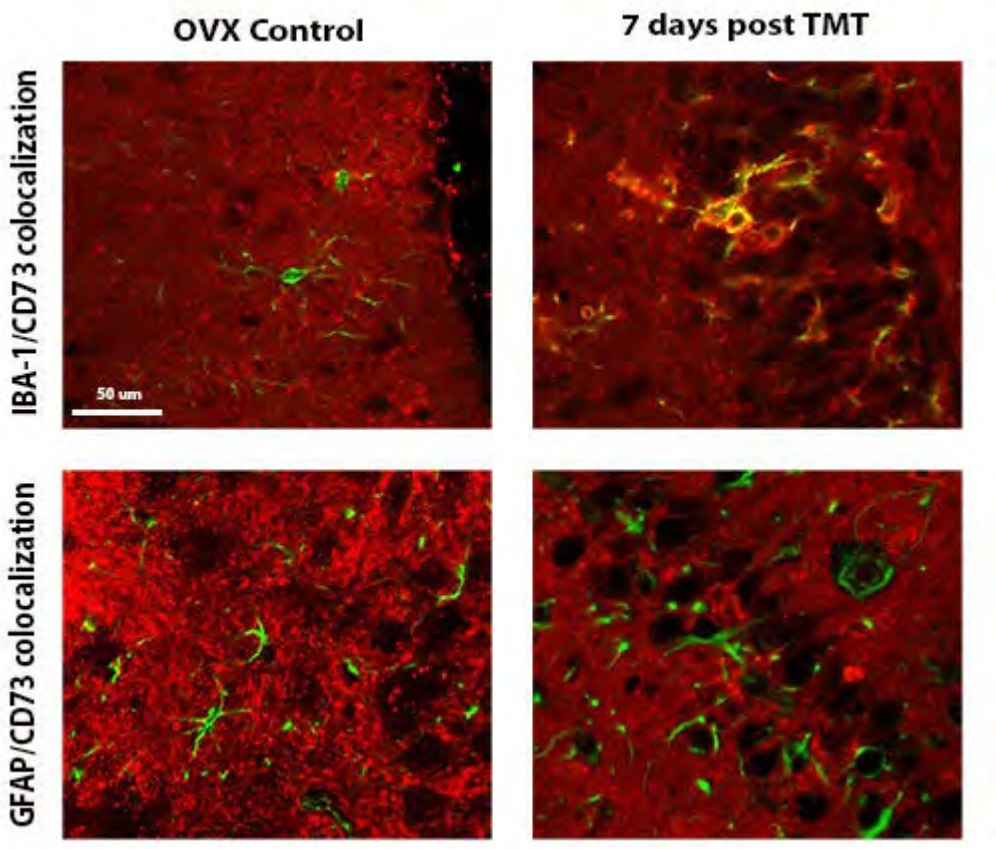
Ecto-5' nucleotidase/cluster of differentiation 73 (CD73) is a membrane enzyme with active site facing extracellular compartment. It is the last and rate-limiting enzyme of the purine catabolic pathway, catalyzing dephosporylation of extracellular adenosine 5'-monophosphate (AMP) to adenosine, which acts via P1 purinoreceptors. Adenosine is potent homeostatic regulator in central nervous system involved in numerous processes such as immunosuppression, control of cell growth, cellular differentiation, etc. Besides hydrolysis of AMP, CD73 was also reported to function as cell adhesion molecule, involved in recognition, migration, synaptic plasticity and remodeling. Although CD73 is broadly expressed on astrocytes, oligodendrocytes and synapses in the mature brain, overexpression of the enzyme on activated astrocytes was found in virtually all models of neuropathologies. One of the tools for obtaining a model of hippocampal neurodegeneration followed by neuroinflammation and gliosis is trimethyltin (TMT) intoxication. Combined with ovariectomy (OVX), these two models create a similar physiological niche seen in dementia and Alzheimer disease. Since our results showed early microglial and astrocyte activation in CA1 region and dentate gyrus, we sought to explore early spatiotemporal changes of CD73 in hippocampus of TMT-treated OVX animals.

Two-month-old female Wistar rats were bilaterally ovariectomized. Three week after surgery they were treated with single intraperitoneal dose of trimethyltin (8 mg/kg) and sacrificed 2, 4 and 7 posttreatment. Brains were prepared for cryosectioning and hippocampal sections were processed for enzyme histochemistry and immunohistochemistry.

AMPase histochemistry revealed biphasic response to TMT intoxication. First, decrease of staining in CA1 synaptic regions 2 days post-treatment was observed, followed by gradual increase of specific labeled glial cells depicted by AMPase histochemistry which infiltrated CA1 pyramidal layer from day 4 to day 7. Spatiotemporal pattern of CD73-immunoreactivity (ir) completely supported data obtained by enzyme histochemistry. To characterize glial cell type infiltrated in the pyramidal layer at the site of neurodegeneration, we co-localized CD73 with astrocytes (GFAP) and microglia (IBA-1) cell markers. Accordingly with reported data, CD73 was present along fine astrocytic processes in OVX controls. Four days post-treatment microglia changed morphology from resting to bushy/amoeboid type as a result of TMT-induced neuronal death. From that time point we observed that CD73 nicely depicted processes of bushy microglia, while amoeboid type completely colocalized with CD73. Furthermore, on day 7 a specific rod microglia in stratum radiatum of CA1 region was completely delineated with CD73. Surprisingly, hippocampal astrocytes did not colocalized with CD73 in any of examined time points.

Our results unexpectedly show gradual up-regulation of CD73 on activated microglia during neurodegeneration followed by neuroinflammation. Degree of CD73 up-regulation correlates with morphologically distinct microglial morphotypes, which suggests that the enzyme may play a role in microglial activation. We hypothesize that CD73 could possibly act as a switch for the morphological transition during neuroinflammation.

This work was supported by the Ministry of Education, Science and Technological development, Republic of Serbia, grants No. 41014 and 173044.



Arbutin promotes functional recovery following lysolecithin-induced demyelination in rat optic chiasm

Forough Ebrahimtabar[1], Fatemeh Ebrahimtabar[2], Atena Nazari[3], Mahdi Pouramir[4], Manuchehr Ashrafpour[5], Fereshteh Pourabdolhossein[6]

[1]Student Research Committee, Neuroscience Research Center, Babol University of Medical Sciences, Babol, Iran, Ganjafrooz street, 47176-47745 Babol, Iran

[2]Student Research Committee, Faculty of veterinary, Azad university of Babol, Babol, Iran

[3]Physiology Departments, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

[4]Biochemistry Departments, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

[5]Neuroscience Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

Introduction

Multiple sclerosis (MS) is an autoimmune and neurodegenerative disease in young adult. Visual disturbance is involved in more than 70% of MS patients as the earliest symptoms. Hydroquinone derivations such as Arbutin has potential role as a free radical scavenger and anti-inflammatory agent which has not been studied in experimental model of demyelination yet. In this study, the effects of Arbutin administration on myelin repair and functional recovery of optic chiasm were investigated.

Methods

Under stereotaxic procedure, local demyelination was induced by administration of LPC (1%, 2µL) into the rat optic chiasm. Rats treated by daily injection of Arbutin (50mg/kg, i.p) until the end of each animal group experiment (3, 7, 14 days after LPC injection) as well an equivalent volume of saline for LPC group animals. Visual-evoked potentials (VEPs) recording was performed for evaluating the function of optic pathway at 3, 7 and 14 days' post lesions. P1-N1 latency and amplitude was measured by e-probe software. Myelin specific staining (Luxol Fast Blue) was also carried out for assessment of myelin repair. To evaluate the anti-inflammatory effect of Arbutin, real time-PCR was used by which inflammatory (IL-1, Il-17, TNF-α) and non-inflammatory (IL-10) cytokines gene expression were investigated. The data was analyzed by two-way ANOVA followed by Bonferroni post-test using Graph pad PRISM software.

Result

Electrophysiological data indicated that Arbutin significantly reduced P1-N1 latency at 7 and 14 days following optic chiasm demyelination compared to the same time points of LPC treated animals ($p<0.05$). Histological study in agreement with VEP recordings data proved that demyelination extension in optic chiasm was considerably reduced in arbutin treated groups at day 7 and 14 in comparison with LPC treated animals ($p<0.001$). q-PCR analyses of optic chiasm samples revealed that Arbutin treatment remarkably reduced the level of IL-1β expression especially at 7 dpi ($p<0.001$) and the expression level of IL17 and TNF-α at 3 and 7 dpi respectively compared to LPC groups ($p<0.05$, $p<0.001$). In addition, Arbutin had notably increased the expression of anti-inflammatory gene (IL-10) at all-time points compared to LPC groups respectively ($p<0.0001$, 0.01).

Conclusions

These data indicate that Arbutin treatment at 50 mg/kg dose have an inhibitory effect on inflammatory cascade of events involved in pathogenesis of MS disease. Our results also demonstrate that Arbutin facilitate myelin repair and restore VEP response in the demyelinated optic chiasm. Thus, Arbutin could have therapeutic potential for demyelinating disorders such as Multiple Sclerosis.

APP gene family members as synaptic adhesion molecules

Simone Eggert[1], Sandra Schilling[1], Jonathan Stephan[2], Mathieu Meleux[1], Marius Zimmermann[1], Alexander August[1], Martin Korte[3], Edward H. Koo[4], Ulrike C. Müller[5], Stefan Kins[1]

[1]TU Kaiserslautern, Human Biology and Human genetics, Erwin-Schrödinger-Str. 13, 67663 Kaiserslautern, Germany
[2]TU Kaiserslautern,Physiology, Kaiserslautern, Germany
[3]TU Braunschweig,Cellular Neurobiology, Braunschweig, Germany
[4]UCSD,Neuroscience, San Diego, USA
[5]University Heidelberg, Pharmacy and Molecular Biotechnology, Heidelberg, Germany

Synaptic dysfunction is a critical step in Alzheimer`s disease (AD) pathogenesis. The amyloid precursor protein (APP), a key player in AD, forms trans-cellular dimers postulated to induce synapse formation. Indeed, we show synaptogenic properties of APP and its family members APLP1 and APLP2 via co-culture assays, similar to other SAMs (synaptic adhesion molecules), such as Neuroligin/Neurexin, indicating that APP/APLPs also belong to the family of SAMs. In line with this, we demonstrate that the APP gene family members exhibit many features of SAMs, including upregulation during synaptogenesis, pre- and postsynaptic localization, and synaptic loss of function in KO animals. About 45 % of APP is dimerized in mouse brains as shown by Blue native (BN) gel analyses. A proteomic screen of these APP BN gel complexes revealed new APP interaction partners, which belong to the family of synaptic adhesion molecules. Interestingly, cortex samples of AD patients showed a significantly reduced amount of APP dimers, indicating that full length APP dimers might play an important role in the disease process. Together, our results show that synaptic stability under physiological and pathological conditions depends on trans-synaptic dimerization of APP in interplay with other SAMs.

Impact of voluntary running and environmental enrichment on learning and memory performance in APP/PS1 mice

Thomas Endres[1], Monique Klausch[1], Georgia-Ioanna Kartalou[1,2], Elke Edelmann[1], Kurt Gottmann[2], Volkmar Lessmann[1]

[1]Otto-von-Guericke University Magdeburg, Institute of Physiology, Leipziger Str. 44, 39120 Magdeburg, Germany
[2]Heinrich-Heine University Düsseldorf, Institute of Neuro- and Sensory Physiology, Universitätsstr. 1, 40225 Düsseldorf, Germany

To identify treatments for Alzheimer's disease (AD) is one of the currently most challenging topics in neuroscience. Here, we analyzed the impact of two non-invasive treatment strategies to counteract the cognitive decline in a mouse model for AD (APP/PS1 mice): voluntary running (i.e. running wheels) and enriched housing conditions. In order to disentangle the potential positive effects of voluntary running from maintaining animals in enriched housing conditions, we created two experimental cohorts. One cohort had continuous access to running wheels in their home cages (voluntary running group), while the other cohort had access to identical but rotation-blocked running wheels (enriched group). We compared the cognitive capacities of these two groups with animals that were kept under standard housing conditions. The treatment regimens started shortly before the first cognitive impairments could be observed in APP/PS1 mice (i.e. 4 months of age) and lasted for two months. Then we tested these animals for episodic memory, spatial memory (Morris water maze) and contextual fear learning. As both treatment regimens have been described to elevate BDNF (brain-derived neurotrophic factor) levels in the brain, we analyzed BDNF and Aβ protein levels in hippocampus and frontal cortices of the tested mice. Furthermore, we analyzed whether these treatments impact on hippocampal long-term potentiation, spines and plaque formation (see poster of Kartalou et al). Our results suggest that both, voluntary running and enriched housing ameliorate cognitive impairments that are observed in APP/PS1 mice that were maintained in standard housing conditions.
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Stimulation of mGluR1/5 improves the defective internalization of AMPA receptors in NPC1 mutant mouse

Xiao Feng[1]

[1]Albrecht-Kossel-Institut für Neuroregeneration, Unimedicine Rostock, Gehlsheimer Straße 20, 18147 Rostock, Germany

Niemann-Pick Type C1 (NPC1) disease is characterized by abnormal accumulation of cholesterol in late endosomal/lysosomal compartments, resulting in neurodegeneration. Although the neural symptoms of NPC1 patients have been described, the pathophysiological mechanisms of neurological disorders in NPC1, however, are not fully understood. In this study, we revealed the defective internalization of GluR2-containing AMPA receptors in both basal and AMPA-stimulated states in NPC1-/- mouse cortical neurons. Further studies show that the cholesterol level decreases in lipid rafts of NPC1 cortex, resulting in the abnormal distribution of the group I metabotropic glutamate receptor (mGluR1/5) in the lipid rafts and their downstream signal of phosphorylated extracellular-signal-activated kinase1/2 MAP kinase (ERK-MAPK). The use of mGluR1/5 agonist 3, 5-Dihydroxyphenylglycine (DHPG) markedly increases the internalization of AMPA receptors in the cortical neurons. As an effective drug for NPC1 disease, β-Cyclodextrin restores the cholesterol level in the lipid rafts and improves the associated mGluR1/5 in lipid rafts, facilitating the recovery of the defective internalization of AMPA receptors, which can be partially blocked by the antagonist of mGluR1/5. On the other hand, calcium imaging experiments also demonstrate that the upregulated calcium influx, due to the defective internalization of AMPA receptors, can be recovered by application of DHPG and β-Cyclodextrin. Finally, we proved that the p-GluR2 and PKC signals might be involved in the dysfunction of the internalization. Our data suggest that the defective internalization of AMPA receptors is a critical reason for neuronal dysfunction in the NPC1 neurons and correcting the abnormal group I mGluRs is a potential therapeutic strategy for NPC1 treatment.

Redox homeostasis modulates axonal microtubule dynamics: Effects on microtubule-dependent transport and tau-microtubule interaction

Chrsitian Gach[1], Benedikt Niewidok[1], Nancy Monteiro Abreu [1], Daniel Villar Romero [1], Lena Schünemann[1], Jacqueline Becker[1], Maike Schober[1], Anna-Sophie Schwarze[1], Roland Brandt[1]

[1]Universität Osnabrueck, Neurobiology, Barbarastrasse 11, 49076 Osnabrueck, Germany

The amount of reactive oxygen species (ROS) within a cell is thoroughly balanced. Deregulation of the ROS homeostasis can disturb various signaling pathways and can cause serious damage to biomolecules, leading to various aberrations such as modifications and aggregation of proteins. Accumulating evidence suggests that the redox state regulates microtubule (MT) dynamics in both physiological and pathological conditions. However, it is unclear whether the change in MT dynamics by an altered ROS homeostasis is a primary effect on MTs or rather mediated indirectly via the action of the microtubule-associated protein (MAP) tau. It is also not known, how this impacts the physiological functions of neurons and what are the molecular mechanisms involved.

Here we employed quantitative live cell imaging of model neurons to investigate the effect of experimentally induced low ROS environment by employing the mitochondria-targeted antioxidant SkQ1 as well as experimentally induced ROS production. We assessed the effect on MT dynamics, on the interaction of the microtubule-associated protein tau with MTs, and on transport dynamics in axon-like processes.

We report that experimentally altered ROS homeostasis leads to a significant change of polymerized tubulin in axon-like processes. Treatment with a microtubule-stabilizing drug reverses the decrease in MTs, indicating that the reduction reflects a destabilization of the microtubule network. Interestingly, the tau-MT interaction and the dynamics of mitochondria were not affected suggesting a primary, tau-independent, effect of the cellular redox environment on MT dynamics. Quantitative phosphoproteomics revealed ROS-induced changes in the phosphorylation of several MT-regulating proteins.

Our results indicate a redox state-dependent MT destabilization in neuronal cells and suggest a role of several MT-regulating proteins in mediating these changes. They provide evidence that MT stabilizing drugs or treatment with mitochondria-targeted antioxidants such as SkQ1 can serve as modulators to target these changes.

Nanobody-based Sensor for Detecting α-Synuclein-Transmission in Parkinson's Disease Models

Christoph Gerdes[1,2], Natalia Waal[1,2], Hannes Verbarg[1], Nora Wender[1], Buket Basmanav[1], Stefan Becker[3], Silvio Rizzoli[1,2], Sebastian Kügler[4], Felipe Opazo[1,2]

[1]Institute of Neuro- and Sensory Physiology, University Medical Center Göttingen, Humboldtallee 23, 37073 Göttingen, Germany

[2]Center for Biostructural Imaging of Neurodegeneration (BIN), University Medical Center Göttingen, von-Siebold-Straße 3a, 37075 Göttingen, Germany

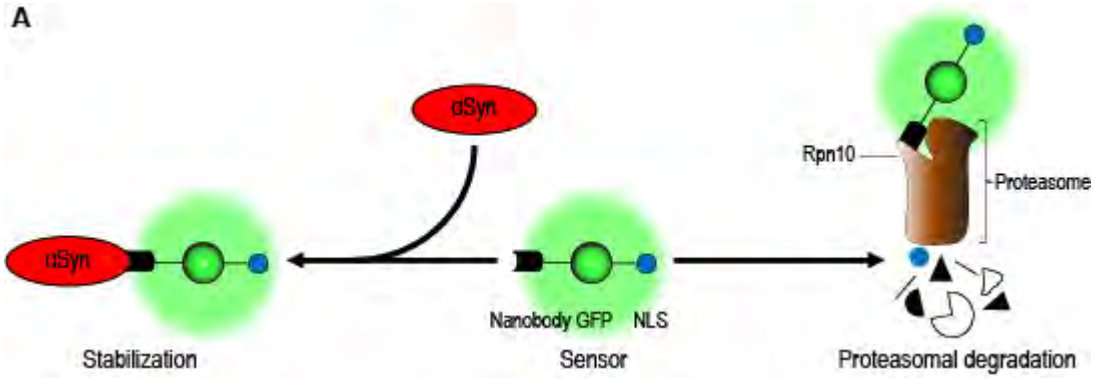
[3]Department for NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

[4]Department of Neurology, University of Göttingen, Medical School, Waldweg 33, 37073 Göttingen, Germany

A-Synuclein (aSyn) seems to play an important role in Parkinson's Disease (PD). The prion-like transmission of aSyn is a relatively new phenomenon and it is regarded to be a central mechanism in the development and progression of PD.

To investigate this, we developed a nanobody-based sensor which consists of a nanobody able to detect aSyn inside living cells and report the amounts of aSyn with an increase in fluorescence. As a proof of concept we first prepared a cell line stably expressing such sensor. We could show that in absence of human aSyn the sensor is dark, whereas in presence of cytosolic aSyn the cell nucleus lightens up. The sensor works by binding aSyn with a relatively strong affinity (Kd ~10 nM), but also to the proteasomal subunit Rpn10, however, with a much weaker affinity. Therefore, sensor is continuously degraded via the proteasome if human aSyn is not present in the cellular cytosol, but is stabilized and accumulates in the nucleus in presence of aSyn. By semi-quantitative Western Blots we determined the detection limit of the sensor in the cell line to be about 120 fg of aSyn in cytosol per cell.

Using this sensor system, it was shown that the uptake of aSyn into the cytosol of both, HEK293 cells and neurons, is significantly more effective when adding a cationic lipid to aSyn. Although we were able to demonstrate that the nanobody also recognizes aSyn-fibrils, we did not observe any uptake of these into the cytosol of neurons. Adding cerebrospinal fluid (CSF) of more than 100 patients – who either suffered from PD or from neurological control diseases – to the sensor cell line revealed that all patients' CSF contained transmittable forms of aSyn. Patients with positive family history for PD showed significantly more transmittable aSyn than patients with negative family history.



Hereditary spastic paraplegia in *Danio rerio*

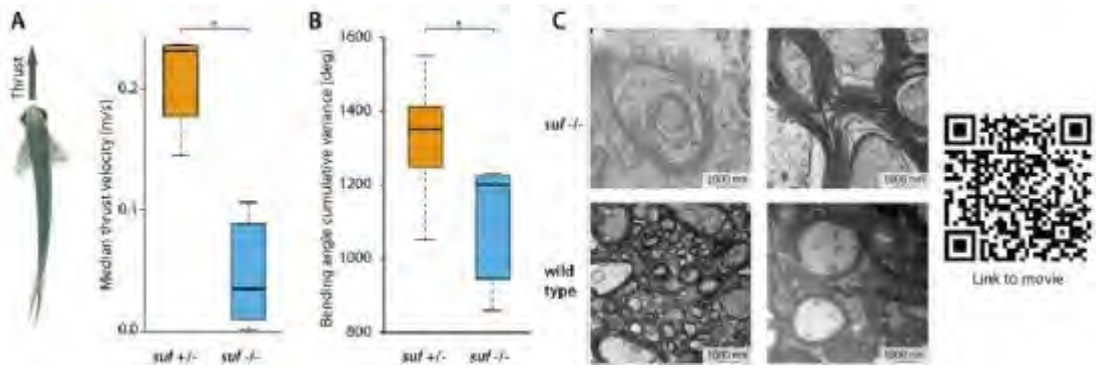
Bart Geurten[1], Gudrun Kracht[2], Wiebke Möbius[3], Torben Ruhwedel[3], Hauke Werner[3], Ralf Heinrich[1], Roland Dosch[2]

[1]Georg-August-University , Cellular Neurobiology, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

[2]Georg-August-University Göttingen, Department of Developmental Biochemistry, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

[3]Max-Planck-Institut für Experimentelle Medizin, Hermann-Rein-Str. 3, 37075 Göttingen

No effective therapy is currently available for hereditary spastic paraplegia (HSP) in humans. HSP causes a swift retrograde degeneration of the longest descending corticospinal tract axons, resulting, inter alia, in a paralysis of the lower extremities. *spastizin* is one of the most prominent candidate genes to be causative to this syndrome. In zebrafishes a knock out of their *spastizin* homologue (*soufflé*) by CRISPR/Cas9 revealed locomotion defects. The Quantification of locomotion phenotypes in *soufflé* mutants, uncovered the onset of the syndrome, as well as coping mechanisms used to reclaim some of the organisms lost agility. Using histology and electron microscopy, we were able to identify the neurons which degenerate during the progression of the syndrome. Quantifying the myelination of those neurons allowed us to gain new insights into its cellular process and the age-dependent progression of HSP. These results suggest that we established the first genetic HSP model in zebrafish. We found further evidence that the Mauthner cells and the flight response of Soufflé deficient fish are affected.



Combination of different approaches for the characterization of a Rodent Alzheimer's Disease Model

Barbara Hinteregger[1,2], Tobias Madl[1], Joerg Neddens[2], Birgit Hutter-Paier[2], Robert Wronski[2]

[1]Medical University Graz, Gottfried Schatz Research Center , Neue Stiftingtalstraße 2, 8010 Graz, Austria

[2]QPS Austria , Parkring 12, 8074 Grambach, Austria

Background
Alzheimer's disease (AD) is a severe neurodegenerative disorder and currently affects more than 27 million people worldwide with numbers expected to grow dramatically as the population ages. Current approaches for monitoring the progression of AD are limited. Thus, there is a critical need for a simple and accurate method to monitor AD pathophysiology quantitatively from early, e.g. prior to the onset of symptoms, to late stages. In this respect, Nuclear Magnetic Resonance (NMR) Spectroscopy-based metabolomics seems to be a promising novel approach. It can monitor global changes in metabolites and provide biomarkers and associated molecular mechanisms that allow monitoring of AD stages. Biomarker identification and longitudinal monitoring of biomarker variation in mouse AD models and control animals promotes the understanding of AD progression and related pathologies.

Methods
In this study, we carried out different methods like immunohistology, behavioral tests, biochemical analysis as well as untargeted NMR-based metabolic phenotyping of Tg4-42 and wild-type mice of different ages. In this integrative approach, immunohistology provides information on the biochemical alterations of brain areas in form of histological quantification of different biomarkers by specific primary antibodies, e.g. GFAP, CD11b and NeuN to receive further information about brain neuropathology, e.g. plaque load, neuroinflammation or synaptic changes. Furthermore, behavioral tests provide phenotypic read-outs related to anxiety, cognition and spatial learning. In addition, biochemical assays give further information e.g. of different amyloid beta levels.
Untargeted NMR spectroscopy is excellently suited to monitor perturbations in a large pool of metabolites in biofluids and tissues and reflects changes downstream of genomic, transcriptomic and proteomic fluctuations.
The combination of these techniques in an integrative approach provides a better understanding of (patho-) physiological alterations in health and disease aiding to further understanding of alterations in complex biological networks involved in AD.

Results
We will present changes in NMR-based metabolite biomarkers of mouse Tg4-42 biofluids and tissues and the integration of these biomarkers with conventional techniques such as immunohistology, biochemical assays as well as behavioral studies.
Histological amyloid beta was found in Tg4-42 transgenic mice only, mainly in the CA1 region of the hippocampus, the CA1 cell layer was thinner and also astrogliosis was detected in this region. Tg4-42 animals showed highly significant spatial learning deficits in the Morris water maze test.
These results provide a deeper insight to understand the (patho-) physiology in this AD mouse model.

Conclusions
By performing behavioral studies, immunohistology, biochemical assays and NMR-based metabolic

phenotyping we present an integrative approach which can be easily extended to other disease models and translated across species since metabolic pathways are conserved through evolution and are essentially similar in rodents and humans.

Deep brain stimulation in the inferior colliculus induces anxiolytic effect and improves haloperidol-induced catalepsy in rats

Hannah Ihme[1], Rainer K. W. Schwarting[1,2], Liana Melo-Thomas[1,2,3]

[1]Philipps-University Marburg, Psychology, Behavioral Neuroscience, Gutenbergstraße 18, 35037 Marburg, Germany
[2]Marburg Center for Mind, Brain, and Behavior (MCMBB), Marburg, Hans-Meerwein-Straße 6, 35032 Marburg, Germany.
[3]Behavioral Neurosciences Institute (INeC), Av. do Café, 2450, Monte Alegre, Ribeirão Preto, 14050-220, São Paulo, Brazil.

Several studies have suggested that the inferior colliculus (IC) is part of the so-called brain aversive system (BAS), which includes amygdala, medial hypothalamus, and dorsal periaqueductal gray. Electrical or chemical stimulation of the IC induces responses such as arousal, freezing and escape that mimic fearful behavior elicited by environmental challenges. Besides that, some reports suggest that electrical deep brain stimulation (DBS) of the IC may also improve haloperidol-induced catalepsy in rats. This latter effect is interpreted as paradoxical kinesia and points to the IC as an alternative target to DBS in order to improve motor deficits in parkinsonian patients. However, the anxiogenic effect of intracollicular DBS limits its use in clinical settings. The present study aims to improve intracollicular DBS parameters in order to avoid anxiogenic side effect but preserving motor improvements in rats treated with haloperidol. To test the effects of DBS in cataleptic rats, male rats received haloperidol (0.5mg/kg, IP); 60 min after injection, the bar test was performed during which a given rat received continuous (5 min) or at regular intervals (5 x 1 min, intervals of 30 s) DBS (30Hz, 600 mA, 100µs). In contrast to the control groups the test group received 5 min of stimulation before performing the bar test. The bar test consists of gently placing the rat with its forepaws on a horizontal bar. The time until it steps down with both forepaws is measured. When quantifying catalepsy time an overall effect of stimulation reducing catalepsy time was seen over the three conditions. However, in comparison to the control groups only continuous DBS with previous stimulation reduced step-down latencies on a significant level indicating the importance of long on holding stimulation for behavioral outcomes. We explored possible aversive side effects in more detail by submitting the same animals to the plus maze test. The results showed that DBS continuous stimulation paired with a prior stimulation phase of 5 min induced more active behavior in rats measured by an increased number of entries and time spent in the open arms and more explorative behavior such as head dipping, risk assessment, end exploring and scanning. This indicates that continuous low amplitude DBS is not only not aversive but has an anxiolytic effect on motor behavior in rats. Intracollicular DBS parameters used at the present study induced motor improvements without any evidence of aversive behavior. Importantly, 30 Hz-DBS of the IC induced anxiolytic effect on the EPM. For the first time, it has been demonstrated that DBS at the IC is anxiolytic instead of aversive. The present study suggest that the IC can be an alternative DBS target to treat anxiety and improve motor deficits observed in some parkinsonian patients.

The role of 5-HT_7-receptor signalling in neurodegenerative diseases

Kathrin Jahreis[1], Josephine Labus[1], Evgeni Ponimaskin[1]

[1]Hannover Medical School, Cellular Neurophysiology, Carl-Neuberg-Straße 1, 30625 Hannover, Germany

A variety of neurodegenerative diseases is characterized by a progressive cognitive decline and typical alterations at the cellular level. Artificial accumulation of different proteins can contribute to cognitive deficits. Among those proteins, the microtubule associated and stabilizing protein Tau is of special interest. Tau localises to the axons and contributes to integrity of axonal cytoskeleton. Pathological increased phosphorylation at various sites of Tau, mediated by different kinases, leads to disordered binding pattern at the microtubule network, decreased solubility and the formation of intracellular accumulated Tau called neurofibrillary tangles (NFTs) in somatodendritic compartments. Accumulation of hyperphosphorylated Tau, associated with synaptic and neuronal dysfunction as well as neuronal cell death, became the common pathological hallmark of so-called tauopathies, including Alzheimer`s disease.

Due to no available causative treatment of tauopathies, it is necessary to understand the pathological mechanism of Tau hyperphosphorylation. In recent years, the serotonergic system has been discussed as a potential target for treating neurodegenerative diseases because of its important role in cognition and behavioural control. In present study we focussed on the serotonin receptor 7 (5-HT_7R). The 5-HT_7R is highly expressed in thalamus, hypothalamus, prefrontal cortex and hippocampus and plays an important role in learning and memory processes. On the cellular level, it is involved in regulation of neuronal morphology, dendritic outgrowth and development of synapses and dendritic spines. This renders 5-HT_7R an interesting target for the treatment of tauopathies. Our previous work has shown that this receptor is critically involved in phosphorylation of Tau. Here, we investigated the effect of different drugs approved for other indications and possessing antagonistic properties toward the 5-HT_7R on hyperphosphorylation of Tau, neuronal cell death and formation of NFTs using primary cultures of cortical neurons as a model.

Modification of the spreading of α-synuclein pathology in vivo

Karina Joppe[1,3], Lars Tatenhorst[1,3], Anna-Elisa Roser[1,2,3], Stefan Becker[4], Mathias Bähr[1,2], Paul Lingor[1,2,3]

[1]University Medical Center Göttingen, Neurology, Waldweg 33, 37073 Göttingen, Germany
[2]Cluster of Excellence Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany
[3]Center for Biostructural Imaging of Neurodegeneration (BIN), Von-Siebold-Str. 3a, 37075 Göttingen, Germany
[4]Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

Parkinson's disease (PD) is a slowly progressing neurodegenerative disorder without cure so far. One of its main pathological characteristics are neuronal protein inclusions called Lewy bodies (LBs), mainly consisting of misfolded and aggregated α-synuclein (α-syn) variants. The mechanisms behind α-syn aggregation and the spreading of α-syn pre-formed fibrils (PFFs) throughout the whole brain in PD are still elusive. Iron levels are increased in PD patients' brains and biophysical studies showed that iron binds to native α-syn fostering its aggregation. Therefore, iron brain load may contribute to the spreading α-syn pathology.

In this study, we thus investigated how iron influences α-syn spreading. We used intrastriatal injections of PFFs in C57BL/6 mice that were perfused at 90 days post injection (dpi). To investigate the influence of iron, we introduced an iron intoxication paradigm to this model: Mouse pups were treated with different concentrations of carbonyl iron from p10 to p17 by oral gavage. Cognition was impaired in mice intoxicated with the highest iron dosage of 240 mg / kg at 3 months post iron intoxication. PFFs-injected mice showed also strong cognitive impairments at 90 dpi. Furthermore, we evaluated the effects of iron load on α-syn PFFs spreading throughout the mouse brain. In a therapeutic approach, we applied Fasudil, a Rho-associated protein kinase (ROCK) inhibitor that previously showed to reduce α-syn aggregation by direct C-terminal binding. Fasudil was applied via drinking water (30 mg / kg) and was well tolerated. In contrast to previous positive results on α-syn aggregation, Fasudil did not affect PFFs spreading in C57BL/6 mice.

Our preliminary data thus demonstrates deleterious effects of α-syn PFFs and iron on cognition and suggest both α-syn and iron as therapeutic targets in PD.

Rescue of dendritic spine pathology in the hippocampus of APP/PS1 mice

Georgia-Ioanna Kartalou[1,2], Thomas Endres[2], Volkmar Lessmann[2], Kurt Gottmann[1]

[1]Institute of Neuro- and Sensory Physiology, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany
[2]Institute of Physiology, Otto-von-Guericke-University, Magdeburg, Germany

The function of the hippocampus in memory formation and consolidation is crucial and the mechanisms underlying this process involve synaptic plasticity. Dendritic spines are postsynaptic sites of excitatory synapses in the brain and they constitute the most important players in synaptic plasticity. Morphological alterations such as changes in dendritic spine density can affect the function of receptors that promote plasticity. Learning and memory impairments are often associated with dendritic spine pathology in Alzheimer's disease (AD). Pathological changes in brain-derived neurotrophic factor (BDNF) availability have also been reported in AD. In this study we investigated potential therapeutic strategies based on the increased BDNF expression that could modulate structural synaptic plasticity in $APP_{KM670/671NL}$ /$PS1_{L166P}$ mouse model of AD. To address our hypothesis we quantified spine density in pyramidal neurons of the CA1 area of the hippocampus of 6 month-old AD mice in the vicinity of amyloid (Aβ) plaques, by immunohistochemical analysis. AD mice showed lower spine density and we further investigated whether treatments that are thought to increase BDNF expression, such as voluntary physical exercise, could prevent the spine deficits in the hippocampus. We observed that voluntary physical exercise prevented spine loss that occurred preferentially in the vicinity of Aβ plaques in the AD hippocampus. Our findings reveal new therapeutic strategies that can ameliorate AD pathology in this animal model of AD.

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Early changes in hippocampal network oscillations and parvalbumin protein expression in a mouse model of Alzheimer's disease

Jochen Kuhse[1], Jan-Oliver Hollnagel[2], Oliver Kann[2], Joachim Kirsch[1], Eva Kiss[3]

[3]University of Medicine and Pharmacy Tirgu Mures, Romania, Dept. of Cellular and Molecular Biology, Gheorghe Marinescu 38, 540139 Tirgu Mures, Romania

[2]Institute of Physiology and Pathophysiology, University of Heidelberg, Germany

[1]) Institute of Anatomy and Cell Biology, University of Heidelberg, Germany

Alterations of expression profiles of synaptic and other neuronal proteins are evident not only in late but also in early presymptomatic stages of Alzheimers disease (AD). Specificaly, we detected that postsynaptic proteins of inhibitory synapses like the scaffold protein gephyrin and the 2 subunit of the GABA(A) receptor are significantly increased as early as 3 months in hippocampus areas of APP-PS1 mouse model of AD, indicating an increased inhibitory input in young APP-PS1 mice. In this study, using immunoblotting and confocal immunofluorescence microscopy, we disclosed a robust increase of parvalbumin protein expression and accentuated projections of parvalbumin expressing interneurons onto principal cells of stratum pyramidale of CA1 and CA3 of 3-months old APP-PS1 mice. Immunofluorescence of gephyrin and the presynaptic vesicular inhibitory amino acid transporter protein VIAAT colocalized with parvalbumin expressing perisomatic projections indicating an increased number of inhibitory synapses in 3 months old APP-PS1 hippocampus. Perisomatic inhibition by parvalbumin positive interneurons is important for the generation of sharp wave-ripples (SPW-Rs) and gamma oscillations in hippocampal networks involved in cognition and memory formation. Thus, we investigated further the impact of the putative increased perisomatic inhibition in these two types of fast neuronal network oscillations in 3-months old APP-PS1 and wild-type (WT) mice. The comparison of CA3 vs CA1 recordings within APP-PS1 and WT sclices revealed that the incidence and amplitude of SPW-Rs was significantly lower in CA1 vs CA3 in APP-PS1 slices whereas the power of gamma oscillations was significantly higher in CA3 vs CA1 in WT slices pointing to a disturbed communication between the CA3 and CA1 networks in APP-PS1 mice. Our data indicate an increased PV+ projections mediated perisomatic inhibition in CA1 and CA3 areas of hippocampus in early stages of AD. This might alter the cross talk between CA3 and CA1 areas and lead to a defective processing of information in the APP-PS1 hippocampus.

Disruption in the hippocampal network function in inducible *FMR1* premutation mice

Ufuk Emre Kul[1,2], Guersel Caliskan[1,3], Renate Hukema[4], Rob Willemsen[4], Monica Santos Santos[1], Oliver Stork[1,3]

[1]Department of Genetics and Molecular Neurobiology, Otto-von-Guericke University, Magdeburg, Germany [2]Leibniz Institute for Neurobiology, Magdeburg, Germany [3]Center for Behavioral Brain Sciences, Magdeburg, Germany [4]Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands

A CGG-repeat expansion in the premutation range (<200 repeats) in the fragile X mental retardation gene (FMR1) is the genetic cause of fragile X-associated tremor/ataxia syndrome (FXTAS). FXTAS is a late-onset neurodegenerative disorder that manifests with intention tremor, ataxia, neuropsychiatric changes and deficits in memory and cognition. Ubiquitin positive intranuclear inclusions are considered a hallmark of the disorder. Our previous work has shown that motor as well as emotional alterations manifest in an inducible transgenic FXTAS mouse model expressing a CGG90 repeat stretch. We have now developed an early transgene induction schedule that does not result in the manifestation of a motor phenotype and allows for studying the cognitive and emotional effects of CGG90 expression. Thereby, we have identified an anxiety-like phenotype that can be reverted with a period without transgene expression (washout phase), and is paralleled by changes in the inclusion load in the dentate gyrus (DG). These changes are accompanied by an equally reversible decrease in the baseline synaptic transmission and a disturbance of long-term potentiation in medial perforant to DG synapses. By contrast, in cornu ammonis area 3 (CA3), we have observed a persistent inclusion load and a progressive phenotype of increased presynaptic excitability at CA3 to CA1 synapses that appears to culminate in an enhancement of trace fear conditioning after the washout phase. These findings demonstrate the early development of hippocampal network changes and emotional disturbances in a model of FMR1 premutation, even in the absence of overt motor deficits. Some aspects of the network and behavioral functions recover upon cessation of the CGG-repeat expression.

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Cortical and subcortical volumetry in patients with Parkinson's disease and cognitive impairment

Martin Kunz[1], Martin Gorges[1], Hans-Jürgen Huppertz[2], Inga Liepelt-Scarfone[3], Alexander Storch[4,5,6], Richard Dodel[7,8], Rüdiger Hilker-Roggendorf[9], Daniela Berg[3,10], Elke Kalbe[11], Hans-Peter Müller[1], Simon Baudrexel[12], Jan Kassubek[1]

[1]Department of Neurology, University of Ulm, Ulm, Germany [2]Swiss Epilepsy Clinic, Klinik Lengg, Zürich, Switzerland [3]German Center of Neurodegenerative Diseases and Hertie Institute for Clinical Brain Research, Tübingen, Germany [4]Department of Neurology, University of Rostock, Rostock, Germany [5]Division of Neurodegenerative Diseases, Department of Neurology, Technische Universität Dresden, Dresden, Germany [6]German Centre for Neurodegenerative Diseases (DZNE) Rostock/Greifswald, Germany [7]Department of Neurology, Philipps University Marburg, Marburg, Germany [8]Department of Neuro-Geriatrics, University Clinic, Essen, Germany [9]Klinik für Neurologie und Klinische Neurophysiologie, Klinikum Vest [10]Department of Neurology, Christian Albrecht University, Kiel, Germany [11]Medical Psychology | Neuropsychology and Gender Studies, Center for Neuropsychological Diagnostics and Intervention (CeNDI), University Hospital Cologne, Cologne, Germany [12]Department of Neurology, J.W. Goethe University, Frankfurt/Main, Germany

In vivo correlates from longitudinal imaging data for progressive cognitive impairment in Parkinson's disease (PD) are still limited.
Here, we analysed morphological patterns from anatomical T1-weighted MR imaging data from PD patients in order to investigate whether the observed patterns are consistent with the Braak hypothesis.
Atlas-based volumetric (ABV) analysis to assess cortical and subcortical volumes from 3D MRI data (MPRAGE) was performed. In addition, Freesurfer was used to determine cortical thickness. Volumetric and cortical thickness data were evaluated over about 4 years; to this end, up to 5 measurements in 140 PD patients and 71 controls from the LANDSCAPE study were subjected to the final statistical analysis.
We could identify three groups of PD patients: group 1 subjects (n=35) presented with volumetric loss of subcortical structures including the striatum. Group 2 subjects (n=89) displayed volumetric loss of group 1 and additionally cortical thinning of parieto-temporal areas and the hippocampus. Group 3 subjects (n=16) were characterized by additional cortical volumetric loss in several prefrontal areas and volumetric loss of limbic structures. The data-driven and investigator-independent classification approach was significantly correlated with the cognitive status. In conclusion, morphological macrostructural cerebral changes in the course of PD with progressive cognitive impairment appear to develop in a sequential manner consistent with the Braak stages 4-6.

Impaired organelle transport in a neuronal cell model derived from Niemann-Pick type C1 patient-specific induced pluripotent stem cells

Maik Liedtke[1], Franziska Peter[1], Christin Völkner[1], Michael Rabenstein[1], Moritz J. Frech[1]

[1]University Medicine of Rostock, Albrecht-Kossel Institute for Neuroregeneration, Gehlsheimer Straße 20, 18147 Rostock, Germany

Niemann-Pick type C1 disease (NPC1) is a rare progressive neurodegenerative disease caused by mutations in the NPC1 gene. A mutation in the NPC1 gene leads to an impaired lipid transport resulting in an accumulation of cholesterol and gangliosides in different cell organelles, like lysosomes, late endosomes and mitochondria. The pathogenic mechanisms ultimately leading to neurological manifestations caused by neuronal dysfunction and cell death are not exactly understood. Recently, we described alterations of the phosphorylation and assembly of the intermediate filaments GFAP and vimentin in NPC1 deficient glial cells, which are known to function as the “cellular railroad system” for different organelles. Interestingly, we observed an upregulation of vimentin in NPC1 deficient neurons. However, it was reported recently that the transport of mitochondria and lysosomes was facilitated in neurofilament light chain deficient cells, suggesting that neurofilaments may function as an intracellular “traffic control system” involved in the transport of organelles. Additionally, an impaired organelle transport, especially of mitochondria is described for different other neurodegenerative diseases like Alzheimer disease (AD) and Amyothrophic Lateral Sclerosis (ALS), indicating to be a general pathophysiological feature of neurodegeneration. However, less is known in regards of the transport of these organelles in NPC1 deficient neurons. Thus, we were interested in the transport of mitochondria and the expression of neurofilaments in NPC1 deficient neurons. We analyzed the transport of mitochondria in NPC1 patient-specific induced pluripotent stem cell (iPSC) derived neurons. For live cell imaging neurons were transfected with baculoviruses to tag mitochondria with GFP. Analysis revealed a significantly reduced speed, as well as a reduced track length in cell lines carrying different NPC1 mutations. Interestingly, the overall track duration was increased. Taken together, we conclude that the transport of mitochondria is generally impaired in NPC1 deficient neurons. Regarding the expression and the phosphorylation of neurofilaments, we found differences in the expression of all three neurofilament subunits, as well as in the phosphorylation pattern of the neurofilament heavy chain. Thus, we hypothesize, that an altered phosphorylation, as well as the overall expression pattern of neurofilament proteins contribute to the impaired mitochondrial transport and thus to the neurodegeneration observed in NPC1. Additionally, our results underline the similarities between NPC1 and other neurodegenerative diseases, like ALS and Alzheimer diseases and thus contribute to a better understanding of pathophysiological mechanisms of neurodegeneration itself.

The NMDA receptor antagonist ketamine transiently reduces thalamocortical spindle and slow oscillations in a rodent model of non-REM sleep

Ali Mahdavi[1,2], Didier Pinault[1], Stefan Rotter[2], Yi Qin[1,3,4], Marine Bertschy[1], Damaris Cornec[1]

[1]Inserm U1114, FMTS, Université de Strasbourg, France
[2]University of Freiburg, Bernstein Center Freiburg (BCF), Germany
[3]University of Amsterdam, Netherlands
[4]Netherlands Institute for Neuroscience, Netherlands

During the early stages of schizophrenia, sleep disturbances are observed, and cortical EEG investigations reveal a deficit in sleep-related, thalamocortical (TC) spindle-frequency (10-16Hz) oscillations. This supports the hypothesis that the thalamus plays a critical role in the etio-pathophysiology of schizophrenia but the underlying neural mechanisms remain to be determined. The thalamus is a key structure for attention-related sensorimotor and cognitive integration processes and for the generation of TC spindle oscillations, which would play a role in plasticity, learning and memory. In the GABAergic thalamic reticular nucleus (TRN) neurons, NMDA receptors are essential in the generation of spindle rhythmic burst firing, which rhythmically hyperpolarizes the postsynaptic TC neurons through the activation of GABA receptors. Moreover, in an in vitro investigation, selective blockade of NMDA receptors significantly shortens spindle-like oscillations.
OBJECTIVE: We hypothesized that the deficit in sleep spindles recorded in schizophrenia involves a reduced function of NMDA receptors.
METHODS: Multisite in vivo electrophysiological cell-to-network recordings were used to investigate the effects of a single administration, at a psychosis-relevant dose (2.5 mg/kg), of the NMDA receptor antagonist ketamine in the somatosensory TC system of pentobarbital-sedated rats.
RESULTS: Under the control condition, spontaneously-occurring sleep-like oscillations were simultaneously recorded in the cortical EEG, in the extracellular field potential of the somatosensory thalamus, and in the juxtacellularly recorded and formally identified TRN cells. TRN cells rhythmically exhibited robust high-frequency bursts of action potentials (4 to 15 at 200-500 Hz). Remarkably, ketamine consistently and transiently reduced TC network spindle and slower oscillations, increased gamma- (30-80 Hz) and higher-frequency oscillations, and switched the firing pattern of TRN cells from the burst mode to the tonic mode. A partial recovery of the ketamine effects was observed at ~60-80 minutes after the systemic administration of ketamine. Dizocilpine (MK-801), a specific NMDA receptor antagonist, but not the cholinesterase inhibitor physostigmine nor the dopamine receptor agonist apomorphine mimicked the ketamine's fleeting psychosis-relevant effects.
CONCLUSION: The present findings are in agreement with clinical data, and they support the hypothesis that the schizophrenia-related deficit in sleep spindles and slower oscillations involves the NMDA receptor hypofunction.
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Misperceptions and performance fluctuations and their relation to resting state functional connectivity in Parkinson patients

Kristina Miloserdov[1,3,4,8], Carsten Schmidt-Samoa[1], Kathleen Williams[1], Christiane Anne Weinrich[2], Kathrin Bürk[5,6], Claudia Trenkwalder[5,7], Mathias Bähr[2,4], Melanie Wilke[1,3,4,8]

[1]UMG, Cognitive Neurology, Rober-Koch Straße 40, 37075 Goettingen, Germany
[2]UMG,Neurology, Robert-Koch Straße 40, 37075 Goettingen, Germany
[3]German Primate Center, Leibniz Institute for Primate Research, Kellnerweg 4, Goettingen, 37077, Germany
[4]DFG Center for Nanoscale Microscopy & Molecular Physiology of the Brain (CNMPB)
[5]Paracelsus-Elena Klinik, Kassel, 34128, Germany
[6]Department of Neurology, University of Marburg, 53043 Marburg, Germany
[7]Department of Neurosurgery, University Medical Center Goettingen, Robert-Koch-Str. 40, Goettingen, 37075, Germany
[8]Leibnitz-Science Campus Primate Cognition, German Primate Center, Goettingen, Germany

Introduction
Patients with Parkinson's disease (PD) frequently suffer from visual misperceptions and hallucinations, which are difficult to objectify and quantify. We aimed to develop an image recognition task to relate misperceptions and performance fluctuations to functional network connectivity in Parkinson patients.
Methods
Parkinson patients (N = 16) performed an image recognition task with real and scrambled images. We assessed whether misperception scores and intra-individual variability in recognition times are related to resting state network abnormalities as measured with functional MRI.
Results
PD patients with self-reported hallucinations exhibited higher proportions of perceptual errors and higher intra-individual variability in recognition times as compared to PD patients without visual hallucinations. While perceptual errors correlated with increased functional connectivity between visual and fronto-parietal networks, intra-individual variability correlated with increased connectivity within the default mode network and between the default mode and executive network.
Conclusions
Our behavioral and functional connectivity results suggest that misperceptions and visual performance fluctuations in Parkinson's disease patients, albeit both related to the occurrence of hallucinations in PD patients, are dissociable phenomena. Both behavioral measures might prove useful in future studies aiming to detect PD patients at risk for hallucinations or cognitive decline.

Optogenetic stimulation inhibits seizure generation in a mouse model of mesial temporal lobe epilepsy

Enya Paschen[1,2], Philipp Janz[1,2], Katharina Heining[3,4], Diego Vieira[3,4], Dr. Ute Häussler[1], Antje Kilias[2,3,4], Prof. Dr. Ulrich Egert[2,4,5], Prof. Dr. Carola Haas[1,4,5]

[1]Experimental Epilepsy Research, Dept. of Neurosurgery, Medical Center - University of Freiburg, Faculty of Medicine, 79106 Freiburg, Germany

[2]Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany

[3]Biomicrotechnology, Department of Microsystems Engineering – IMTEK, Faculty of Engineering, University of Freiburg, 79110 Freiburg, Germany

[4]Bernstein Center Freiburg, University of Freiburg, 79104 Freiburg, Germany

[5]BrainLinks-BrainTools Cluster of Excellence, University of Freiburg, 79110 Freiburg, Germany

Mesial temporal lobe epilepsy (MTLE) is the most common focal epilepsy in adults and is characterized by spontaneous, recurrent epileptic seizures and hippocampal sclerosis. Due to pharmacoresistance, surgical resection of the epileptogenic focus often represents the only therapeutic option in MTLE.
Previous *in vitro* studies have shown that low-frequency stimulation (LFS) can interfere with the generation of spontaneous seizures. Hence, we aimed at identifying stimulation parameters for successful seizure control in an *in vivo* mouse model of MTLE.
Firstly, we established a model of variable MTLE disease severity by injecting different concentrations of kainate (KA) unilaterally into the hippocampus of C57BL/6 mice. Secondly, we targeted principal cells in the entorhinal cortex using a channelrhodopsin2-encoding viral vector to enable perforant path stimulation in chronically epileptic mice. During local photostimulation of afferent fibers from the entorhinal cortex into the sclerotic hippocampus, we recorded epileptic activity at different positions in the hippocampal formation to assess the epileptogenicity and the effects of LFS on subclinical as well as behavioral epileptic seizures.
We found that i) the severity of experimental MTLE – described as the variability in hippocampal sclerosis and seizure characteristics – can be modified by increasing KA concentrations; ii) optogenetic LFS of entorhinal input can interfere with both, spontaneous recurrent seizures originating from the KA-injected hippocampus and evoked convulsive seizures in several degrees of MTLE severity; and iii) optogenetic 1 Hz LFS appears most effective in seizure prevention while leaving the animals mobility unaffected.
Our results suggest that LFS of entorhinal input reliably reduces seizure frequency most likely due to “preconditioning” processes that prevent seizure generation and generalization. Thus, this may constitute a promising approach for seizure control in MTLE.

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Apolipoprotein D-mediated regulation of lysosomal membrane integrity preserve lysosomal function and promotes cell survival in Niemann-Pick Type A disease.

Raquel Pascua-Maestro[1], María D. Ganfornina[1], María D. Ledesma[2], Diego Sanchez[1]

[1]IBGM, Dpto. Bioquímica y Biología Molecular y Fisiología, Valladolid, Spain
[2]Centro de Biología Molecular Severo Ochoa, CSIC, Madrid, Spain

Lysosomal Storage Diseases (LSDs) are genetic, early onset, neurodegenerative diseases that result in poor survival and both systemic and nervous system dysfunction. All LSDs are associated to leukodystrophy or myelination problems.

We have recently discovered that the Lipocalin Apolipoprotein D (ApoD) is essential for the maintenance of lysosomal functional integrity in glial cells. ApoD function ensures processes as diverse as cell survival upon oxidative stress (by reverting membrane permeabilization and loss of pH gradients), adequate compaction of myelin (by controlling glycolipid recycling processes), or proper phagocytic activity after nervous system injury. The crucial role of ApoD within the lysosome led us to study the potential effects of ApoD on a particularly devastating LSD, the Niemann Pick type A disease (NPA), caused by loss of function mutations in the gene encoding for acid sphingomyelinase, which results in sphingomyelin accumulation in lysosomal and plasma membranes. NPA patients rapidly develop progressive neurodegeneration, cerebral and cerebellar atrophy, significant Purkinje cell loss, and myelin deficiencies. No treatment is available today, in spite of various attempts with pharmacological, enzyme-replacement, or cell-based strategies.

Using two independent NPA-patient derived fibroblasts cell lines and two healthy control lines, we here demonstrate that, as in glial cells and neurons, ApoD is targeted to lysosomes of NPA fibroblasts. While oxidative stress induces an accelerated entry of ApoD into the lysosomal compartment of healthy cells, such accelerated targeting is lost in the diseased cells, contributing to the vulnerability of NPA lysosomes. We assessed lysosomal functional integrity and cell survival, using L-leucyl-L-leucine methyl ester as positive control for lysosomal membrane rupture, and pre-treatment of healthy control cells with sphingomyelin as a phenocopy of NPA disease.

By measuring cathepsin B activity (Magic Red assay), galectin-3 subcellular location, and lysosomal pH (Lysosensor Yellow/Blue DND-160 ratiometric assay), we demonstrate that exogenously added ApoD is able to significantly reduce lysosomal permeabilization and NPA-promoted lysosomal alkalinization. ApoD addition reverts the accumulation of oxidized products in lysosomes (revealed by lipofuscin signal) and of lipid peroxidation (4-hydroxynonenal signal) in NPA cells, resulting in a significant increase in cell survival.
Our results reveal that ApoD protection of lysosomal integrity is able to counteract biological deterioration in NPA cells, and open therapeutic opportunities for this devastating disease.

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Replicative reprogramming in the context of physiological CNS aging and age-related neurodegeneration

Diane Penndorf[1], Alessandro Ori[2], Ivonne Heinze[2], Joanna Kirkpatrick[2], Otto W Witte[1], Alexandra Kretz[1]

[1]Jena University Hospital, Hans Berger Department of Neurology, Am Klinikum 1, 07747 Jena, Germany
[2]Leibniz Institute on Aging – Fritz Lipmann Institute, 07745 Jena, Germany

Cellular senescence plays a crucial role in the aging process, particularly in proliferating cells, which respond with cell cycle arrest. However, aging mechanisms in post-mitotic neurons, where the paradigm of replicative senescence fails to a wide extent, are not well understood. In mature neurons, active cell cycle suppression appears mandatory to avoid atypical cell cycle re-itineration, a deregulation entailing neuronal dysfunction and apoptosis. The process of 'replicative reprogramming' and cell cycle-related neuronal death (CRND) is currently discussed broadly in neurodegeneration and substantiated for Alzheimer's disease. However, the importance for other age-related CNS pathologies and the physiological aging process of the central nervous system (CNS) itself is unknown.
In this study, parameters of aberrant cell cycle re-induction were assessed in the context of age-related amyotrophic lateral sclerosis (ALS), a lethal neurodegenerative disorder characterized by a progressive loss of motor neurons, and in physiological as compared to pathological CNS aging.
As model systems, the ventral spinal cord of ALS-mimicking hSOD1^{G93A} mice, and the neocortex of highly aged (27-30 months) C57BL/6 and progeroid Klotho mutants were investigated and compared with physiological control conditions. Our qPCR-based analyses illustrate broad alterations in the expression patterns of phase-specific cell cycle regulators both in ALS as well as in healthy aged and progeroid animals. In support, on the protein level we detected a strong increase of the G_1 phase cell cycle regulator CyclinD1 under disease-like conditions. Furthermore, an alteration in the subcellular location of different cell cycle regulators, e.g., the CNS-specific cell cycle inhibitor Cdk5 was observed. A nuclear Cdk5 loss, which is inductive for cell cycle re-initiation, was detected in hSOD1^{G93A} motor neurons already early in the disease process. Diminished nuclear Cdk5 was accompanied by reduction of its co-activator p35. Apart from immunofluorescence and western blot techniques, such down-regulation of Cdk5 was further confirmed using systematic mass spectrometry. In neurons, p35 is mandatory for the cell cycle-suppressive nuclear maintenance of Cdk5. This Cdk5/p35 interaction is disturbed by the calpain-dependent cleavage of p35. Using the quantitative Simple Western™ technique, we found an increased calpain activity along with a nuclear p35 degradation and Cdk5 depletion. As a putative so far undescribed upstream regulator for the induction of such a replicative reprogramming, we investigated the growth arrest-specific Gas2, which is involved in mediating replicative arrest, calpain inhibition and caspase 3-dependent apoptosis. Interestingly, we found a down-regulation of Gas2 under ALS-like and CNS aging conditions. According to these results, we hypothesize that the deregulation of Gas2-driven calpain activity triggers Cdk5/p35-dependent atypical cell cycle re-induction in the aging-related ALS pathology. Further analysis will prove if this axis is similarly affected under physiological aging conditions.

In summary, we introduce a novel molecular pathway as putative explanation for the phenomenon of replicative reprogramming and subsequent CRND in aging and ALS-related neurodegeneration.

scRNAseq analysis of brain organoids to study molecular mechanism of Leigh syndrome

Tancredi Massimo Pentimalli[1], Agnieszka Rybak-Wolf[1], Nikos Karaiskos[1], Gizem Inak[1], Alessandro Prigione[1], Nikolaus Rajewsky[1]

[1]Max Delbrück Center for Molecular Medicine (MDC)
Systems Biology of Gene Regulatory Elements, Robert Roessle Str 10

Leigh syndrome is incurable and early-onset neurodegenerative disorder associated with defects in mitochondrial enzymes. The disease mechanisms remain elusive because of the lack of an experimental model that accurately recapitulates the human condition.
To create an experimental model which exactly captures patient genetics and allows the study of numerous CNS cells types in a 3D brain tissue-like microenvironment, we generated LS patient-derived brain organoids.
Modifying a protocol originally published by Lancaster et al., 2013, we could generate early stage forebrain organoids from iPSC lines carrying SURF1 mutations and compared them to CRISPR-corrected isogenic iPSC lines. Combining the latest cutting-edge technologies such as single cell RNA sequencing (scRNA-Seq), total/ circRNA RNA sequencing with immunohistochemistry; we identified novel disease-associated changes in cell type composition and global alterations in proliferative and differentiative pathways.
Preliminary data and approaches to translating these data into new therapeutic strategies will be presented.

Mitochondrial and lysosomal dysfunction have opposite effects on lipid biosynthesis

Leonardo Gabriel Pereyra[1], Nuno Raimundo[1]

[1]University Medical Center Goettingen, Institute of Cellular Biochemistry, Humboldtallee 23, 37073 Goettingen, Germany

Organelles enable compartmentalization of reactions involved in cellular processes. Crosstalk pathways between different organelles ensure coordination of cellular activities. In this study, we focus on the impact of lysosomal and mitochondrial malfunction on cellular metabolism. To induce the mitochondrial and lysosomal malfunction in HeLa cells, we reduced the expression of complex III subunit of the respiratory chain (UQCRC1) and of lysosomal enzymes cathepsin B and acid alpha glucosidase (CTS and GAA), respectively. We analyzed the cells by RNA sequencing, founding that the synthesis of cellular lipids is affected by mitochondrial and lysosomal dysfunction, albeit in opposite ways. We confirmed these results using mouse embryonic fibroblasts and *in vivo* mouse model of lysosomal and mitochondrial dysfunction. Further research is required to understand the mechanisms involved.

Axonal changes upon toxin-induced myelin remodeling

Friederike Pfeiffer[1], Petra Fallier-Becker[2]

[1]Werner Reichardt Centre for Integrative Neuroscience (CIN), University of Tübingen
[2]Institute of Pathology and Neuropathology, University Hospital Tübingen, Germany

In Multiple Sclerosis, oligodendrocytes and myelin are lost leaving axons demyelinated. Remyelination restores nerve conduction and protects axons from degeneration. Remyelination can be performed by oligodendrocyte precursor cells (OPCs), if they receive the appropriate signals that induce their differentiation into mature myelinating oligodendrocytes. The nature of these signals is currently under intensive investigation. Thereby, it has become clear that neurons form synaptic contacts onto OPCs and are able to regulate myelination through electrical activity. Although remyelination occurs in adult organisms, many features of developmental myelination seem to be recapitulated during remyelination. In this study, we induce demyelination by feeding Cuprizone pellets in transgenic mice with cre-inducible GFP-expression in oligodendrocyte lineage cells, which can be traced after cre-activation. This enabled us to visualize myelin remodeling after 5 weeks of Cuprizone feeding and after 5 weeks of Cuprizone feeding followed by 1 or 5 weeks of feeding with normal food. We analyzed the distribution of the sodium channel subunit Nav1.6 in corpus callosum of all treatment groups and found the number of nodes of Ranvier to be significantly enhanced after 5 weeks of Cuprizone treatment followed by 5 weeks of recovery, when compared to the control group. In addition, ultrastructural analysis of axons after Cuprizone-treatment and during recovery was performed, showing morphological changes in unmyelinated axons. We also measured compound action potentials (CAPs) in order to assess the physiological function of affected axon bundles. Taken together, by investigating axon morphology and function upon myelin remodeling with different methods, we revealed axonal changes that occur simultaneously to re-movement of mature oligodendrocytes followed by enhanced generation of new oligodendrocytes. Understanding how axons adapt to demyelination in order to restore myelination will help to identify targets for new therapies aiming at improving the success of remyelination.

GABAergic Synaptic Input to Cerebellar Purkinje Cells is Affected in a Niemann-Pick Type C1 Mouse Model

Michael Rabenstein[1], Christin Völkner[1], Maik Liedtke[1], Arndt Rolfs[1], Moritz J. Frech[1]

[1]University Medicine of Rostock, Albrecht-Kossel-Institute for Neuroregeneration, Gehlsheimer Str. 20, 18147 Rostock, Germany

Niemann-Pick Type C1 disease (NPC1) is a rare progressive neurodegenerative disease caused by mutations in the NPC1 gene, leading to accumulation of cholesterol in the late endosomes and lysosomes. Besides a described variety of morphological alterations of neurons, a detailed knowledge about the pathophysiological processes in neurons leading to dysfunction and degeneration is still missing. Of special interest is the degeneration of cerebellar Purkinje cells (PCs) resulting in ataxia. Previously, we reported alterations of the intrinsic activity of PCs in NPC1-deficient mice. Since the intrinsic activity is modulated by, amongst others, GABAergic synaptic input, we asked ourselves, if alterations of the GABAergic synaptic transmission to PCs, as well as its regulation, are present in NPC1. By means of patch clamp experiments we recorded spontaneous inhibitory postsynaptic currents (sIPSCs) of PCs in acute cerebellar slices of p19-p25 NPC1-deficient and wild type mice. We found a higher sIPSC frequency in PCs of NPC1-deficient mice. Applying NMDA to activate interneurons, we recorded significantly less often an increase of the sIPSC frequency in PCs of NPC1-deficient mice, showing an impaired regulation of synaptic transmission. Furthermore, NMDA-induced rise in sIPSC frequency was reduced in PCs of NPC1-deficient mice. In summary, we observed alterations in the inhibitory synaptic transmission and regulation in PCs of NPC1-deficient mice. In interneurons, NMDA induces a calcium influx, activating the protein kinase C (PKC) and subsequently increasing the surface expression of GluR2-positive AMPA-receptors (AMPAR). Thus, excitability of interneurons is increased and consequently their activity and GABA release. Based on previous studies we assumed that due to an impaired PKC function in NPC1 the expression of AMPAR is altered. Therefore, we checked the cerebellar expression of GluR2. Although the total level of GluR2 was unaffected, the amount of Ser880-phosphorylated GluR2 was significantly reduced in NPC1-deficient mice. Since the Ser880-phosphorylation leads to an internalization of GluR2-positive AMPAR, this hints to an increased surface expression of these receptors, like we reported previously in our NPC1-mutated neuronal cells. The higher excitability of interneurons, which results from this increased AMPAR expression, could explain the higher sIPSC frequency, observed in PCs. Additionally, a further rise in the surface expression of GluR2-positive AMPAR could be limited, explaining the reduced effect of NMDA application in NPC1-deficient mice.

We hypothesize, that an impaired PKC function alters the surface expression of AMPAR in cerebellar interneurons. On the one hand, this can result in an increased excitability and increased release of GABA. But on the other hand, the regulation of their excitability can be defected. If such alterations of the GABAergic synaptic transmission to PCs contribute to the decline of the PCs will be object of further studies.

Specific Mutations in Presenilin 1 have a Differential Role on Mitochondrial Phenotype and Function

Liliana Rojas-Charry[1], Laura Heikaus[2], Harmut Schlueter[2], Christian Hagel[1], Markus Glatzel[1], Diego Sepulveda-Falla[1]

[1]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[2]Institute for Clinical Chemistry, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Introduction: Mitochondria are well known for its role as the “power center” of the cells. But they hold more functions such as cellular homeostasis, apoptosis, iron processing, calcium buffering and steroid synthesis, to name a few. The importance of mitochondria in cellular homeostasis is unquestionable, and they cannot be set aside when promoting healthy aging. In fact, the relationship between mitochondria and neurodegenerative diseases is widely known and has been discussed extensively. Presenilin 1 (PS1), one of the proteins whose mutations cause Familial Alzheimer´s Disease, has been found in different cellular locations, including mitochondrial associated membranes, but its possible role in mitochondrial function is not well understood.

Objective: The aim of this study is to characterize the role of mutant PS1 overexpression in mitochondrial morphology and function.

Methods: Transgenic mice overexpressing human PS1 mutations G384A and E280A were used as models. Primary neurons were grown in co-culture with astrocytes for immunofluorescence experiments, evaluation of mitochondrial morphology and for respiration assays using the Seahorse system to evaluate oxygen consumption under different stimuli. Whole brains were dissected from male mice at different age groups (1, 4, 6, 9 and 12 months) to isolate intact mitochondria in a Percoll gradient. The mitochondrial fraction was prepared for proteomics using LC-MS/MS analysis.

Results: Significant differences were found in mitochondrial morphology in primary neurons carrying the hPS1E280A mutation, in contrast to hPS1G384A and control mitochondria. PS1 colocalized more with the mitochondrial marker Tom20 in cortical neurons, not in hippocampal. Proteomic analysis showed variation in the expression of proteins especially related to respiration and mitochondrial ribosomal function in pure mitochondria extracted from hPS1E280A mouse brains. Finally, mitochondrial oxygen consumption showed functional impairment in primary neurons from hPS1E280A mutants, but not in the hPS1G384A mutants, confirming a differential mitochondrial phenotype.

Conclusions: Until now it is not known if mitochondrial dysfunction is a cause or a consequence in Alzheimer’s neurodegeneration, but increasing evidence has shown its relevance in cellular processes directly related to neurodegeneration. Our results demonstrate that PS1 overexpression of specific mutations modulate mitochondrial morphology, oxygen consumption and the expression of key ribosomal and respiratory mitochondrial proteins. It is remarkable that the mitochondrial phenotype was different between mutations, being more dramatic in the hPS1E280A mutation, which generates similar concentrations of Aβ40 and AB42, contrary to what it is observed in the mutation hPS1G384A which tends to increase the Aβ42/ Aβ40 ratio, suggesting a mutation-specific mitochondrial pathology in familial Alzheimer’s disease.

Combination of sesamin and alpha-mangostin attenuates hydrogen peroxide-induced neurodegeneration

Waralee Ruankham[2], Wilasinee Suwanjang[1], Virapong Prachayasittikul[2], Supaluk Prachayasittikul[3], Kamonrat Phopin[1,2]

[1]Mahidol University, Center for Research and Innovation, Faculty of Medical Technology, Phuttamonthon 4 Road, 73170 Nakorn Pathom, Thailand

[2]Mahidol University, Department of Clinical Microbiology and Applied Technology, Faculty of Medical Technology, Phuttamonthon 4 Road, 73170 Nakorn Pathom, Thailand

[3]Mahidol University, Center of Data Mining and Biomedical Informatics, Faculty of Medical Technology, Phuttamonthon 4 Road, 73170 Nakorn Pathom, Thailand

Sesamin is the most prominent lignan found in sesame (*Sesamum indicum* L.) seed and oil. It is catered to be a nutritional supplement that confers antioxidant and anti-inflammatory effects. Alpha-mangostin, a xanthone of *Garcinia mangostana* L., possess broad biological properties, such as antioxidant, anticancer, antimicrobial, and anti-inflammatory like sesamin. The aim of the study was to investigate the neuroprotective effects of sesamin in combination with alpha-mangostin against oxidative stress in hydrogen peroxide (H_2O_2)-induced human neuronal cells. The neuronal cells were treated with 1 μM sesamin, 1 μM alpha-mangostin, or a combination of sesamin (1 μM) and alpha-mangostin (1 μM) for 3 h prior to H_2O_2 exposure 24 h. The levels of cell viability, apoptosis profiles, and ROS productions were estimated by MTT, annexin V/7-AAD, and DCFDA assays, respectively. Treatment of H_2O_2 significantly increased cytotoxicity, total apoptosis, and ROS production. Interestingly, neuronal cells treated with a combination of sesamin and alpha-mangostin significantly increased cell viability about 1.2 time and decreased total apoptosis (1.3 time) and ROS production (1.5 time) compared to the H_2O_2 alone. Therefore, sesamin and alpha-mangostin with potent antioxidant properties have an ability to prevent the neurodegeneration caused by oxidative stress. The combination of both sesamin and alpha-mangostin can potentially attenuate oxidative damages occurred in neuronal cells. These data suggest that sesamin and alpha-mangostin might be promising natural compounds for prevention and/or treatment of neurodegenerative diseases.

Alterations of neurogenesis in dentate gyrus precede development of Alzheimer's disease-like pathology in OXYS rats

Ekaterina Rudnitskaya[1], Tatiana Kozlova[1], Alena Burnyasheva[1], Nataliya Kolosova[1], Natalya Stefanova[1]

[1]Institute of Cytology and Genetics SB RAS, Laboratory of Molecular mechanisms of aging, Lavrentieva 10, 630090 Novosibirsk, Russia

Alzheimer's disease (AD) is age-related neurodegenerative disorder that causes dementia as a result of atrophic changes in the brain, leading to disruption of attention, memory, and executive function. There is a growing body of evidences that AD-related neurodegenerative changes are associated with alterations of the neuronal plasticity in brain areas crucial for learning and memory: hippocampus and frontal cortex. Alterations of neurogenesis in the dentate gyrus (DG) already at the early postnatal age lead to disruption of neuronal plasticity in the hippocampus and thus may contribute to AD manifestation late in life. However, the precise mechanisms of the process are not fully understood. To investigate the link between early-life changes of neurogenesis and development of AD we used OXYS rats that simulate all key signs of the most common sporadic form of AD.

Methods. 3-10-, 20-day-, 3-5- and 18-month-old male OXYS and Wistar (control) rats were used. Immunohistochemistry was used to identify the number of neuronal cells at different stages of maturation and the number of apoptotic cells. The RNA-seq data were used to analyze differentially expressed genes involved in neurogenesis. ELISA and western-blot analysis were used to quantify the levels of neurotrophins (NGF, BDNF) and its receptors (TrkA, TrkB, phosphoTrkB, $p75^{NTR}$). Development of the neonatal reflexes (forelimb grasp, righting, negative geotaxis and cliff avoidance) was investigated.

Results. OXYS pups demonstrate delayed development of negative geotaxis and righting reflexes as compared to Wistar pups; the observation may point out to retardation of hippocampal development. Indeed, we showed increased density of neuroblasts and immature neurons at 10 days and increased number of apoptotic cells at 20 days of age in the DG of OXYS rats as compared to Wistar rats. These parameters may reflect delay of hippocampal development and alterations of DG neurogenesis at an early age prior to appearance of AD signs in OXYS rats. Manifestation of AD-like pathology in OXYS rats (to the age of 3-5 months) is accompanied by changes in expression of genes involved in neurogenesis that associated with angiogenesis and regulation of extracellular microenvironment against background of imbalance of neurotrophins: increased BDNF level and decreased NGF level in the hippocampus. Progression of AD-like pathology in OXYS rats (from 3-5 to 18 months of age) is accompanied by changes in expression of genes involved in neurogenesis in the hippocampus which are associated with angiogenesis, response to oxidative stress and negative regulation of cell death against background of imbalance in activation of neurotrophin receptors: decrease of BDNF receptor's TrkB activation (that's mean phosphoTrkB/TrkB ratio) reflecting decrease of prosurvival BDNF effects with simultaneous increase of TrkA/$p75^{NTR}$ ratio reflecting shift of NGF effects from proapoptotic to prosurvival.

Conclusion. Alteration of DG neurogenesis at early age may contribute to the emergence of AD signs late in life. Manifestation and progression of the disorder are accompanied by changes in expression of genes associated with angiogenesis and imbalance of neurotrophic support of the hippocampus. These events may point out to disturbance of microenvironment in neurogenic niche of hippocampus and lead to alterations of neurogenesis.

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Epigenetic profiling in experimental models entailing mitochondrial and non-mitochondrial toxins - implications for movement disorders

Ranganayaki Sathyanarayanan[1], Srinivas Bharath MM[1]

[1]National Institute of Mental Health and Neurosciences, Neurochemistry, Hosur road, 560029 Bangalore, India

Movement disorders such as Parkinson's disease (PD), Huntington's disease (HD) and Manganism involve neurodegeneration of the nigrostriatum. However, their clinical manifestation is different indicating that the underlying degenerative pathways are different. While biochemical and genetic mechanisms could contribute to this at the cellular level, genome wide studies have identified epigenetic mechanisms governing the same. The comprehensive epigenetic code defining a particular disease is still not known. This could be analyzed in neurotoxic models that mimic the three movement disorders using Methyl Phenyl Pyridinium (MPP+) (Mitochondrial complex I inhibitor and PD toxin), 3-nitropropionic acid (3-NPA) (Mitochondrial complex II inhibitor and HD toxin) and Manganese (Mn) (Non-mitochondrial toxin and Manganism toxin).

Aim- To outline the epigenetic changes induced by 3-NPA, MPP+ and Mn in N27 dopaminergic neuronal cells and mice models with implications for neurodegeneration and movement disorder.

Methods- Whole genome transcriptome analysis and methylation microarray was carried out in N27 cells treated with Mn/ MPP/ 3- NPA at their respective LD50 concentrations. Histones were extracted from the toxin treated cells/toxin injected mice, followed by western blot based acetylation profiling of H3 and H4 histone against various lysine modifications. ChIP- seq was performed on immunoprecipitated DNA from H3K56Ac antibody and the differentially acetylated genes were validated by PCR.

Results- Gene ontology analysis of the microarray dataset showed enrichment of molecular functions, with autophagy being very exclusive to 3-NPA toxicity via mTORC2, while this was not observed in the other two models. Methylomics identified 2 autophagy genes to be methylated only in the 3-NPA model and not the others. Histone acetylation profile showed dose and time- dependent increase in acetylation of H3K56, only in 3-NPA and not in Mn and MPP models. These results were evident in vivo with the 3-NPA model showing significant increase in H3K56Ac in the striatum, compared to control. Chip- seq experiment with H3K56Ac antibody revealed a unique gene profile regulated by H3K56Ac in the 3-NPA model.

Conclusion- Complex II inhibition induced neurotoxicity followed a distinct set of molecular and epigenetic signatures which were not seen in case of complex I inhibition or non-mitochondrial toxin. This highlights the complex regulatory mechanisms involved in neurodegenerative mechanisms underlying movement disorders.

Astrocyte – T-lymphocyte communication under neuroinflammatory conditions

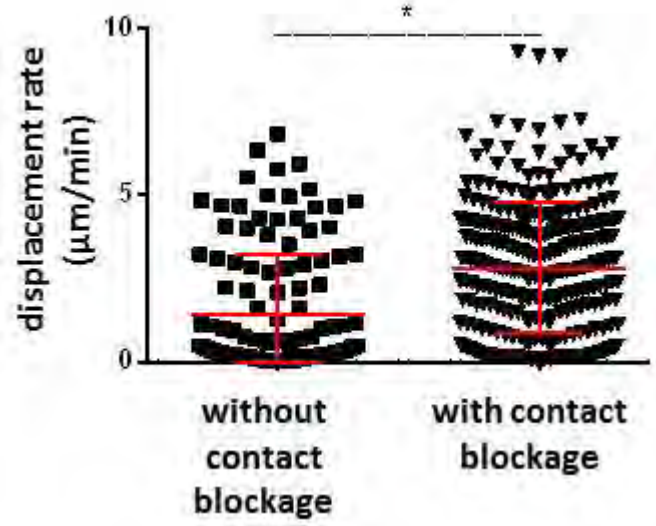
Samantha Schmaul[1], Julian Loeffel[1], Dirk Luchtman[1], Stefan Bittner[1]

[1]University Medical Center Mainz, Department of Neurology, Langenbeckstr. 1, 55131 Mainz, Germany

From an evolutionary point of view, the immune system and the central nervous system (CNS) developed hand-in-hand. Therefore, communication pathways have always been tightly linked and interconnected. For example, it has been shown before that the presence of T-cells is necessary for a healthy brain in order to develop correctly. Mice that are T cell deficient show e.g. learning and memory as well as social behaviour deficits. These CNS -immune system interactions become even stronger during neurological diseases. During diseases like Parkinson's disease, Th17 are recruited into the substancia nigra pars compacta, where they have a potentially detrimental effect on the disease course. A similar increase in Th17 cells within the CNS can be observed in Alzheimer's disease as well as in multiple sclerosis. The reasons for recruitment may be as diverse as the functions these Th17 execute but their basic interaction mechanisms in the CNS are potentially comparable.

Astrocytes are increasingly recognized as active participants in modulating and driving neuroinflammatory responses, integrating specific signals from the extracellular environment. However, it is currently unknown which signals drive these astrocytic responses and it remains under debate whether astrocytes are able to directly pass these signals on to immune cells.

In order to address these questions, we have established a live view of astrocytes in their cellular context to analyse their functional properties in more depth using an Aldh1l1-eGFP reporter mouse in an organotypic hippocampal slice culture model. To mimic neuroinflammatory conditions, proinflammatory T cells were added to the slice cultures and their interactions with astrocytes were studied in-depth using two-photon live imaging. In this context, we have found that highly pro-inflammatory Th17 cells show a distinctly different behaviour when contacting astrocytes in comparison to those that do not contact astrocytes. They remain more stable in their contact, show less meandering, which implies less scanning behaviour. Overall, this subset has significantly decreased displacement rates. When blocking astrocyte - T cell interactions with specific blocking antibodies, the astrocyte-contacting Th17 cells change their behaviour dramatically towards those that initially do not contact astrocytes at all (Fig. 1). These findings imply a specific and functionally relevant interaction between astrocytes and proinflammatory T-cells in the context of neuroinflammation. Therefore, we considered whether this interaction may additionally be antigen-dependent. To this purpose, we analysed CNS-specific antigen recognising versus CNS-unspecific antigen-recognising Th17 cells. The general behaviour of both cell types is comparable. When looking at the astrocyte-contacting subset, CNS-unspecific antigen-recognising Th17 cells show differential behaviour towards CNS-specific antigen recognising Th17. The contact duration is largely decreased and fewer stable contacts could be found. This let us to the conclusion that under neuroinflammatory conditions, these contacts are mediated by antigen-presentation as well as antigen-specificity.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with contact blockage

Linking cognition to amyloid-β burden in the brain of a non-human primate (*Microcebus murinus*)

Daniel Schmidtke[1,2], Elke Zimmermann[1,2], Stéphanie G. Trouche[3], Pascaline Fontès[3], Jean- Michel Verdier[3], Nadine Mestre-Francés[3]

[1]University of Veterinary Medicine Hannover, Intitute of Zoology, Bünteweg 17, 30559 Hannover, Germany
[2]Center for Systems Neuroscience Hannover, Hannover, Germany
[3]MMDN, University of Montpellier, EPHE, INSERM, U1198, PSL Research University, Montpellier, F-34095 France

In the non-human-primate (NHP) *Microcebus murinus*, the grey mouse lemur, many morphological, physiological, and cognitive traits are age-dependent, comparable to humans. Extensive studies on brain aging in different laboratory colonies of *M. murinus* demonstrated the natural development of pathological brain alterations in a sub-population of aged individuals, including hippocampal and septal atrophies, the aggregation of phosphorylated Tau protein, and deposits of amyloid-β peptide (Aβ) and its precursor. They may be markers of a neurodegenerative disease comparable to Alzheimer's disease (AD) in humans. To further explore this hypothesis, we submitted aged individuals that took part in a cognitive study on visual pairwise discrimination (PD)/discrimination reversal (PDR) learning to neuro-immunohistochemistry (Aβ and Tau) once they had died from a natural death and investigated possible links between Aβ (and Tau) burden and cognitive performance.

In total we trained 37 old grey mouse lemurs (12 males, 25 females; age range: 5-11 years) in a standardized, touchscreen-based PD/PDR task. Out of these 37 subjects, 20 completed the entire criterion-based PD/PDR training. Of the remaining 17 subjects, two died from a natural death during the course of the study, while 15 dropped out of the experiment as they failed to complete the pre-training procedure that precedes the actual PD/PDR task. Interestingly, where neuro immunohistochemistry was possible, we found that pre-training dropouts presented significantly more often with intraneuronal accumulations of Aβ as compared to NHPs that completed the pre-training (Fisher's Exact Test, N_{total} = 31, odds ratio estimate: 0.129; 95% confidence interval: 0.011, 0.868; p-value = 0.023). Performance in visual pairwise discrimination learning (number of trials to criterion), on the other hand, was linked to the quantity (low vs. high burden) of extracellular Aβ deposits in the cortex (Wilcoxon Rank Sum Tests, N_{total} = 16, W = 52, p-value = 0.0196). A statement about the exact spatial pattern of the Aβ proteopathy at the time of cognitive testing cannot be made, as the histopathology was performed after subjects had died from a natural death, i.e. often a long time after the cognitive testing. Since intracellular Aβ depositions are considered to precede and catalyze AD-like extracellular proteopathology in both transgenic animal models and humans, it is possible that the linkage we found between extracellular Aβ burden at death and cognitive performance in the PD is also a reflection of intracellular Aβ burden at the time of testing. All tested animals proved negative for phosphorylated Tau.

In summary, our study provides the first evidence for a direct link of a naturally developed cortical Aβ burden and cognitive performance in an NHP. To solve the matter of the exact link between intra- and extracellular Aβ burden and cognition in future studies on mouse lemur AD-like pathology, it will be necessary to combine cognitive testing with *in vivo* assessment of Aβ buildups in this species. However, the here-presented findings, together with recent methodological advances in mouse lemur genetics, *in vivo* imaging, and cross-species comparable cognitive testing, are in strong support for the idea that the grey mouse lemur is exceptionally suited as a natural NHP model in biomedical research, especially in the field of healthy and pathological brain aging.

Effect of novel acetylcholinesterase inhibitors 3-nitro-6-amino-substituted imidazo[1,2-b] pyridazine derivative compounds on mitochondrial physiology

Rakesh Kumar Sharma[1], Manisha Singh[1], Sridhar Pulavarthi[2], Sabbasani Rajasekhara[2], Ravi Shankar Akundi[1]

[1]Neuroinflammation Research Laboratory, Faculty of Life Science and Biotechnology, South Asian University, Akbar Bhavan, Chanakyapuri, 110021 New Delhi, India.

[2]Department of Chemistry, School of Advanced Sciences, Vellore-632014, Tamil Nadu, India

Cognitive dysfunction and memory loss is a characteristic feature of Alzheimer's disease (AD) which is caused by degeneration of neurons in the cerebral cortex. Loss of cholinergic neurons, low levels of neurotransmitter acetylcholine and deficit of choline acetyltransferase (ChAT) has been reported in patients with AD and is responsible for the deterioration in cognitive function. In the early stages of the disease, acetylcholinesterase inhibitors reduce the progression of AD by maintaining the levels of acetylcholine within the synaptic cleft. Although acetylcholinesterase inhibitors are commercially available, there is a need to identify compounds with enhanced activity and additional properties that would address some of the drawbacks of the current drugs. In this study we worked on a group of novel acetylcholinesterase inhibitors which are derivatives of 3-nitro-6-amino-substituted imidazo [1,2-b] pyridazine compounds. These compounds show a moderate-to-strong inhibition of acetylcholinesterase – between 34% for compound 5k to 82% for compound 5c. We further tested the toxicity of these compounds and elucidated downstream intracellular signaling pathways in the human neuroblatoma cell-line, IMR-32.

There is a differential level of inhibition among the various substituted imidazopyridazine compounds tested. Except for compound 5k, none of the other compounds tested showed toxicity up to 100μM though there is a significant increase in cells arrested in G1 phase. This is further confirmed with a decrease in the levels of phosphorylated cdc2 in cells treated with substituted imidazopyridazine compounds. There is also reduction in cellular ATP levels, an increase in the phosphorylation of AMP-activated protein kinase (AMPK), and higher expression of LC3-B, responsible for autophagy. Furthermore, except for compound 5k, we found increased oxidative stress in cells treated with these compounds using the mitochondria-specific dye, Mitosox. We further tested the role of these compounds in mediating inflammation in the murine macrophage cell-line, RAW264.7. While compound 5c was shown to induce the expression of the pro-inflammatory molecule, cyclooxygenase 2 (COX-2), compounds 5h and 5k instead inhibited lipopolysaccharide (LPS)-induced COX-2 synthesis in these cells. We also did Molecular docking studies which showed compound 5k to be an effective inhibitor of beta-secretase (PDB Entry: 3BRA) with a CDOCKER energy of -20.64 compared to the drug verubecestat, which showed a CDOCKER energy of -12.46. These results show a promising role for compound 5k which does not show oxidative stress, decreases inflammation and may inhibit beta-secretase. These additional properties of compound 5k make it a novel drug as an acetylcholinesterase inhibitor which requires further study.

Baseline neuronal-activity dependence of Aβ-induced dysfunction

Manuel Simon[1], Benedikt Zott[1], Arthur Konnerth[1]

[1] Institute of Neuroscience and the Munich Cluster for Systems Neurology, Technical University of Munich, 80802 Munich, Germany

Even though Aβ plaques can be found in many brain regions of patients suffering from Alzheimer's disease (AD), the function of certain brain areas seems to be more affected by AD pathology than others. Thus, the well-characterized neuronal hyperactivity is more pronounced in areas like the hippocampus even though hippocampal plaque load is lower than neocortical amyloid deposition in very early stages of AD. One major difference between hippocampus and cortex is that the neuronal activity levels at baseline are much higher in hippocampal neurons. To test, whether neuronal activity indeed determines susceptibility to Aβ, we developed an experimental protocol in which the baseline activity of murine hippocampal slices could be pharmacologically controlled. In this study, we used two-photon calcium population imaging in the hippocampal CA1 area to determine the relationship between neuronal baseline activity and susceptibility to the application of soluble Aβ.

During our initial experiments, we were puzzled by the fact that Aβ application can induce activity in hippocampal CA1 neurons in vivo but not in in vitro hippocampal slices. We then discovered that the ineffectiveness of Aβ in brain slices was due to the very low levels of spontaneous activity in such in vitro preparations. We could demonstrate that under in vitro conditions, Aβ can activate neurons only after raising the level of spontaneous neuronal activity to that observed in vivo. This was achieved by applying glutamate, by increasing extracellular potassium levels or by blocking GABAergic inhibition. Another remarkable finding of these experiments was that, on the single cell level, the degree of Aβ-dependent hyperactivity was promoted by the baseline activity of the respective neuron. Thus, cells with high levels of baseline activity had even higher levels of hyperactivity upon Aβ injection than neurons with low baseline activity.

We next asked which Aβ species is mostly responsible for neuronal dysfunction. We tested different preparations of synthetic Aβ including monomers, covalently crosslinked dimers and a combination of multiple forms of dimers and oligomers. We demonstrate that amyloid dimers as well as the preparation including dimers and oligomers are the most potent inducers of neuronal hyperactivity, while monomers are largely ineffective.

In summary, our results demonstrate that Aβ-induced neuronal hyperactivity is baseline activity dependent and requires a 'threshold' level of afferent glutamatergic synaptic activity. We also show that the most toxic species of Aβ are dimers and oligomers, but not monomers. Thus, our findings can explain why certain brain regions with high levels of baseline activity are more susceptible to Aβ- induced dysfunction both in humans and mice. Considering that neuronal activity promotes a further increase of Aβ secretion, our data suggest a vicious cycle of hyperactivity and Aβ deposition in the brains of AD patients.

Increase of neuronal activity by 4-Aminopyridine in vivo, improves sensory-motor dysfunction in a mouse model of SMA

Christian Marc Simon[1], John G. Pagiazitis [2], Emily V. Fletcher[3], Beatriz Blanco-Redondo[4], George Z. Mentis[2]

[1]Leipzig University, Carl-Ludwig-Institute for Physiology, 04103 Leipzig, Germany
[2]Center for Motor Neuron Biology and Disease, Columbia University, NY, 10032, USA
[3]Cambridge Institute for Medical Research, University of Cambridge, UK.
[4]Leipzig University, Rudolf-Schönheimer - Institute for Biochemistry, 04103 Leipzig, Germany

Dysfunction of neuronal circuits are important determinants in neurodegenerative diseases. Spinal muscular atrophy (SMA) — caused by deficiency in the ubiquitously expressed SMN protein — is characterized by loss of central synapses, motor neuron death and skeletal muscle atrophy. SMA vulnerable motor neurons exhibit a reduced firing frequency as a response to impaired premotor synapses early in the disease process in mice. Strikingly, genetic restoration of SMN in premotor proprioceptive neurons improved the firing frequency in SMA motor neurons and the disease phenotype, suggesting that a pharmacological increase of neuronal activity could be a therapeutic strategy. The FDA approved 4-aminopyridine (4-AP) increases neuronal activity by block of voltage activated K+ channels. 4-AP was reported to correct locomotor dysfunction in a fly model of SMA, suggesting that it might alleviate the SMA disease phenotype in mice.

To address whether an increase of neuronal activity could be beneficial to the SMA phenotype in vertebrates, we injected daily SMAΔ7 mice with 4-AP (1mg/kg, BID). The treated SMA mice displayed slightly increased lifespan and bodyweight compared to vehicle-treated SMA mice. Furthermore, 4-AP treated SMA mice show an improvement in motor behavior at the end stage of disease, resulting in better righting times and ability to walk. To probe the cellular mechanisms for the improved motor function, we investigated the neuromuscular junction (NMJ) innervation extent in the quadratus lumborum (QL) muscle using immunohistochemistry. Strikingly, NMJ innervation in 4-AP treated SMA animals was significantly improved compared to untreated mutant littermates, with no rescue of vulnerable motor neurons, suggesting that 4-AP either prevents NMJ denervation or induces sprouting. To investigate whether increased NMJ innervation benefits muscle function, we conducted electromyography from the QL muscle. 4-AP treated SMA animals exhibited an increased amplitude in the compound muscle action potential compared to controls. Furthermore, 4-AP treatment resulted in significant increase in the amplitude of the monosynaptic spinal reflex. Morphological analysis revealed an increased number of proprioceptive synapses onto vulnerable motor neurons, suggesting that the improved amplitude of the spinal reflex is a result of a higher number of functional proprioceptive synapses following 4-AP treatment.

Here, we show that in vivo injection of 4-AP in SMA mice results in significant improved central (proprioceptive) and peripheral (NMJ) synaptic connectivity and motor function in SMA mice. Our study reveals that sufficient increase of neuronal activity may have a beneficial long-term effect for SMA patients.

Aberrant nitric oxide and redox signalling induces glycation activity resulting in enhanced neuronal dysfunction in neurodegeneration

Joern Steinert[1], Julie Bourgognon [1], Jereme Spiers [1]

[1]University of Leicester, Neuroscience, Psychology and Behaviour, University Road, LE9 2ES Leicester, United Kingdom

NO produced by Nitric Oxide Synthases (NOS) mediates a wide range of biological signalling with low physiological concentrations, NO-mediated activation of soluble guanylyl cyclase predominates in the brain. At higher concentrations, NO induces post-translational modifications (PTM) and when essential inactivation/buffering mechanisms are overwhelmed nitrergic toxicity may dominate. PTMs include protein thiol S-nitrosylation (SNO) and 3-nitrotyrosination (3-NT) which affects protein-protein interactions, structure and function. In neurodegenerative diseases neuroinflammatory nitrergic signalling is enhanced resulting in increased SNO and 3-NT formation of neuronal proteins due to higher NO production and/or insufficient buffering, which exacerbates pathological processes, making it important molecule in disease.
We employed a mouse model of neurodegeneration induced by misfolded prion protein. Mice were inoculated with infectious scrapie prion at 3 weeks of age and neurodegenerative markers were assessed between 6 and 9 weeks post inoculation (wpi). We performed immunocytochemistry and immunoblotting, RTqPCR and electrophysiology studies and tested disease reversibility by targeting nitrergic signalling. We characterised neuronal activity of hippocampal pyramidal neurons by recording miniature and Schaffer collateral-evoked excitatory postsynaptic currents (EPSCs), changes in synaptic protein levels and nitrergic PTM. To specifically assess effects of NOS antagonism on disease pathology, we injected mice daily between 6-9 wpi with an NOS inhibitor (L-NAME, 20mg/kg).
Our studies showed that mice which develop prion disease-induced neurodegeneration exhibit increased NO signalling. We identified elevated hippocampal and cortical NOS activity in conjunction with increased oxidative stress levels at 6 wpi and compromised glutamatergic activity and neuronal function suggesting a broad (pre- and/or post-) synaptic decline. Expression of synaptic proteins was reduced for Munc18, complexin and SNAP25 at 10 wpi. Our data further suggest that 3-NT signalling enhances glycation signalling, a non-enzymatic PTM of proteins which generates advanced glycation endproducts (AGE). This irreversible PTM is responsible for protein dysfunction and aggregation thereby augmenting existing neurodegenerative signalling. Importantly, several phenotypes of neurodegeneration, including 3-NT and AGE signalling and neuronal dysfunction were diminished in mice treated with the NOS inhibitor.
The data suggest that suppressing NO production during early disease stages has positive impacts on glutamatergic synaptic transmission and neuronal function, thereby slowing prion disease progression. Our findings further indicate that aberrant NO-signalling is closely linked to protein glycation and thus suppression of NO toxicity reduced neurodegenerative insults, a mechanism potentially applicable to other neuropathologies.

The neurovascular unit in Alzheimer's disease

Stephanie Lauren Taylor[1], Gabor Petzold[1]

[1]German Center for Neurodegenerative Diseases, AG Petzold Neurovascular Diseases, Sigmund-Freud-Strasse 27, 53127 Bonn, Germany

Alzheimer's disease (AD) is the most common form of dementia affecting the aging population worldwide. Dysfunction of the brains' vasculature has been linked with pathology of the disease, and indeed small brain bleeds (CMBs) seen on MRI and after death are reported to be higher in patients with AD than the general population. When vessels in the brain rupture they release toxic substances, which can increase tissue damage. Fortunately the brain possesses immune cells (microglia and astrocytes) that constantly survey the microenvironment for hints of damage, though they have been identified as important neurotoxic effectors in different disease models. Thus, the 1st goal of my work it to investigate how the progression of AD effects these immune cells responsiveness to microvessel damage, and whether there are behavioral deficits or consequence of impaired response on vessel integrity (leakiness) and repair (survival). My 2nd goal will be to test whether different pharmacological blockers of astrocytes and microglia can improve functional outcome. I hypothesize that microglia and astrocyte based repair of damaged microvessels in the brain will be impaired in AD, and this impairment will exacerbate dysfunction/loss of microvessels that could lead to impaired neuronal circuits. Over time, we suspect the accumulation of micro-vascular insults and poor repair responses in AD exacerbates behavior. 3 stages of AD will be investigated to access progression of pathology and pharmacological intervention; 1: (pre) 4-6 months – soluble Aβ; elevated, no plaques; 2: (early) 6-8 months – Aβ; plaques present, little CAA; 3: (late) 10-12 months – plaques and CAA. For aim 1, adult transgenic mice (APP/PS1tg/wt:Cx3cr1GFP/wt; AD mouse with eGFP microglia) or wild-type littermates (APP/PS1wt/wt:Cx3cr1GFP/wt; eGFP microglia) will undergo surgery to implant a chronic cranial window over motor cortex for in vivo multi-photon imaging. This will allow imaging of the same cortical areas prior to and after induction of CMBs using a high power femtosecond laser, immediately (0-1hr) and repeatedly (days-weeks) to assess microglia dynamics and accumulation as well as vessel permeability and repair. We expect to see a decrease in microglia around sites of lesions, which will lead to dysfunction of the vessels and increased permeability of fluorescently labeled dyes injected IV. Behavioral tests will be run concurrently to access cognitive (Barnes Maze) and motor deficits (skilled reaching). For aim 2, similar procedures will be used, except mice will also receive an astrocyte-targeted adeno-associated virus expressing Ca2+ indicators, allowing for simultaneous imaging of astrocyte activity in response to Aβ; and CMBs. Inhibitors will be chosen based on whether microglial activation/dysfunction precedes or follows astrocytic hyperactivity. Hence, this study will be the first to elucidate the role of astrocyte-microglia dysfunction in vascular pathology during AD progression.

Two-photon glutamate imaging suggests mechanism for amyloid-β-dependent neuronal dysfunction

Felix Gabriel Unger[1], Benedikt Zott[1], Hsing-Jung Chen[1], Arthur Konnerth[1]

[1]Technische Universität München, Institut für Neurowissenschaften, Biedersteiner Straße 29, Bau 601, 80802 München, Germany

Over the last years, accumulating evidence from mouse models of Alzheimer's disease (AD) has established neuronal hyperactivity to be a hallmark of early stages of AD. Our group recently demonstrated that this hyperactivity can be triggered by soluble amyloid-beta (Aβ) (Busche et al., 2012). However, the exact mechanisms how soluble Aβ can trigger this hyperactivity still remain unknown. In line with reports from previous studies (Hefendehl et al., 2016; Lei et al., 2016), we found ample evidence that one possible mechanisms of Aβ-dependent neuronal dysfunction is impaired glutamate uptake. In our study, we used two-photon imaging of the genetically encoded glutamate sensor SF-iGluSnFr (Marvin et al., 2013) and intracellular electrophysiology in acute murine hippocampal slices to analyze glutamate dynamics and NMDA-receptor mediated EPSCs after synaptic stimulation.

The imaging experiments indicate that soluble Aβ increases extracellular levels of glutamate after synaptic stimulation. Thus, Aβ prolonged the decay time as well as the maximum amplitude of the synaptically evoked glutamate transients. These findings are independently supported by patch-clamp recording experiments of NMDA- receptor mediated EPSCs in CA1 pyramidal neurons. In these experiments, we demonstrated that Aβ application increased the decay time of the EPSC as well as the full width at half maximum. This effect was fully reversible after wash-out of Aβ. Remarkably, in the iGluSnFr experiments as well as the patch clamp recordings, the application of the glutamate-reuptake inhibitor TBOA led to the same effects as the application of Aβ. Just like Aβ, TBOA prolonged the decay time of the fluorescent glutamate signal as well as the NMDA-receptor mediated EPSC. Additionally, the application of TBOA can occlude the action of Aβ, suggesting that both substances act through the same mechanism.

In conclusion, two different approaches independently demonstrated increased extracellular glutamate after synaptic stimulation in the presence of Aβ. Taken together, our results provide substantial evidence that soluble Aβ inhibits glutamate reuptake and offer a mechanistic explanation for the Aβ-dependent induction of neuronal hyperactivity in the brains of AD mouse models and patients. This might have beneficial implications for the development of disease-modifying drugs for AD.

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Evaluation of potential pharmacological chaperones in a neuronal cell model derived from Niemann-Pick type C1 patient-specific induced pluripotent stem cells

Christin Völkner[1], Franziska Peter[1], Maik Liedtke[1], Michael Rabenstein[1], Moritz J. Frech[1]

[1]University Medicine of Rostock, Albrecht-Kossel-Institute for Neuroregeneration, Gehlsheimer Straße 20, 18147 Rostock, Germany

Niemann–Pick Type C1 disease is an autosomal recessive neurodegenerative disorder caused by mutations in the NPC1 gene, which encodes for a lysosomal membrane-bound protein involved in the cholesterol transport. A mutation in the NPC1 gene disrupts efflux of cholesterol from late endosomes and lysosomes, leading to a clinically heterogeneous phenotype that includes neurological and systemic manifestations.

The disease is most commonly caused by missense mutations leading to amino acid substitutions. For the prevalent mutation I1061T it has been shown that the protein is identified as misfolded by the endoplasmic reticulum (ER) control machinery leading to its subsequent degradation by the proteasome. It was demonstrated, that overexpressed mutant protein can transit to late endosomes and lysosomes, restoring physiological level of cholesterol in these compartments. Thus, strategies increasing the level of mutant protein in relevant compartments, display promising approaches in the treatment of lysosomal storage disorders.

An emerging strategy for the treatment of NPC1 is the use of pharmacological chaperones (PCs). PCs induce or stabilize the proper conformation of the misfolded but catalytically active enzyme, preventing its degradation by ER-associated degradation (ERAD). This strategy holds great potential, since small molecules are able to cross the blood brain barrier.

We used our recently developed neuronal differentiated cells (NDCs) derived from NPC1 patient-specific induced pluripotent stem cells (iPSCs) to evaluate the effect of potential pharmacological chaperones. Amongst others, we used 25-hydroxycholesterol (25-HC) that has been described as a pharmacological chaperone for NPC1 in patient-specific fibroblasts. The ability of the PCs to increase the levels of mature NPC1 protein was assessed by Endoglycosidase H (Endo H) assay. Endo H removes immature N-linked glycans from proteins. In control NDCs we observed an Endo H resistant, slow migrating species, indicating that the protein was properly folded and efficiently transported out of ER. In contrast, $NPC1^{I1061T}$ was present as an Endo H-sensitive, more rapidly migrating species, indicating that the mutant protein was retained in the ER prior to its degradation. After treatment with 25-HC, $NPC1^{I1061T}$ showed a detectable increase in the Endo H resistant band, suggesting that a small portion of the mutant protein folded correctly and fluxed through the Golgi and probably to the lysosomes.

To gain support for this interpretation, we visualized NPC1 protein by immunofluorescence and assessed its colocalization by confocal microscopy with CellLight® Lysosomes-RFP, BacMam 2.0, a fusion construct of Lamp1 (lysosomal associated membrane protein 1) and emRFP, enabling specific targeting to cellular lysosomes. In control NDCs, we observed WT NPC1 protein as a strong punctate pattern that colocalized with lysosomes, whereas mutant NDCs showed a weak and diffuse staining pattern and a lower colocalization with lysosomes. Treatment with 25-HC increased the staining intensity of the mutant protein and resulted in an increase of colocalization with lysosomes.

These findings suggest that pharmacological chaperones, that stabilize the proper conformation of the mutant NPC1 protein in the ER, can be therapeutically beneficial for some Niemann–Pick Type C1 patients.

Cytoskeletal alterations contributing to synapto-axonal dysfunction in Parkinson´s disease.

Carmina Carelia Warth Perez Arias[1], Lucas Caldi Gomes[1], Kim-Ann Saal[2], Christof Lenz[3], Henning Urlaub[3], Silvio Rizzoli[2], Paul Lingor[1]

[1]University Medical Center Göttingen, Dept. of Neurology, Von Siebold Straße, 37073 Göttingen, Germany
[2]University Medical Center Göttingen, Dept. of Neuro- and Sensory Physiology, Humboldtallee, 37073 Göttingen, Germany
[3]Max Planck Institute for Biophysical Chemistry, Bioanalytical Mass Spectrometry, Am Fassberg, 37077 Göttingen, Germany

Parkinson´s diseases (PD) is characterized as a movement disorder with a prominent and selective degeneration of dopaminergic nigrostriatal neurons. Motor symptoms such as bradykinesia and rigor arise in later stages of the diseases, once approximately 30% of the dopaminergic neurons have been lost. Stronger affected by this point are however the hydroxylase positive striatal dopaminergic terminals, with a degeneration above 50%. A synapto-axonal degeneration preceding the loss of dopaminergic somata in a dying back manner is hence, as in many neurodegenerative diseases, an early pathological step in PD. Accordingly, a quantitative analysis of the protein composition of the synapto-axonal compartment and it´s changes in PD, would lead to a better understanding of the disease and it´s progression. The aim in this project is to understand the alterations that the synapto-axonal compartment undergoes in PD to contribute to a quantitative description of pathological changes in the synapse and to identify novel therapeutically relevant molecular targets.

In a pilot study, we quantified the differential proteome of human post mortem midbrain tissue of a PD patient and compared it to an age-matched control subject (AMC). Using the Selected Window Acquisition of All Theoretical Precursors (SWATH)-MS technique, 2532 proteins were quantified, out of which approximately 10% were found to be differentially expressed. Amongst these, we identified several synapto-axonal proteins: ROCK2 integrates inhibitory cues and modulates the actin cytoskeleton and it's inhibition increases neuronal survival and axonal regeneration of dopaminergic neurons. Synaptogyrin-3 is involved in exocytosis as well as an indirect regulation of the SLC6A3 dopamine transporter. Syntaphilin, which inhibits the SNARE complex formation by absorbance of free Syntaxin 1 and Neuromodulin, a major component of growth cones and involved in axonal and dendritic filopodia induction, were also differentially regulated. Other differentially expressed proteins include Synaptohysin, possibly involved in structural functions such as organizing membrane components, as well as targeting the vesicles on the plasma membrane, Rabphilin-3A, responsible for protein transport and neurotransmitter release by regulating membrane flow in the nerve terminal and Synapsin-2, a neural phosphoprotein coating synaptic vesicles that regulates neurotransmitter release.

Based on the outcome of the pilot study, which corroborated the feasibility of a proteomic approach on human post-mortem tissue, we now expanded our differential proteomic analysis including post mortem hippocampal sections of 17 PD patients, 15 AMC and 19 younger Multiple Sclerosis (MS) patients. To correlate our cross-sectional post-mortem data with information on the longitudinal development of the pathophysiology in an animal model, we included the differential protein quantification of three time points of a PD mouse model expressing the mutated form of human alpha-Synuclein. Our data thus supports the idea that synapto-axonal alterations contribute to PD pathogenesis and identifies molecular correlates that could represent future therapeutic targets.

Effects of cannabidiol on diabetes outcomes and chronic cerebral hypoperfusion comorbidities in middle-aged rats

Rúbia Maria Weffort de Oliveira[1], Amanda Santiago[1], Marco Aurelio Mori[1], Humberto Milani[1], Francisco Silveira Guimarães[2]

[1]Universidade Estadual de Maringá, Departamento de Farmacologia e Terapêutica. Av. Colombo 5790, K-68, 104a, 87020-900, Maringá, Paraná, Brasil

[2]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Departamento de Farmacologia, Av. Bandeirantes, 3900, 14049-900, Ribeirão Preto, São Paulo, Brasil

Diabetes and aging are risk factors for cognitive impairments after chronic cerebral hypoperfusion (CCH). Cannabidiol (CBD) is a phytocannabinoid present in the Cannabis sativa plant. It has beneficial effects on both cerebral ischemic diseases and diabetes. We have recently reported that diabetes interacted synergistically with aging to increase neuroinflammation and memory deficits in rats subjected to CCH. The present study investigated whether CBD would alleviate cognitive decline and affect markers of inflammation and neuroplasticity in the hippocampus in middle-aged diabetic rats submitted to CCH. Diabetes was induced in middle-aged rats (14 months old) by intravenous streptozotocin (SZT) administration. Thirty days later, the diabetic animals were subjected to sham or CCH surgeries and treated with CBD (10 mg/kg, once a day) during 30 days. Diabetes exacerbated cognitive deficits induced by CCH in middle-aged rats. Repeated CBD treatment decreased body weight in both sham- and CCH-operated animals. Cannabidiol improved memory performance and reduced hippocampal levels of inflammation markers (inducible nitric oxide synthase, ionized calcium-binding adapter molecule 1, glial fibrillary acidic protein, and arginase 1). Cannabidiol attenuated the decrease in hippocampal levels of brain-derived neurotrophic factor induced by CCH in diabetic animals, but it did not affect the levels of neuroplasticity markers (growth-associated protein-43 and synaptophysin) in middle-aged diabetic rats. These results suggest that the neuroprotective effects of CBD in middle-aged diabetic rats subjected to CCH are related to a reduction in neuroinflammation. However, they seemed to occur independently of hippocampal neuroplasticity changes.

Endocytic defects impair lysosomal and mitochondrial function

Katarzyna Wieciorek[1,2], King Faisal Yambire[1], Lorena Fernández Mosquera[1], Ángela Sánchez Guerrero[1], Ira Milosevic[1,2], Nuno Raimundo[1]

[1]University Medical Center Göttingen, Germany
[2]European Neuroscience Institute (ENI), Göttingen, Germany

Several links between endocytosis and the maintenance of mitochondrial structure and function have been reported, yet the underlying mechanisms remain poorly understood. Endocytic proteins, such as membrane curvature sensor and inducer endophilin B, GTPases dynamin-2 and dynamin-related protein-1, are necessary for proper mitochondrial function. Moreover, inhibitors of clathrin-mediated endocytosis, endosidin9 (inhibitor of vesicle recycling at the plasma membrane) and tyrosine kinase inhibitor tyrphostin A23, uncouple mitochondrial oxidative phosphorylation. Our main goal was to explore effects of impaired endocytosis using the mammalian models without key endocytic proteins, endophilin-A and phosphatase synaptojanin-1, on mitochondrial function.

We analysed the Next Generation Sequencing (NGS) and Mass Spectrometry (MS) data to find out which signaling pathways and organelles are significantly affected by the lack of endophilin-A and synaptojanin-1. We evaluated mitochondrial function and structure based on the following criteria: morphology, membrane potential, oxygen consumption rate and reactive oxygen species production. Moreover, due to the interdependence of mitochondria and lysosomes, we also assessed lysosomal capacity by measuring proteolytic activity and lysosomal mass.

The NGS and MS data revealed that the PI3K/AKT/mTOR pathway was the most affected signalling network. We then determined experimentally that this pathway is inhibited in endophilin-TKO and synaptojanin-KO brains.

Overall, it was studied if endophilin-A and synaptojanin-1 are necessary for proper mitochondrial and lysosomal function, and how lack of them impacts signaling pathways.

Role of the subthalamic nucleus in impulse control

Zifeng Xia[1], Franziska Richter[2], Frank W Ohl[1], Kentaroh Takagaki[1]

[1]Leibniz Institute for Neurobiology, Brenneckestraße 6, 39118 Magdeburg, Germany
[2]Institute for Pharmacology, Pharmacy and Toxicology, University of Leipzig

The subthalamic nucleus (STN) is the most commonly targeted region for deep brain stimulation (DBS), which improves dyskinesia and motor fluctuations, as well as quality of life in patients with advanced Parkinson disease. Despite its benefits on core PD symptoms, however, DBS to the STN also causes complex behavioral changes, for instance, acute STN DBS might also induce impulsive behaviours, such as pathological gambling, hypersexuality, compulsive shopping and binge eating. Moreover, STN plays an important role in regulating decision thresholds, according to current models of decision-making and learning. The mechanisms behind these side effects are poorly understood.
In this project, we study the role of the STN in regulating decision thresholds and how STN stimulation contributes to changes in impulsivity with molecular roughened and carbon nanotube coated tetrodes. To answer this question, we will approach the mechanisms of impulsivity by studying a complex gerbil shuttle box stop task which is designed to model human impulsivity and impulse control, and to investigate these behaviors on a single-unit basis, in a large number of healthy mammalian subjects.

Lysosomal and mitochondrial crosstalk: a case for neurodegeneration in LSDs?

King Faisal Yambire[1,2], Ira Milosevic[2], Nuno Raimundo[1]

[1]Universitätsmedizin Göttingen, Institute of Cellular Biochemistry, Humboldtallee 23, 37073 Göttingen, Germany
[2]Synaptic Vesicle Dynamics, European Neuroscience Institute, Grisebachstr. 5, 37077 Göttingen, Germany

Metabolic defects result in various diseases, with neurodegeneration as one of the most prevalent consequences. Mitochondria and lysosomes are essential for cellular metabolism. In addition to being cellular 'powerplants', mitochondria play crucial roles in cellular signaling by contributing to cellular stress responses like autophagy, apoptosis or cell proliferation. Lysosomes have evolved beyond their role as cellular 'incinerators' to coordinate major processes such as autophagy and nutrient sensing. Contrary to previous views, recent evidence suggest the existence of functional interdependent networks between lysosomes and mitochondria. In this study, we elucidate mechanisms of crosstalk beyond autophagy (mitophagy) between lysosomes and mitochondria, and show that lysosomal defects affect mitochondrial function.

To identify the mechanism(s) regulating lysosomal and mitochondrial crosstalk, we employed two biochemically similar models of lysosomal lipid storage disorders (LSDs) with distinct etiologies: Acid sphingomyelinase- and Niemann-pick type C1- deficient mouse tissues, which are respective models for Niemann-Pick types A and C diseases. We also evaluated the effects of chronic lysosomal malfunction on mitochondrial fitness and function in cells from patients of these disorders.

We found in Niemann-Pick disease patient cells that impaired S1PR1 signaling engages transcriptional programs, via KLF2 and ETV1, to repress mitochondrial biogenesis and function. Moreover, *in silico* experiments from microarray datasets of brain and liver samples of a mouse model of Niemann-Pick disease confirmed the induction of KLF2 and ETV1, and the concomitant repression of mitochondrial biogenesis. Interestingly, mechanisms of KLF2 and ETV1 downregulation, including siRNA-mediated silencing or enhanced S1PR1 signaling, are enough to promote mitochondrial biogenesis. These findings highlight the involvement of a transcriptional network in the regulation of lysosomal and mitochondrial crosstalk and the therapeutic potential of modulating S1PR1 signaling in Niemann-Pick disease.

Vulnerability of highly active brain regions in Alzheimer's disease

Benedikt Zott[1], Arthur Konnerth[1]

[1]Institute of Neuroscience and Munich Cluster for Systems Neurology, Technical University of Munich, Biedersteiner Str. 29, 80802 München, Germany

Accumulating evidence, both from observations in mouse models and humans, indicates that an early dysfunction in Alzheimer's disease (AD) is an amyloid-β (Aβ)-dependent hyperactivity in a subset of neurons. However, not all brain regions seem to be affected to the same extent. Thus, we recently demonstrated that, in mouse models of AD, neuronal hyperactivity can be detected much earlier in hippocampal than in the neocortical neurons even though there is no evidence for higher Aβ levels in the hippocampus [1]. However, the reason for this difference in susceptibility remained unclear. Since functional MRI data from AD patients has suggested that in early AD, brain areas with particularly high levels of baseline activity and connectivity are extremely susceptible to Aβ-induced neuronal dysfunction [2], we asked whether baseline activity is indeed what drives neuronal dysfunction in AD. In our study, we used in vivo two-photon calcium imaging to determine the relationship between baseline activity and susceptibility to the application of soluble Aβ in different brain regions.

Several lines of evidence support the notion of a baseline activity-dependence of the Aβ action on neurons. First, we compared the action of Aβ on hippocampal CA1 neurons that show high levels of baseline activity and layer 2/3 cortical neurons with low levels of ongoing activity. Indeed, application of the same concentration of Aβ triggered hyperactivity in hippocampal neurons but was ineffective in layer 2/3 of the neocortex. Since recent advances in the methodology of two-photon imaging have enabled us to perform in vivo imaging in the deep cortical layers [3], we next tested whether soluble Aβ can trigger hyperactivity in cortical layer 5, which has higher levels of baseline activity than layer 2/3. Remarkably, Aβ application could robustly trigger a strong hyperactivity in these neurons. Finally, we asked, whether we could abolish Aβ-dependent hyperactivity in the hippocampus by blocking ongoing activity. Indeed, suppressing neuronal activity through blocking synaptic excitation by antagonists of glutamatergic transmission (CNQX, APV) or blocking neuronal firing with TTX both made the application of Aβ in hippocampal CA1 ineffective.

Taken together, our results reveal a tight correlation between the level of baseline neuronal activity and the susceptibility to Aβ-dependent dysfunction. We provide a cellular explanation for the clinical observation that, in AD patients, brain areas with particularly high levels of baseline activity and connectivity are more susceptible to functional impairments [2].

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Neuroinflammation in a mouse model of amyotrophic lateral sclerosis with FUS gene mutation and effects of standard and new therapies.

Diana Ivanovna Babaevskaia[1], Johannes de Munter[2,3], Alexander Trofimov[1,2], Joao Costa-Nunes[1], Dmitry Pavlov[1,2], Ekaterina Veniaminova[1,2], Margarita Oplatchikova[1], Anna Gorlova[1,2], Klaus-Peter Lesch[1,2,4], Erik Wolters[3], Daniel Anthony[5], Tatyana Strekalova[1,2,4]

[1]I.M. Sechenov First Moscow State Medical University (Sechenov University), Laboratory of Psychiatric Neurobiology, Institute of Molecular Medicine, Moscow, Russia

[2]Department of Neuroscience, School for Mental Health and Neuroscience, Maastricht University, Maastricht, Netherlands

[3]Neuroplast, Maastricht Netherlands

[4]Division of Molecular Psychiatry, Laboratory of Translational Neuroscience, Department of Psychiatry, Psychosomatics and Psychotherapy, University of Wuerzburg, Wuerzburg, Germany

[5]Department of Pharmacology, Oxford University, Oxford, UK

A novel transgenic mouse line, which is based on the mutation of Fused in sarcoma protein (FUS), DNA/RNA-binding factor, was recently proposed and validated as a paradigm of amyotrophic lateral sclerosis (ALS). Here, we investigated behavioral and physiologic parameters, alone with inflammatory markers in the CNS and blood of FUS-transgenic (FUS-tg) female mice and their wildtype littermates. Subgroups of mice corresponding to a pre-symptomatic age of FUS-tg mutants, where treated with chronic dosing with riluzole (8 mg/kg/day, p.o.), a standard therapy of ALS, or selective COX2 blocker celecoxib (30 mg/kg/day p.o.) during six weeks, or a single i.c.v. administration of human stem cells (Neuro-Cells, 500 000 CD34 in 10 mkl). Separate groups were acutely challenged with low dose 0.1mg/kg of lipopolysaccharide (LPS). We found multiple emotional and cognitive aberrations in FUS-tg mice at their pre-symptomatic stage, plasma interleukin 1-beta and interleukin-6 were elevated. There was increased behavioral response to the LPS challenge, as well as greater increases of pro-inflammatory cytokines, e.g. interleukin 1-beta, as compared with wild type controls. Pro-inflammatory changes were more pronounced in the brain than in the spinal cord. In naïve mutant mice studied at their symptomatic phases of the ALS pathology, profound increases of brain and spinal cord levels of pro-inflammatory markers including Iba-1, were accompanied by increases of apoptotic markers such as GSK3 alpha and beta. FUS-tg mice treated with celecoxib revealed a reduction of peripheral levels of cytokines, while pro-inflammatory changes in the spinal cord were unaltered. Neuro-Cells-treated FUS-tg group displayed normalized CNS and plasma levels of pro-inflammatory markers. Among treated FUS-tg groups, Neuro-Cells-treated animals, but not riluzole- and celecoxib-treated mice showed marketable improvement of motor, emotional, cognitive behavior and basic physiological functions. Thus, new therapies such as stem cells (Neuro-cells) diminish neuro-inflammation during the ALS syndrome and thus, can be beneficial.

a-Synuclein Aggregation Mechanisms and the Role of Lysosomal Cathepsins in Parkinson's Disease

Josina Bunk[1], Alice Drobny[1], Susy Prieto Huarcaya[1], Friederike Zunke[1]

[1]Institute of Biochemistry, University of Kiel

The formation of insoluble a-synuclein aggregates is considered as key event in the development of Parkinson's disease (PD) and related synucleinopathies. Recent studies indicate that a-synuclein exists as both natively unfolded monomers and folded multimers under physiological conditions, however the relationship of these species to pathological aggregates is not well understood. We were now able to show that lysosomal glycosphingolipids seem to play an important role in a-synuclein aggregation pathways and can cause a reversible structural change in a-synuclein, promoting its aggregation and toxicity [1]. We here show different therapeutic approaches targeting lysosomal lipid levels, which were able to restore physiological a-synuclein and diminished pathology in induced pluripotent stem cell (iPS)-derived midbrain neurons of PD patients [1-3]. Moreover we investigate the role of lysosomal cathepsins on a-synuclein aggregation pathways, since cathepsins D, B and L have been mechanistically and genetically linked to lysosomal a-synuclein degradation and PD pathology [4]. We here characterize various cathepsin D variants associated with neurodegeneration as well as PD and speculate on their effects on a-synuclein homeostasis and PD pathology. Vice-versa we are also interested in the effect of pathological a-synuclein on maturation and function of lysosomal cathepsins. Since a-synuclein aggregates have been shown to interfere with intracellular protein trafficking [5], we propose an impairment of lysosomal protease activity of cathepsins, followed by a decrease in a-synuclein degradation resulting in a negative feedback loop feeding the pathological a-synuclein aggregation mechanisms.
We propose that therapeutic strategies reducing lysosomal lipid levels and/or initial a-synuclein level, may be applicable to PD as we as other synucleinopathies.

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Poster Topic

T12: Neuroimmunology, Inflammation, and Neuroprotection

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Distinct carbohydrate content is responsible for differences in enzymatic and adhesive properties of eN/CD73 in rat cortical astrocyte cultures when exposed to factors commonly up-regulated in states of brain injury, inflammation or degeneration

Marija Adzic[1], Nadezda Nedeljkovic[2]

[1]The Centre for Laser Microscopy, Department for General Physiology and Biophysics, Institute for Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Studentski trg 3, 11000 Belgrade, Serbia Montenegro

[2]Department for General Physiology and Biophysics, Institute for Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Studentski trg 3, 11000 Belgrade, Serbia Montenegro

Ecto-5'-nucleotidase (eN/CD73), a GPI-anchored membrane protein, is the main source of extracellular adenosine and membrane receptor for extracellular matrix proteins. In different CNS pathologies, including ischemia, traumatic brain injury, neuroinflammatory and neurodegenerative disorders, glioma and neuroblastoma, astrocytes develop reactive phenotype, characterized by enhanced expression of eN/CD73, among many other structural and functional molecules. Adenosine produced by eN/CD73 acts at P1 receptors subtypes, mainly A1R, A2AR and A2BR, expressed on neurons, astrocytes, microglia, vascular endothelium, and infiltrated leukocytes to induce a number of modulatory actions during the course of neuroinflammation.

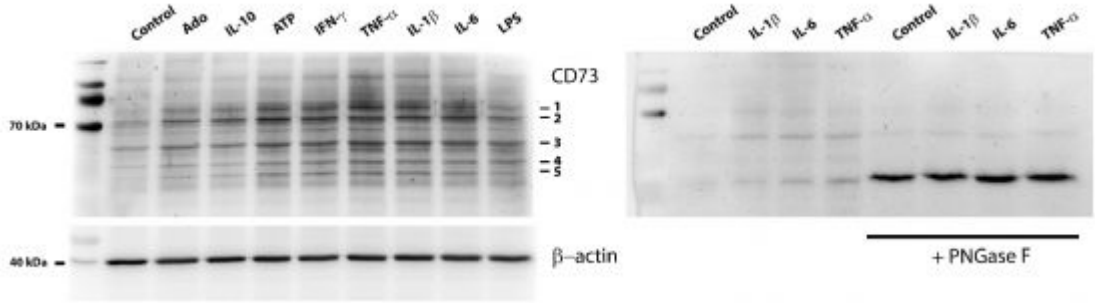
Rat eN/CD73 molecule is a glycoprotein with five N-glycosylation sites, that may be fully or partially modified with different glycan molecules, resulting in several eN/CD73 glycoisoforms. Since the enzyme has no structural isoforms, the cell-type and stimulus-specific pattern of eN/CD73 glycosylation account for the variations in observed enzymatic and biochemical properties. In our previous study, we have demonstrated that under resting conditions cultured cortical astrocytes express distinct eN/CD73 glycoforms, whereas the pattern of CD73 glycosylation may affect astrocytes behavior in neuroinflammatory conditions.

The aim of present study was to explore whether inflammatory mediators typically involved in neuroinflammation affect pattern of eN/CD73 glycosylation, the rate of adenosine production and cells adhesive properties of eN/CD73. Thus, rat cortical astrocytes treated with TNF-α, IFN-γ, LPS, IL-1β, IL-6, IL-10, ATP or adenosine for 24 h were assayed for 5'-AMP phosphohydrolase activity, while the cell adhesive properties of eN/CD73 were tested in the scratch wound migration assay. Additionally, expression of eN/CD73-mRNA and eN/CD73 glycosylation patterns were analyzed by qRT-PCR and Western blot, respectively.

Proinflammatory cytokines IL-1β, TNF-α and IL-6, up-regulate eN/CD73 enhancing its abundance by cultured astrocytes, while the immunomodulatory factor IL-10 does not induce such change. Western blot analysis demonstrates the appearance of the protein bands with slightly different molecular weights. Processing the same samples with PNGase F which removes N-linked oligosaccharides from glycoproteins, resulted in a single protein band, confirming that distinct eN/CD73 bands represent different glycoforms. In accordance with the results obtained with qRT-PCR and Western blot analysis, astrocytes treated with IL-1β, TNF-α and IL-6 exhibit markedly enhanced 5'-AMP phosphohydrolase activity. To shed more light on the physiological relevance of altered eN/CD73 glycosylation in neuroinflammatory conditions and signaling pathways that mediate these alterations, expression of adenosine receptor subtypes A1R, A2AR and A2BR have been determined, along with the activation of NFκB and STAT3 signaling pathways, responsible for a development of the „harmful“ A1 and „beneficial“ A2 astrocyte phenotypes, respectively.

In summary, our data demonstrate that regulation of eN/CD73 by reactive astrocytes may be installed at

posttranslational level, by different pattern of N-glycosylation which generates functionally different eN/CD73 glycoforms, thus, opening a question about a regulation of the complex enzymatic machinery that executes glycosylation in endoplasmic reticulum during the course of neuroinflammation *in vivo*.



Pituicyte Cues Regulate the Development of Permeable Neuro-Vascular Interfaces.

Savani Anbalagan[1], Ludmila Gordon[1], Janna Blechman[1], Ryota L. Matsuoka[2,3], Preethi Rajamannar[1], Einav Wircer[1,4], Jakob Biran[1,5], Adrianna Reuveny[1], Dena Leshkowitz[6], Didier Y.R. Stainier[2], Gil Levkowitz[1]

[1]Department of Molecular Cell Biology, Weizmann Institute of Science, PO Box 26, Rehovot 7610001, Israel

[2]Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, 61231 Bad Nauheim, Germany

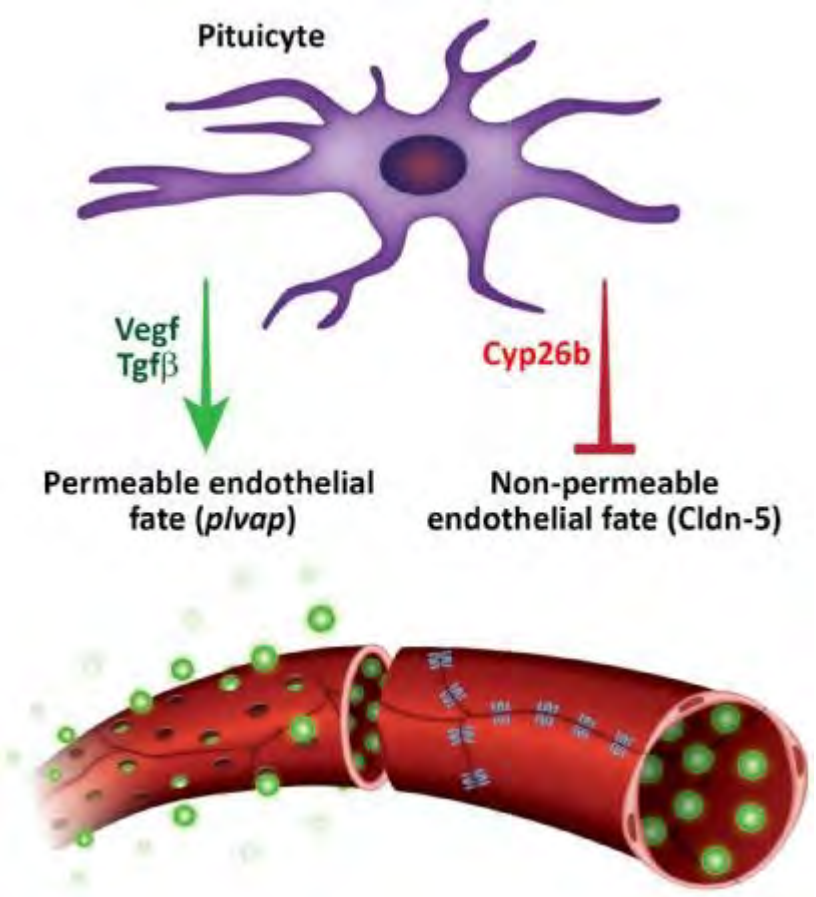
[3]Present address: Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA

[4]Present address: The Musculoskeletal Genetics Laboratory, Azrieli Faculty of Medicine, Bar Ilan University, Safed, Israel.

[5]Present address: Department of Poultry and Aquaculture, Agricultural Research Organization, Rishon Letziyon 7528809, Israel

[6]Bioinformatics Unit, LSCF Weizmann Institute of Science, PO Box 26, Rehovot 7610001, Israel

The hypothalamo-neurohypophyseal system (HNS) regulates homeostasis through passage of neurohormones and blood-borne proteins via permeable blood capillaries that lack blood-brain-barrier (BBB). The basic components of the HNS are the hypothalamic axonal projections, endothelial blood vessels and astroglial-like cells, termed pituicytes. Why do neurohypophyseal blood vessels become permeable while the neighboring blood vessels of the brain form a tight BBB remains unclear. We present the first vertebrate pituicytes transcriptome and show that pituicytes express genes that are associated with BBB breakdown during neuroinflammation. We tested the hypothesis that pituicyte-derived signals instruct the developmental cellular decision to form a permeable neuro-vascular conduit that bypass the BBB. Thus, we provide evidence that pituicyte-derived cues (Vegfa, Tgfβ3, Cyp26b) regulate normal development and maintenance of permeable neuro-vascular interfaces via dual mechanism. Finally, the mechanism by which a permeable endothelial fate is maintained in the developing neurohypophysis resembles previously reported pathophysiological conditions in the brain.


Pituicyte
Vegf
Tgfβ
Cyp26b
Permeable endothelial fate (*plvap*)
Non-permeable endothelial fate (Cldn-5)

In-vivo electrocorticography recordings in awake mice after stroke as a tool for assessing early disruption of cortical connectivity

Jonatan Mathis Biskamp[1,2], Tobias Ewert[1], Christian Gerloff[1,3], Tim Magnus[1,2]

[1]University Medical Center Hamburg-Eppendorf, Department of Neurology
[2]Experimental Research in Stroke and Inflammation Laboratory (ERSI)
[3]Experimental Electrophysiology and Neuroimaging (xENI)

Ischemic stroke disrupts cortical organization and leads to widespread changes in neuronal activity as well as in interregional connectivity between cerebral networks[1]. In the past years first achievements have been accomplished to elucidate pathophysiological cascades following stroke that could be therapeutically modified to improve functional outcome in patients[2,3,4]. However, a more detailed analysis of the underlying pathophysiology, e.g. the role of neuroinflammatory processes and their impact on electrophysiological and behavioral parameters, yet needs to be evaluated in order to identify further possible therapeutic options in stroke treatment.

In this study we monitored spontaneous neural oscillatory activity in awake non-anesthetized mice following stroke induced by transient middle cerebral artery occlusion (tMCAO) over the acute (0-3 days), subacute (3-7 days) and early chronic (7-14 days) stages. Using an array of 8 electrocorticography (ECoG)-electrodes per hemisphere covering olfactory, somatosensory, retrosplenial and visual cortices we show spatiotemporal changes in oscillatory power and coherence across brain regions namely in the theta frequency band (6-9 Hz) over the ipsilesional and contralesional hemisphere. These functional changes are region specific and correlate with stroke volume as well as behavioral deficits. Theta oscillations in rodents are a prime example of a slow activity pattern that establish neuronal synchrony over long distances[5,6,7]. Therefore our approach might serve in future studies as an indicator to observe the effects of (neuroinflammatory) variables on the recovery of physiological activity patterns and functional connectivity in cortical areas after stroke.

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Intranasal administration of mesenchymal stem cell-derived exosomes loaded with phosphatase and tensin homolog small interfering RNA enables functional recovery in rats after complete spinal cord injury

Shaowei Guo[1], Nisim Perets[2], Oshra Betzer[3], Shahar Ben-Shaul[1], Anton Sheinin[4], Izhak Michaelevski[5], Rachela Popovtzer[3], Daniel Offen[2], Shulamit Levenberg[1]

[1]Biomedical Engineering Department, Technion Israel Institute of Technology, Haifa, Israel
[2]Sagol School of Neuroscience, Tel Aviv University, Israel
[3]Faculty of Engineering and the Institute of Nanotechnology & Advanced Materials, Bar-Ilan University, Israel
[4]Department of Human Molecular Genetics and Biochemistry, Felsenstein Medical Research Center, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
[5]Department of Molecular Biology, Ariel University, Israel

Complete spinal cord injury (SCI) is a debilitating disease which usually leads to permanent functional impairments, with various complications and limited spontaneous recovery or efficient treatment. Here, we report that in rats with complete SCI, intranasal administrations of mesenchymal stem cells-derived exosomes (MSC-Exo) could penetrate the blood brain barrier, home selectively to the spinal cord lesion, and show affinity to neurons within the lesion. When these exosomes were loaded with phosphatase and tensin homolog small interfering RNA, termed ExoPTEN, they migrated from the nose and silenced PTEN expression in the lesion. Furthermore, the loaded exosomes promoted robust axonal regeneration and angiogenesis, accompanied with decreased astrogliosis and microgliosis. Moreover, the intranasal ExoPTEN treatment partially restored electrophysiological and structural integrity, and, most importantly, enabled remarkable functional recovery. This rapid, non-invasive approach, using cell-free nano-swimmers carrying molecules to target pathophysiological mechanisms suggests novel strategy for clinical translation to SCI and beyond.

Epo-Induced Neuroprotection: Crucial Role for Orthologues of the Orphan Cytokine Receptor CRLF3

Nina Hahn[1]

[1] Department of Cellular Neurobiology, Georg-August-University Goettingen, Goettingen, Germany

The cytokine Erythropoietin (Epo) is mainly known for its function in erythropoiesis. However, Epo plays additional roles in cell protection in various tissues including the nervous system. While the signaling pathway of its erythropoietic function is understood in great detail, the receptor mediating neuroprotection remains enigmatic. Erythropoiesis is stimulated by circulating Epo binding to the homodimeric classical Epo receptor (EpoR) on erythroid progenitors. The nature of the “alternative” Epo receptors involved in neuroprotection is currently under discussion. Knowledge about the signaling pathway of Epo’s neuroprotective effect is essential in order to judge its potential therapeutical benefits against neurodegenerative diseases. In this study, we provide evidence the orphan cytokine receptor-like factor 3 (CRLF3) is involved in Epo-mediated neuroprotection.

Although insects seem to lack orthologues of Epo and EpoR, we demonstrated a neuroprotective effect of recombinant human Epo on primary brain cells of the beetle *Tribolium castaneum* and the locust *Locusta migratoria* under challenging conditions (e. g. hypoxia or serum-deprivation). In order to knock down the orthologues of the type I cytokine receptor CRLF3 in these cells, we established soaking RNAi as a convenient method for loss-of-function studies in insect primary brain cell cultures. Knock down of CRLF3 abolished the neuroprotective effect of Epo and its non-erythropoietic variants demonstrating its necessity in Epo-induced neuroprotection *in vitro*.

In addition, we currently broaden our research to mammalian cell lines from various tissues investigating the importance of CRLF3 in cellprotection and its expression in response to harmful stimuli. Our studies aim to support the development of Epo derivatives that specifically activate neuroprotective mechanisms.

Manipulating microglia to enhance anti-viral activity in the CNS – implications for multiple sclerosis and viral encephalitis

Lorna Hayden[1], Tiia Semenoff[1], Julia Edgar[1], Marieke Pingen[1], Xiaohong Shi[2], Christopher Linington[1]

[1]Institute of Infection, Immunity and Inflammation, Neuroimmunology Group, University Place, G12 8TA Glasgow, United Kingdom

[2]MRC-University of Glasgow Centre for Virus Research, Glasgow G61 1QH, United Kingdom

The presence of oligoclonal IgG bands in cerebrospinal fluid is a hallmark of multiple sclerosis (MS). Recently it was reported that intrathecal synthesis of lipid-specific IgM is associated with a reduced risk of Natalizumab-treated MS patients developing progressive multifocal leukoencephalopathy (PML)1; an often fatal demyelinating disease caused by infection of oligodendrocytes by John Cunningham virus (JCV). This led us to speculate that components of this anti-lipid IgM response may enhance anti-viral activity in the CNS. Consequently, we investigated the anti-viral properties of a sulfatide-reactive2 mouse monoclonal IgM (O4) in primary CNS cultures.

Microarray analysis identified the anti-viral type-I interferon response as the most upregulated pathway in O4-treated cultures. Using RT-qPCR, we found *Ifnb1* was upregulated 4 to 8 hours post-treatment with expression of downstream interferon-stimulated genes (ISGs) (*Rsad2, Ifit2, Oasl, Mx1, Cxcl10, Ccl5*) increasing between 8 and 24 hours post-treatment. ISG expression was dependent on Interferon-β (IFN-β) activation of the interferon-α/β receptor (IFNAR) as demonstrated using *Ifnar*-/- mice and an IFN-β neutralising antibody.

Due to the inability of JCV to infect murine cells, Bunyamwera virus (BUNV) was selected as a model neurotropic virus to test the functional anti-viral properties of this anti-lipid IgM response. Using immunocytochemistry, plaque assay and RT-qPCR, pre-treatment with O4 was found to significantly reduce percent of virally infected cells, supernatant virus titre and number of viral transcripts in rat and mouse cultures. This protective effect was abolished in *Ifnar*-/- cultures.

Using fluorescent in situ hybridisation, microglia were identified as the main producers of *Ifnb1*, whilst ISG encoding transcripts were detected in neurons, astrocytes and oligodendrocytes, as well as microglia. This highlights the ability of O4-mediated Ifnb1 induction to act in a paracrine manner to support a global anti-viral response.

In conclusion, we have identified a novel mechanism whereby the binding of lipid-specific IgM to the oligodendrocyte membrane induces a type-I interferon dependent anti-viral response in primary CNS cultures. Microglia are the master regulators of this response, upregulating ISG expression in all major CNS cell types and providing robust protection against BUNV. This provides a logical explanation for the decreased incidence of PML reported in MS patients with intrathecal synthesis of anti-lipid IgM. Our results have profound implications for risk stratification in MS and other diseases associated with PML and may also have therapeutic potential in the treatment of viral encephalitis.

1 VILLAR, et al. 2015. Ann Neurol: 77(3), 447-57.

2 BRENNAN, et al. 2012. J Neuroimmunol: 238(1-2), 87-95.

NMDAR dependent and independent plasticity in a model of anti-NMDAR encephalitis

Timo Kirschstein[1], Roman Blome[1], Willi Bach[1], Xiati Guli[1], Christian Bien[2], Rüdiger Köhling[1]

[1]University of Rostock, Institute of Physiology, Gertrudenstr. 9, 18057 Rostock, Germany
[2]Epilepsy Center Bethel, Krankenhaus Mara, 33617 Bielefeld, Germany

Cerebrospinal fluid (CSF) from patients with anti-NMDAR encephalitis contains autoantibodies against NMDAR which have been assumed to be pathogenic since in previous studies using patient CSF, NMDAR-dependent processes such as long-term potentiation (LTP) were compromised. Here, we tested the specificity of this autoantibody by comparing NMDAR-dependent and NMDAR-independent LTP within the same hippocampal subfield, CA3. To this end, we used CSF samples from four anti-NMDAR encephalitis patients and three control patients. Patient-derived cell-free CSF with proven presence or absence of NMDAR-antibodies was stereotactically injected into the rat hippocampus in vivo. In hippocampal brain slices prepared 1-8 days after intrahippocampal injection, NMDAR-dependent LTP at the associational-commissural (A/C) fiber-CA3 synapse and NMDAR-independent LTP at the mossy fiber-CA3 synapse were analyzed. LTP in CA3 induced by stimulation of A/C fibers was significantly higher in control slices than in slices from NMDAR-CSF-treated animals, although some variation between the individual CSF samples was observed. Residual LTP in NMDAR-CSF-treated tissue could be abolished by the NMDAR inhibitor D-AP5 indicating NMDAR dependence. Moreover, the CA3 excitatory postsynaptic field potential (fEPSP) was followed by epileptiform afterpotentials in 5% of slices from control-CSF-treated animals, but in 26% of slices from NMDAR-CSF-treated animals ($P<0.01$). In contrast to the A/C-fiber-CA3 synapse, stimulating the mossy fiber (MF) input to CA3 induced significant LTP magnitudes in both control-CSF and in NMDAR-CSF-treated tissue suggesting that NMDAR-independent MF-LTP is intact in NMDAR-CSF-treated tissue. These findings indicate that anti-NMDAR containing CSF impairs LTP at the A/C fiber-CA3 synapse, although there is substantial variation among CSF samples suggesting different epitopes among patient-derived antibodies. The differential inhibition of LTP at this synapse in contrast to the MF-CA3 synapse points to the antibody specificity and underlines the pathophysiological role of the NMDAR-antibody.

Ancient functions of “erythropoietin-like” neuroprotective signaling in insects: receptors, transduction pathways and anti-apoptotic effects

Debbra Yasemin Knorr[1], Nina Hahn[1], Bita Massih[1], Franziska Schmitt[1], Nicola Schwedhelm-Domeyer[1], Stephanie Pauls[1], Ralf Heinrich[1]

[1]University of Göttingen, Cellular Neurobiology, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

The cytokine erythropoietin (Epo) mediates protective and regenerative functions in mammalian nervous systems via activation of poorly characterized receptors, that differ from the “classical” homodimeric Epo receptor (EpoR) expressed on erythroid progenitor cells. Epo genes have been identified in vertebrate species ranging from human to fish, suggesting that Epo signaling evolved earlier than the vertebrate lineage.
However invertebrate genomes lack orthologs of *Epo* and *EpoR*. Nevertheless, recombinant human Epo and the non-erythropoietic Epo splice variant EV-3 protect insect neurons from apoptotic cell death and support regeneration of dendritic and axonal processes. These beneficial functions are mediated through cytokine receptor-like factor 3 (CRLF3, an orphan cytokine receptor in humans), activation of JAK/STAT transduction pathways and expression of factors that prevent the activation of pro-apoptotic caspases. Orthologs of CRLF3 have been detected in various tissues (including nervous system, hemocytes, skeletal muscles) of locusts, crickets and beetles but not in Drosophila, which also lacks Epo-mediated neuroprotection. We explore the structural and functional characteristics of the Epo-binding receptors, partly shared transduction pathways that prevent apoptosis and the functional implication in neuroprotective and neuroregenerative processes of insects to further characterize common principles of cell protection mediated by “epo-like cytokines” that were already established in the last common ancestor of insects and vertebrates.

Microglia-related increase in NTPDase1 expression during EAE

Danijela Laketa[1], Marija Jakovljevic[2], Iva Bozic[2], Ivana Bjelobaba[2], Danijela Savic[2], Sanja Pekovic[2], Nadezda Nedeljkovic[1], Irena Lavrnja[2]

[1]Faculty of Biology, University of Belgrade, Institute of Physiology and Biochemistry, Studentski trg 3, 11001 Belgrade, Serbia
[2]University of Belgrade, Institute for Biological Research “Sinisa Stankovic”, Department of Neurochemistry, Blvd Despota Stefana 143, 11001 Belgrade, Serbia

Ectonucleoside triphosphate diphosphohydrolase 1 (NTPDase1/CD39) is the main ATP- and ADP-degrading enzyme in extracellular fluid of the central nervous system. In the hydrolysis cascade NTPDase1 removes ATP and ADP and produces AMP, which is hydrolysed by ecto-5'-nucleotidase to adenosine. During neuroinflammation, increased extracellular ATP levels exert proinflammatory effects at microglia as resident immune cells, while adenosine effects are antiinflammatory. Literature data indicate involvement of purinergic signaling in experimental autoimmune encephalomyelitis (EAE), while decreased number of NTPDase1/CD39+ regulatory T-cells was evidenced in multiple sclerosis. Downregulation of NTPDase1 expression was observed in proinflammatory activation phenotype of macrophages. However, data on the role of NTPDase1 on glial cells in neuroinflammation are still scarce. We have shown increase in ATP-, ADP- and AMP-hydrolysis, together with upregulated mRNA and protein expression of NTPDase1 in lumbar spinal cord, correlated to the disease course during EAE. In this study we aimed to explore contribution of particular cell subsets to the observed changes in NTPDase1 expression.

Acute monophasic EAE was induced in female rats of Dark Agouti strain by active immunization with a mixture of spinal cord homogenate in complete Freund’s adjuvant. Immunized animals were sacrificed at the onset, peak and end of symptoms, while naïve animals were used as control. Significant increase of NTPDase1 immunofluorescence in lumbar spinal cord cross-sections was related to prominent infiltrates at the peak of EAE and increased expression of NTPDase1 among isolated mononuclear cells. Analysis of triple-labeled Arginase1/NTPDase1/Iba1 and iNOS/NTPDase1/Iba1 immunofluorescent micrographs showed prevalent contribution of Arginase1+ microglia in comparison to iNOS+ microglia in NTPDase1 immunofluorescence, at the peak of EAE. Further studies are needed to reveal possible association of NTPDase1 with antiinflammatory phenotype in microglia.

Identification and characterization of novel autoantigens of autoimmune neuropathies

Christian P. Moritz[1,5], Oda Stoevesandt[2], Yannick Tholance[1,3], Evelyne Federspiel[1,3], Karine Ferraud[1,3], Martin Jung[4], Carole Rosier[3], Mike Taussig[2], Jean-Philippe Camdessanché[1,3,5], Jean-Christophe Antoine[1,3,5]

[1]Institut NeuroMyoGène INSERM U1217/CNRS UMR 5310, Synaptopathies and Autoantibodies, 10, rue de Marandière, 42270 Saint-Etienne / Lyon, France

[2]Cambridge Protein Arrays Ltd., Babraham Research Campus, Cambridge, UK

[3]Centre Hospitalier Universitaire de Saint-Étienne, 42055 Saint-Étienne Cedex 2, France

[4] Medical Biochemistry and Molecular Biology, Universitätsklinikum des Saarlandes, Homburg, Germany

[5]Faculty of Medicine, Synaptopathies and Autoantibodies, 10, rue de Marandière, 42270 Saint-Etienne / Lyon, France

Several neuropathies are caused by autoimmune misfunctioning, such as autoantibodies that are directed against own nerve tissue. Patients with these diseases suffer from motor issues (loss/disturbance of voluntary movement) and/or sensory issues (paresthesia up to chronic pain). Identifying the autoantigens of this disease will improve its diagnosis, treatment, and understanding. Our lab has improved and applied an immunoblotting-based technique to screen the blood sera from ca. 100 patients with neuropathies based on their immunological fingerprints. A selected set of sera from 34 patients-of-interest (biggest groups: chronic inflammatory demyelinating polyneuropathy, CIDP; sensory neuronopathies, SNN) and 9 healthy controls (HC) in order to identify their autoantigens via HuProt protein arrays containing >16,000 different bate proteins. We identified promising antigens strongly reacting with up to 36% of CIDP sera and up to 41% of the SNN sera. We have performed ELISA to verify and quantify the data with a bigger patient cohort. Further, we have developed a novel idea to study the total repertoire of antigens in a more holistic way. Already the size of the antigen repertoire appears to depend on the clinical situation: e.g., patients with pain target 2x more autoantigens than pain-free patients. A part of the autoantibody set specifically targets axon-, glia-, immune system-, and neuronal disease-related antigens. Our project will help to understand the role of autoantibodies in neuropathy patients by providing new biomarkers and by addressing the set of autoantigens in a holistic way.

Anti-FGFR3 antibody: a biomarker of sensory neuronopathies or an active player of neuron degeneration?

Yara Nasser[1,2,3], Christian Moritz[1,3], Evelyne Reynaud-Federspiel[1,3], Jean Philippe Camdessanche[1,2,3], Jean Christophe Antoine[1,2,3], Nadia Boutahar[1,2,3]

[1]INSTITUT NEUROMYOGÈNE, INSERM U1217/ CNRS UMR 5310 - Université de Lyon - Université Claude Bernard Lyon 1.
[2]UNIVERSITY HOSPITAL OF SAINT ETIENNE, FRANCE.
[3]JEAN MONNET UNIVERSITY, SAINT ETIENNE, FRANCE.

Sensory neuronopathies (SNN) are multifactorial diseases characterized by neuron cell death in the dorsal root ganglia. In 2015, Antoine et al. have identified an autoantibody, directed toward the fibroblast growth factor receptor 3 (anti-FGFR3), as a serum biomarker of a subgroup of SNN. Some of those patients also presented a dysimmune background. Preliminary results in our lab on neuron cultures have shown that a rabbit anti-FGFR3 antibody recognizing the intracellular domain of FGFR3 induced neuron cell death while the control rabbit IgGs did not. Therefore, we decided to study the role of this rabbit anti-FGFR3 antibody as well as the molecular mechanism involved in neuronal cytotoxicity. The two main signaling pathways associated with FGFRs are MAPK-ERK1/2 and MAPK-p38. Our analysis revealed an increased expression of FGFR3 receptor genes and glutamatergic AMPA and NMDA receptor subunits in anti-FGFR3 antibody-treated neuronal cultures, suggesting a potential excitotoxic neuronal death. This overexpression is prevented when adding U0126 or SB230580, inhibitors of the two MAPK pathways. The inhibition of FGFR3 activation with Dovinitib, a tyrosine kinase inhibitor and a drug used in cancer treatment, showed similar results to those previously obtained with anti-FGFR3 antibody. This corroborates the hypothesis that neuron degeneration may be due to the inhibition of the FGFR3 tyrosine kinase domains by anti-FGFR3 antibodies. We also tested the potential activation of autophagy after the internalization of antibodies by neurons. The mRNA expression of Optineurin and p62 genes, two key markers of autophagy, were increased in the presence of anti-FGFR3 antibodies meaning that autophagy activation may also play a role in neuron degeneration.

COMPLEX REGULATION OF ECTO-5'-NUCLEOTIDASE/CD73 DURING NEUROINFLAMMATION: UNDERLYING MECHANISM LEADING TO ALTERED ADENOSINE GENERATION

Nadezda Nedeljkovic[1], Danijela Laketa[1], Irena Lavrnja[2], Marija Adzic[3]

[1]Faculty of Biology, University of Belgrade, Department for General Physiology and Biophysics, Studentski trg 3, 11001 Belgrade, Serbia

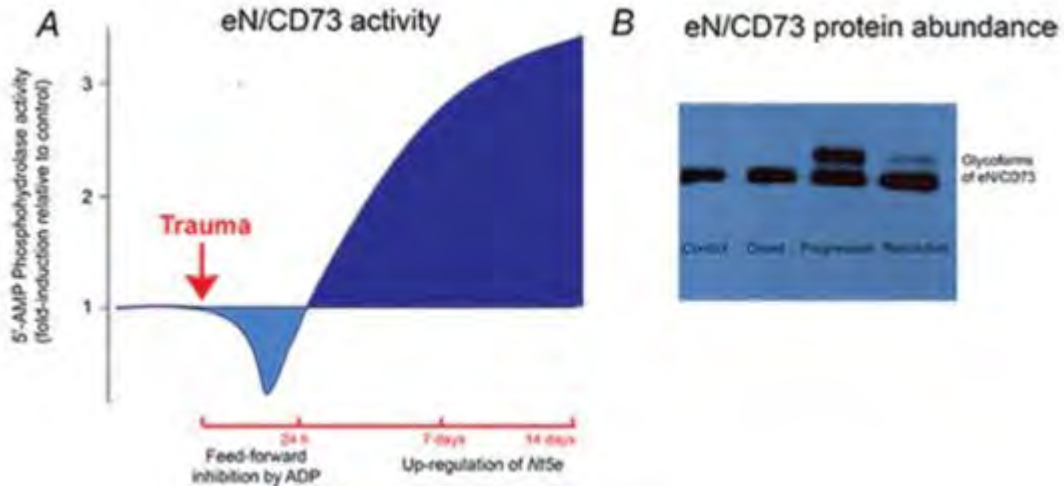
[2]Institute for Biological Research “Sinisa Stankovic”, University of Belgrade, Despota Stefana 143, Belgrade;

[3]Centre for laser microscopy, Faculty of Biology, University of Belgrade.

Neuroinflammation is the innate immune response of the CNS to brain trauma, with a physiological purpose of restoring brain homeostasis. Acute neuroinflammation emerges as a response to danger signals and occurs through three interdependent phases, termed initiation, progression, and resolution. Danger signals are molecules associated with a pathogen or cell damage and evidence are showing that extracellular ATP, released in high concentrations from damaged or dying cells, functions as a danger signal. ATP induces microglial and astrocyte activation by acting at P2X7R and P2Y1R subtypes. ATP is rapidly degraded by ectonucleotidase enzymes, which catalyze the sequential hydrolysis of ATP to adenosine. E-NTPDases hydrolyze ATP and ADP to AMP, while ecto-5’-nucleotidase (eN), also known as CD73, catalyzes the final and the rate-limiting step of adenosine formation. The unique property of this cascade is that elimination of ATP creates adenosine, with essentially opposite actions. Thus, in contrast to ATP, adenosine functions as an anti-inflammatory signal, acting upon adenosine receptors, located at astrocytes, microglia, endothelial cells, and immune cells, to suppress the neuroinflammatory reaction and to strengthen the integrity of the blood-brain barrier (BBB). Since eN/CD73 is the main source of extracellular adenosine, it is of a major pharmacological interest. Thus, the involvement of eN/CD73 in neuroinflammation and its complex regulation in cells comprising neurovascular unit (NVU) provide the focus of our work.

Ecto-5'-nucleotidase/CD73 is a glycoprotein, bound to the external side of a cell membrane by the GPI anchor. The principal function of eN/CD73 is hydrolysis of 5’-AMP and generation of adenosine. The catalytic activity of eN/CD73 can be competitively inhibited by high levels of ATP and ADP. The activity of eN/CD73 may be also diminished by enzymatic cleavage of GPI anchor and release of the soluble molecule, as well as by altered pattern of enzyme glycosylation.

Studies in a large number of experimental models of human neuropathologies demonstrate dynamic changes in eN/CD73 activity in cells comprising the NVU. Based on the temporal analysis and expression profiling of eN/CD73 in two distinct models of brain injury, cortical stab injury and experimental autoimmune encephalomyelitis, we were able to postulate the mechanism of eN/CD73 regulation during neuroinflammation. Given that adenosine acts as a strong immunosuppressive factor, it comes as no surprise that regenerative pathways share a common strategy of postponed up-regulation of eN/CD73. However, initiation of neuroinflammation and remodeling of the BBB require transient shutdown of eN/CD73 activity at NVU. Thus, in neuroinflammatory conditions eN/CD73 exhibits a pattern of a biphasic regulation, with an early decrease in the enzyme activity at the onset, followed by its strong induction through the resolution phase of neuroinflammation (Fig.1*A*). Early attenuation of eN/CD73 activity is achieved by direct enzyme inhibition by ATP and ADP, or by cleavage of the enzyme molecule from the cell surface, while late induction of the enzyme activity is accomplished by transcriptional up-regulation of *Nt5e* gene and by posttranslational variations in *N*-glycosylation (Fig.1*B*). Our data point out that the regulation of eN/CD73 is complex and include allosteric, biochemical, transcriptional and post-translational modifications, together with tissue-specific regulators.


A
eN/CD73 activity
5'-AMP Phosphohydrolase activity
(fold-induction relative to control)
3
2
1
Trauma
24 h
7 days
14 days
Feed-forward
inhibition by ADP
Up-regulation of Nt5e
B
eN/CD73 protein abundance
Glycoforms
of eN/CD73

Ceftriaxone pretreatment modulates brain energy metabolism after focal permanent ischemia

Yasmine Nonose[1], Andressa Wigner Brochier[1], Jussemara Souza da Silva[1], Rodrigo Vieira Apel[1], Roberto Farina Almeida[1], Fernanda Urruth Fontella[1], Diogo Onofre Gomes Souza[1,2], Adriano Martimbianco de Assis[1]

[1]Postgraduate Program in Biological Sciences: Biochemistry, ICBS, Universidade Federal do Rio Grande do Sul (UFRGS), Rua Ramiro Barcelos, 2600 - Anexo. Porto Alegre/RS 90035-000, Brazil.

[2]Department of Biochemistry, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre/RS 90035-003, Brazil.

Cerebral ischemia is a leading cause of death and disability worldwide. The absence of effective neuroprotective agents in the clinic has increased the interest in understanding endogenous neuroprotection such as those trigged by preconditioning strategies. Ceftriaxone (CTX), a beta-lactam antibiotic, is known to stimulate the expression of GLT-1 and prevent glutamate excitotoxicity. However, CTX preconditioning studies lack information on its effects on brain energy metabolism. Therefore, this study evaluated CTX pretreatment effects on energy substrates oxidation rate in a model of focal permanent ischemia (FPI). Adult
male Wistar rats were submitted to a pretreatment with CTX (200 mg/kg, i.p.) or vehicle (saline) for 5 days previously to FPI induction or sham-operated surgery. Neurochemical and behavioral assessments were performed 2 days after. We observed that CTX pretreatment diminished infarct volume, but motor performance on the cylinder test and neurological score were unnafected.
GLT-1 immunocontent was maintained in control levels in CTX pretreated group. Also, CTX enhanced glutamate uptake activity, but not its release from synaptic vesicles. FPI injury increased glutamate and lactate oxidation rate, which was prevented by CTX pretreatment. HPLC analysis of amino acid levels in cerebrospinal fluid demonstrated an increase in Asp, which can suggest a deceleration of the citric acid cycle. Our study shows for the first time that CTX pretreatment may have effects beyond glutamate transport modulation that impact brain energy metabolism and corroborate to the hibernation hypothesis of brain preconditioning. In addition, it further demonstrates the coupling between the glutamatergic system and brain energy metabolism shortly after cerebral ischemia.

Effect of Fingolimod on neuronal architecture and activity

Abhisarika Patnaik[1], Maria Fezzari[2], Eleonora Spiombi[2], Nicoletta Landsberger[2], Martin Korte[1], Marta Zagrebelsky[1]

[1]Zoological Institute, Division of Cellular Neurobiology, TU Braunschweig, Germany
[2]Rett Research Unit, Division of Neuroscience, Faculty of Medicine, University of Milan, Italy

Brain Derived Neurotrophic Factor (BDNF) promotes cell survival, regulates neuronal architecture and modulates synaptic plasticity in the developing and in the adult central nervous system (CNS). Due to its trophic and plasticity promoting functions, low BDNF-levels associated with various neurological disorders, could augment disease progression. From a therapeutic perspective, increasing BDNF levels to improve CNS function seems a promising approach. However, effective delivery of this poorly-diffusing molecule, across the Blood Brain Barrier presents major limitations. Hence, the prospect of increasing endogenous BDNF via drug-administration is especially interesting. One such drug candidate is *Fingolimod* (FTY720), the first FDA approved oral drug for MS therapy. The pro-drug has been shown to diffuse into the CNS where it is phosphorylated intracellularly into its active form; Fingolimod-Phosphate (Fingo-P) signaling via Sphingosine-1-phosphate receptors (S1PRs) is expressed in several CNS cell types.

In cortical cultures, low doses of Fingo-P increase BDNF mRNA and protein in an activity and MAPK-dependent manner. However, whether Fingolimod influences neuronal morphology and activity in a BDNF-dependent manner, in the intact hippocampus remains rather elusive. Differential effects of Fingolimod and Fingolimod-Phosphate have been reported in recent studies. Thus, we applied different concentrations of both forms of the drug for 24 hours to mature wild-type (WT) primary hippocampal cultures. Neurons treated for 24h display a mild, however significant increase in dendritic complexity and in spine density. Surprisingly, lower Fingo-P concentrations exert stronger effects. Also, the co-application of BDNF-scavenging, TrkB-Fc receptor bodies completely prevents these alterations suggesting a BDNF-mediated effect of Fingolimod in regulating the neuronal architecture of mature healthy hippocampal neurons.

Next, we investigated whether Fingolimod might prevent or rescue structural alterations in two neurological conditions linked to low BDNF levels: Rett Syndrome (RTT) & Alzheimer's (AD). Cortical cultures from MeCP2 knockout (KO) or CDKL5 KO, mouse models for RTT were treated with Fingo-P until DIV7 and analyzed for changes in neurite outgrowth. The treatment of MeCP2 (but not CDKL5) KO cultures rescues neurite length and complexity to similar levels as WT cultures. Next, to mimic early-AD phenotype at spines, mature WT hippocampal cultures are exposed for 6h to soluble $A\beta_{1-42}$. Neurons exhibit a concentration-dependent loss of dendritic spines associated to an increase in spine length and decrease in head width. Subsequently, a possible protective effect of 24h pre-treatment, against $A\beta_{1-42}$ induced spine alterations is currently being tested.
Overall our results demonstrate that Fingolimod modulates the architecture of healthy mature hippocampal neurons, mediated by BDNF. Moreover, we show that Fingolimod might be able to rescue the typical structural alterations of hippocampal neurons observed under certain pathological conditions.

Differential interaction patterns of antisera to the oral cavity bacteria *Porphyromonas gingivalis* and *Streptococcus mutans* on a human first trimester fetal brain multiprotein array

Bernhard Reuss[1]

[1]Universitary Medical Center Göttingen, Institute for Neuroanatomy, Kreuzbergring 36, 37075 Göttingen, Germany

Due to molecular mimicry, prenatal maternal bacterial infections can cause pathological changes in fetal brain development, resulting for the offspring in an increased schizophrenia risk later in life.* Accordingly, it has previously been shown that antibodies directed to *Neisseria gonorrhoeae* interact on a human fetal brain multiprotein array (HFBMPA) with known schizophrenia candidates. The present study now extends these investigations on antibodies to the oral cavity bacteria *Porphyromonas gingivalis* (anti-*P. gingivalis*) and *Streptococcus mutans* (anti-*S. mutans*). For anti-*P. gingivalis*, 27 different proteins could be identified on the HFBMPA including the schizophrenia candidates phospholipase C beta3 (PLCb3) and Set binding protein 1 (SETbP1). Interactions of anti-*P. gingivalis* with PLCb3 and SETbP1 could be confirmed by Western blot analysis using recombinant protein samples, and the high expression levels of SETbP1 in prefrontal cortex and the fetal brain suggest a role of these interactions for fetal brain development. For anti-*S. mutans*, 31 different proteins could be identified on the HFBMPA including schizophrenia candidates like paralemmin-1 (Palm1) and chromogranin-A (CgA). Interactions of anti-*S. mutans* with Palm1 and CgA could be confirmed by Western blot analysis using recombinant protein samples, and the high expression levels of Palm1 in prefrontal cortex, amygdala and fetal brain suggest a role of these interactions for fetal brain development. These findings confirm and extend previous results on the role of molecular mimicry for protein interactions of antibacterial antisera in the fetal brain, functional consequences of which could contribute to the characteristic neurodevelopmental deficits in the brains of schizophrenic patients. *see: Babulas et al., 2006 and Sørensen et al., 2009.

HERV-K is a ligand for TLR8 and mediates glioblastoma invasiveness

Christine Römer[1], Manvendra Singh[1], Alice Buonfiglioli[1], Omar Dzaye[1], Seija Lehnardt[2], Zsuzsanna Izsvák[1]

[1]Max-Delbrück Center for Molecular Medicine, Mobile DNA, Robert Rössle Str. 10., 13092 Berlin, Germany

[2]Institute of Cell Biology and Neurobiology, Center of Anatomy, Charité – Universitaetsmedizin Berlin, 10117 Berlin, Germany

Endogenous retroviral elements are remnants of ancient retroviral infections in human genome that can become transcriptionally active in cancer. Single-stranded RNA molecules are sensed by an intracellular toll-like receptor 8 (TLR8). Recently, immunotherapeutic approaches involving both, TLR8 and human endogenous retrovirus K (HERV-K) have shown promise in cancer and might bring new hope to glioblastoma patients with generally very poor prognosis. In this project, we investigate whether HERV-K is an endogenous ligand for the TLR8 in glioblastoma and if and how this ligand-receptor complex affects glioblastoma invasion.

We analyzed publicly available RNA-sequencing data (GEO accession: GSE79338) and found that both, HERV-K (HML-2) and TLR8 transcripts are upregulated in glioblastoma compared with healthy brain tissue. We use quantitative polymerase chain reaction (qPCR) and flow cytometry to quantify and compare the expression levels of TLR8 in glioblastoma patients versus control brains. To investigate the role of HERV-K and TLR8 interaction in glioblastoma, we compare early-passage primary patient-derived cell-lines with long-term cultured commercially available ones, stimulating these with HERV-K, scramble RNA, TLR8 agonist CL075 and vehicle and performing invasion and apoptosis assays.

Initial results show that the expression of TLR8 transcripts is higher in glioblastoma tissue (N=4) compared to control brains (N=3). Although the fraction of glioblastoma cells expressing TLR8 is only around 3%, its stimulation with a specific agonist CL075 has a strong positive effect on glioblastoma cell invasion. Similarly, stimulation of TLR8 with the HERV-K transcript increased invasion.

Together, these results suggest that HERV-K and TLR8 ligand-receptor complex promotes glioblastoma invasion. Further experiments are needed to reveal the downstream mechanisms behind this.

Ferritin in primary murine Microglia

Melanie Schürz[1], Karin Oberascher[1], Nikolaus Bresgen[1], Hubert H. Kerschbaum[1]

[1]University of Salzburg, Department of Biosciences, Hellbrunnerstr. 34, 5020 Salzburg, Austria

Ferritin is a multimeric iron storage protein, which can bind up to 4500 iron atoms. Intracellular ferritin is pivotal to cellular homeostasis by sequestering an excess of free “labile” iron which is prone to promote the iron-dependent generation of reactive oxygen species (ROS) and stimulation of lipid peroxidation (LPO) especially in conditions of inflammation, where a gradual increase of iron and ferritin is seen (Thomsen et al., 2015). In addition, secreted ferritin isoforms may also deliver iron to other cells. Ferritin has also been identified in the blood plasm (serum ferritin) and is increased under certain pathological conditions, such as inflammation or malignancy. Moreover, small amounts of ferritin are also present in in the cerebrospinal fluid (CSF). Recently, it has been proposed that ferritin serves as iron transporter across the blood-brain-barrier as well as iron shuttling between neural cells in the brain. In particular, the increased iron needs of oligodendrocytes are considered to be covered by ferritin uptake and not by transferrin featured by only two iron binding sites. The ferritin molecule is composed of 24 heavy (FTH) and light (FTL) subunits, the FTH: FTL ratio defining tissue specific ferritin isoforms of either basic (FTL-rich) or acidic (FTH-rich) character. The brain tissue isoferritin is generally considered to be FTH-rich; however, little is known on the distinct ferritin composition of different cell types. Here we present an investigation on the composition of ferritin in microglial cells which play a significant role in different neuroinflammatory processes. By employing isoform-specific monoclonal antibodies we could demonstrate differences of ferritin expression between the BV2 cell line and primary microglia which could point at transformation- related changes of microglial ferritin composition. Additionally, immunocytochemical staining revealed that ferritin is present in microglia in a distinct spotted pattern and not uniformly distributed throughout the cell. Moreover, evidence exists that ferritin may also bind other metals including cadmium (Pead et al., 1995). Due to its long biological half- life Cd accumulates in the environment and Cd exerts toxic effects in the brain. In order to address a potential effect of Cd on ferritin abundance we also examined ferritin expression in primary microglia as well as BV2 cells in the presence of Cd.

Pead, S., Durrant, E., Webb, B., Larsen, C., Heaton, D., Johnson, J., & Watt, G. D. (1995). Metal ion binding to apo, holo, and reconstituted horse spleen ferritin. J Inorg Biochem, 59(1), 15-27.
Thomsen, M. S., Andersen, M. V., Christoffersen, P. R., Jensen, M. D., Lichota, J., & Moos, T. (2015). Neurodegeneration with inflammation is accompanied by accumulation of iron and ferritin in microglia and neurons. Neurobiol Dis, 81, 108-118. doi:10.1016/j.nbd.2015.03.013

LGI1 antibodies from patients with autoimmune encephalitis alter K_V1.1 and AMPA receptors changing synaptic excitability, plasticity and memory

Josefine Sell[1], Mar Petit-Pedrol[2], Holger Haselmann[1], Mihai Ceanga[1], Jesús Planagumà[2], Francesco Mannara[2], Marija Radosevic[2], Marianna Spatola[2,3], Josep Dalmau[2,4,5,6], Christian Geis[1]

[1]Jena University Hospital, Hans-Berger-Department of Neurology, Jena, Germany
[2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Universitat de Barcelona, Barcelona, Spain
[3]University of Lausanne (UNIL), Lausanne, Switzerland
[4]Department of Neurology, University of Pennsylvania, Philadelphia, USA
[5]Centro de Investigación Biomédica en Red Enfermedades Raras (CIBERER), Valencia, Spain
[6]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Background:
Leucine-rich glioma inactivated protein 1 (LGI1) is a target of autoantibodies associated with autoimmune limbic encephalitis. The neuronal secreted protein LGI1 interacts presynaptically with the disintegrin and metalloproteinase domain-containing protein 23 (ADAM23) and K_V1.1 potassium channels, and postsynaptically with ADAM22 and AMPA receptors, providing a trans-synaptic complex. Despite evidence that this disease is immune-mediated, the underlying LGI1 antibody-mediated mechanisms are quite unknown.
We aimed to identify the epitope regions of LGI1 and whether the antibodies disrupt the interaction of LGI1 with its binding partners. Additionally, we were interested in pathophysiological mechanisms of altered synaptic transmission, synaptic plasticity and memory deficits induced by anti-LGI1 antibodies.
Methods:
LGI1 deletion constructs served to determine the binding epitope regions. HEK293 cells expressing ADAM22 or 23 preincubated with patients' IgG were exposed to soluble LGI1 to investigate if autoantibodies interfered the LGI1/ADAM binding. Purified patient-derived immunoglobulin G antibodies (control- and anti-LGI1-IgG) were used in a mouse model of continuous 14-day cerebroventricular infusion via osmotic pumps. Using this model, the hippocampal K_V1.1 and AMPAR density was analyzed by confocal imaging and memory deficits were tested. The physiological effects of the IgGs were examined with whole-cell patch-clamp and field potential recordings in hippocampus with electrical stimulation in presynaptic fiber tracts. Dendritic arborization and spine density of IgG-infused mice were studied with Golgi-Cox staining and subsequent Sholl analysis and spine counting.
Findings:
LGI1 IgGs reacted with the LRR as well as the EPTP domains. The antibodies prevented the binding of LGI1 to ADAM23 and ADAM22. Mice infused with LGI1 IgG, but not control IgG, showed a decreased level of K_V1.1 and AMPA receptors. In acute slices of infused mice, patch-clamp recordings of hippocampal neurons showed hyperexcitability with increased glutamatergic transmission. Paired-pulse facilitation and the synaptic failure-rate upon minimal stimulation were reduced indicating a higher presynaptic release probability. Analysis of synaptic plasticity showed a severe impairment of long-term potentiation along with a prolonged but reversible memory loss. Investigation of Golgi-Cox stained neurons revealed that dendritic complexity and spine density is unchanged.
Interpretation:

Our results demonstrate that LGI1 antibodies disrupt pre- and postsynaptic LGI1 signaling by targeting ADAM22/23 interaction with LGI1 domains. The observed antibody-induced reduction of $K_V1.1$ and AMPAR corroborates previous observations in mouse models of genetic alteration of LGI1. Patients' antibodies affect presynaptic function by increasing neurotransmitter release and synaptic excitability. Since dendrite and spine morphology is unchanged after antibody transfer, the enlarged glutamatergic transmission is not mediated by defective synapse maturation. Antibody-induced disturbance of presynaptic $K_V1.1$ function may underlie the observed presynaptic hyperexcitability. Overall, the findings support the pathogenicity of patients' antibodies.

Poster Topic

T13: Cognitive, Emotional, Behavioral State Disorders and Addiction

T13-1A Persistent increase in ventral hippocampal long-term potentiation by juvenile stress: A role for astrocytic glutamine synthetase
Anne Albrecht, Sebastian Ivens, Gürsel Caliskan, Uwe Heinemann, Oliver Stork

T13-2A Neuropeptide S receptor polymorphism (I107N) facilitates fear extinction in sex- and salience-dependent manner
Xabier Bengoetxea, Jasmin Remmes, Lena Goedecke, Peter Blaesse, Hans-Christian Pape, Kay Jüngling

T13-3A Pharmacological and non-invasive electrostimulation approaches to enhance learning in rats overexpressing the dopamine transporter
Nadine Bernhardt, Maike Kristin Lieser, Bettina Habelt, Henriette Edemann-Callesen, Christine Winter

T13-4A Functional analysis of a triplet deletion in the gene encoding the sodium glucose transporter 3, a potential risk factor for ADHD
Frank Döring, Nadine Schäfer, Maximilian Friedrich, Morten Egevang Jørgensen, Sina Kollert, Hermann Koepsell, Erhard Wischmeyer, Klaus-Peter Lesch, Dietmar Geiger

T13-5A Evaluation of the therapeutic effect of tween 80 modified Chitosan nanocapsules loaded with thymoquinone in a reserpine-induced model of depression in Wistar rats
Amena Sayed El-Feky, Heba Mohamed Fahmy, Taiseer Mohamed Abd El-Daim, Amera Abdelkrem Abd Rabo, Amira Bahaa El-Din Mostafa, Eslam Tarek Mostafa, Yasser Ashry Khadrawy

T13-6A FMRP modulates activity-dependent spine plasticity by binding cofilin1 mRNA and regulating localization and local translation
Jonas Feuge, Franziska Scharkowski, Martin Korte, Kristin Michaelsen-Preusse

T13-1B The effect of deep-brain stimulation of the medial forebrain bundle on sleep in the FSL rat model of depression
Wilf John Jago Gardner, Laura Durieux, Fanny Fuchs, Chantal Mathis, Volker A Coenen, Máté Döbrössy, Lucas Lecourtier

T13-2B A functional role for the Neuropeptide-S receptor polymorphism NPSR1 I107N in fear and anxiety
Lena Goedecke, Jasmin Remmes, Xabier Bengoetxea, Sion Park, Hans-Christian Pape, Kay Jüngling

T13-3B Blockade of endogenous opioids enhances threat learning by social observation
Jan Haaker, Jonatan Yi, Predrag Petrovic, Andreas Olsson

T13-4B 5-HTT deficient mice after experiencing prenatal stress: Gene expression study focusing on genes related to the vasopressin and oxytocin brain systems
Catharina Sophia Hamann, Karla-Gerlinde Schraut, Gabriela Ortega, Lisa Seeberger, Klaus-Peter Lesch, Angelika Schmitt-Böhrer

T13-5B Contribution of CRF and 5-HT in the anterodorsal BNST to phasic and sustained fear in freely behaving mice
Thomas Seidenbecher, Margarita Hessel

T13-6B Methylphenidate (MPH) produces conditioned place preference (CPP) in marmoset monkeys and cannabidiol exposure during extinction do not inhibit the reinstatement of MPH-induced CPP
Adel Kashefi, Renata B Duarte, Fernando M Jesus, Abbas Haghparast, Carlos Tomaz

T13-7B Proteasomal degradation of KCa2.2 channels is involved in emergence of acute epileptiform activity
Rüdiger Köhling, Steffen Müller, Xiati Guli, Victor Sudmann, Timo Kirschstein

T13-1C Spatial memory Impairments Following Imunotoxic Lesion of GABAergic Neurons of The basal Forebrain*A*
Lali Kruashvili

T13-2C The recognition memory in mice: standardization of behavioural tests and application of the method to study effects of a mycotherapy substance.
Veralice Lanaia, Paola Rossi

T13-3C Impact of the ASM/ceramide system on hippocampal neuronal excitability
Chih-hung Lin, Fang Zheng, Maria J. Valero, Johannes Kornhuber, Christian Alzheimer

T13-4C Immediate and persisting effect of toluene chronic exposure in adult and adolescent rats: the structure of the hippocampus and learning and memory
Nino Pochkhidze, Mzia Zhania, Manana Dashniani, Nadezhda Japaridze, Nino Chkhikvishvili, Lia Gelazonia

T13-5C The characterisation of ultrasonic communication in rats lacking brain serotonin.
Agnieszka Potasiewicz, Agnieszka Nikiforuk, Joanna Golebiowska, Malgorzata Holuj, Natalia Alenina, Michael Bader, Piotr Popik

T13-6C Effects of an acute decrease of central serotonin on decision making, impulsivity and social abilities of a novel line of rats with inducible serotonin depletion in the brain.
Marion Rivalan, Natalia Alenina, Michael Bader, York Winter, Lucille Alonso

T13-7C Sign- versus Goal-Tracking Behavior in Haploinsufficient Cacna1c Rats
Nivethini Sangarapillai, Markus Woehr, Rainer K. W. Schwarting

T13-1D Resting state fMRI based target selection for personalized brain stimulation temporarily alters the default mode network in healthy subjects
Aditya Singh, Tracy Erwin-Grabner, Grant Sutcliffe, Andrea Antal, Walter Paulus, Roberto Goya-Maldonado

T13-2D Cocaine preference in *Drosophila melanogaster.*
Raquel Suárez-Grimalt, David Owald

T13-3D Psychophysiological and Epigenetic Markers of Fear Generalization in Anxious and Non-Anxious Depression
Catherina Wurst, Carolin Leistner, Felix Nitzschke, Saskia Stonawski, Martin Herrmann, Paul Pauli, Jürgen Deckert, Andreas Menke

T13-4D Involvement of D1/D2 dopamine receptors within the nucleus accumbens and ventral tegmental area in the development of sensitization to antinociceptive effect of morphine A
Leila Zarepour

T13-5D Human stem cell-based model of alcohol use disorders
Annika Zink, Gizem Inak, Pawel Lisowski, Erich Wanker, Josef Priller, Alessandro Prigione

T13-6D Brain-computer interface (BCI) based communication with the completely paralysed
Niels Birbaumer, Andres Jaramillo Gonzalez, Ujwal Chaudhary

Persistent increase in ventral hippocampal long-term potentiation by juvenile stress: A role for astrocytic glutamine synthetase

Anne Albrecht[1,2,3], Sebastian Ivens[4,5], Gürsel Caliskan[1,2,4], Uwe Heinemann[4], Oliver Stork[1,2]

[1]Otto-von-Guericke University Magdeburg, Institute of Biology, Department of Genetics and Molecular Neuroscience, Leipziger Str. 44, 39120 Magdeburg, Germany

[2]Center for Behavioral Brain Sciences, Universitätsplatz 2, 39106 Magdeburg, Germany

[3]Leibniz Institute of Neurobiology, Brenneckestr. 6, 39122 Magdeburg

[4]Institute for Neurophysiology, Charité-Universitätsmedizin Berlin, Augustenburger Platz 1, 13353 Berlin, Germany

[5]Department of Psychiatry and Psychotherapy, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

A traumatic childhood is among the most important risk factors for developing stress-related psychopathologies such as posttraumatic stress disorder or depression later in life. In rodents, childhood adversity can be modeled by juvenile stress, resulting in increased anxiety and impaired coping with stressful challenges in adulthood.

In the current study, we found that juvenile stress led to increased LTP in the ventral CA1. This was paralleled by reduced mRNA expression levels glutamine synthetase (GLUL) in the ventral CA1 Stratum radiatum, a glutamate degrading enzyme specifically expressed in astrocytes. Indeed, the pharmacological inhibition of GLUL in slices of naïve rats mimicked the effect of juvenile stress on ventral CA1-LTP, while supplying glutamine to slices from juvenile stressed rats normalized their LTP levels.

Together, juvenile stress lastingly increased LTP in the ventral hippocampus, a region associated with anxiety and emotional memory processes. Impairments in the astrocytic glutmine/glutamate metabolism in astrocytes appear critically involved in these processes and further support a role for neuron-glia interaction in mediating stress effects on plasticity.

Neuropeptide S receptor polymorphism (I107N) facilitates fear extinction in sex- and salience-dependent manner

Xabier Bengoetxea[1], Jasmin Remmes[1], Lena Goedecke[1], Peter Blaesse[1], Hans-Christian Pape[1], Kay Jüngling[1]

[1] Institute of Physiology I, Westfälische Wilhelms-University, Münster, Germany

Aims:: The neuropeptide-S (NPS) and its G-protein coupled receptor (NPSR1), modulate a variety of autonomous and cognitive functions (e.g. stress, fear and anxiety). In humans, a single-nucleotide polymorphism (SNP) in the NPSR1 gene has been linked to panic disorders and increased stress levels. We hypothesize that introduction of the human SNP I107N in mice will impact the activity of the NPS system by altering the NPSR1 functionality, leading to alterations in fear-related circuits and behavior.

Methods: A novel mouse model is generated, in which an amino acid substitution of isoleucine (I) by arginine (N) at position 107 in the NPSR1 protein was induced by CRISPR/Cas9-mediated gene editing, mimicking the human SNP. Different homozygous cohorts of this mouse line (i.e. ii and NN groups), will be tested into two paradigms. First, to determine the innate fear expression status, startle responses against different white noise bursts intensities will be evaluated. Then, new groups of mice will be tested in a classical Pavlovian fear conditioning test. Both sexes will be tested under different shock intensities.

Results: No neat effect in fear startle responses was observed between the genotypes in any of the sexes in the startle response test. In contrast, the effects in the fear conditioning paradigm were strongly dependent on the sex and the saliency applied. Sex was shown to be a critical factor in order to detect the effect of the genotype. Not only that, but also the saliency of the paradigm shown to have a strong influence in the outcome of the experiment.

Conclusion: The genetic manipulation of mice to induce the human relevant NPSR1 variation reproduces the main behavioral characteristics known from the clinical studies. We conclude that the saliency is a critical factor in order to recruit the NPS system into the fear related circuits, in order to elicit the modification of the behavior.

Pharmacological and non-invasive electrostimulation approaches to enhance learning in rats overexpressing the dopamine transporter

Nadine Bernhardt[1], Maike Kristin Lieser[1], Bettina Habelt[1], Henriette Edemann-Callesen[1,2,3], Christine Winter[1,2]

[1]Department of Psychiatry and Psychotherapy, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany

[2]Department of Psychiatry and Psychotherapy, Charité Universitätsmedizin Berlin, Germany

[3]International Graduate Program Medical Neurosciences, Charité Universitätsmedizin Berlin, Germany

Dopamine functioning with its capacity to modulate motor control and motivation as well as cognition has been implicated for numerous neurological and psychiatric diseases. An imbalance in dopamine homeostasis as evident in the dopamine overexpressing rat model (DAT-tg), results in a predisposition for repetitive tic-like behaviour thus representing a model of cortico-striatal circuit dependent repetitive symptoms. In line Tourette syndrome specific medication (Hadar et al., 2016) and frontal transcranial brain stimulation (tDCS) has been shown to successfully reduce such behavioural alterations (Edemann-Callesen et al., 2018). In addition DAT-tg rat's exhibit learning and memory deficits associated with changes in adult hippocampal neurogenesis (Bernhardt et al., 2018). When subjected to the Morris water maze (MWM) DAT-tg rats show a striking inability to acquire information and deploy spatial search strategies. The aversive environment, which is a well-known stress-inducing factor, may thus prompt repetitive behaviour, manifesting in disproportionate thigmotactic swimming in the MWM. In an effort to test this hypothesis pharmacological and anodal tDCS treatments and their capacity to enhance behavioural performance during MWM were tested. Results will be presented and discussed.

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Functional analysis of a triplet deletion in the gene encoding the sodium glucose transporter 3, a potential risk factor for ADHD

Frank Döring[1,5], Nadine Schäfer[2], Maximilian Friedrich[3], Morten Egevang Jørgensen[2], Sina Kollert[1,4], Hermann Koepsell[2], Erhard Wischmeyer[1,5], Klaus-Peter Lesch[3,4,6], Dietmar Geiger[2]

[1]Universität Würzburg, Physiologisches Institut - AG Molekulare Elektrophysiologie, Röntgenring 9 , 97070 Würzburg, Germany

[2]Department of Molecular Plant Physiology and Biophysics, Julius-von-Sachs-Institute, University of Würzburg, Würzburg, Germany

[3]Division of Molecular Psychiatry, Center of Mental Health, University Hospital of Würzburg, Würzburg, Germany

[4]Laboratory of Psychiatric Neurobiology, Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Moscow, Russia

[5]Department of Psychiatry, Psychosomatics and Psychotherapy, Center of Mental Health,University Hospital of Würzburg, Würzburg, Germany

[6]Department of Neuroscience, School for Mental Health and Neuroscience (MHeNS), Maastricht University, Maastricht, The Netherlands

Sodium-glucose transporters (SGLT) belong to the solute carrier 5 family, which is characterized by sodium dependent transport of sugars and other solutes. In contrast, the human SGLT3 (hSGLT3) isoform, encoded by SLC5A4, acts as a glucose sensor that does not transport sugar but induces membrane depolarization by Na^+ currents upon ligand binding. Whole-exome sequencing (WES) of several extended pedigrees with high density of attention-deficit/hyperactivity disorder (ADHD) identified a triplet ATG deletion in SLC5A4 leading to a single amino acid loss (ΔM500) in the hSGLT3 protein imperfectly co-segregating with the clinical phenotype of ADHD. Since mutations in homologous domains of hSGLT1 and hSGLT2 were found to affect intestinal and renal function, respectively, we analyzed the functional properties of hSGLT3[wt] and [ΔM500] by voltage clamp and current clamp recordings from cRNA-injected *Xenopus laevis* oocytes.

The cation conductance of hSGLT3[wt] was activated by application of glucose or the specific agonist 1-desoxynojirimycin (DNJ) as revealed by inward currents in the voltage clamp configuration and cell depolarization in the current clamp mode. Almost no currents and changes in membrane potential were observed when glucose or DNJ were applied to hSGLT3[ΔM500]-injected oocytes, demonstrating a loss of function by this amino acid deletion in hSGLT3. To monitor membrane targeting of wt and mutant hSGLT3, fusion constructs with YFP were generated, heterologously expressed in *Xenopus laevis* oocytes and analyzed for membrane fluorescence by confocal microscopy. In comparison to hSGLT3[wt] the fluorescent signal of mutant [ΔM500] was reduced by 43% indicating that the mutant phenotype might mainly result from inaccurate membrane targeting. As revealed by homology modeling, residue M500 is located in TM11 suggesting that in addition to the core structure (TM1-TM10) of the transporter, the surrounding TMs are equally crucial for transport/sensor function.

In conclusion, our findings indicate that the deletion [ΔM500] in hSGLT3 inhibits membrane targeting and thus largely disrupts glucose-induced sodium conductance, which may, in interaction with other ADHD risk-related gene variants, influence the risk for ADHD in deletion carriers.

Evaluation of the therapeutic effect of tween 80 modified Chitosan nanocapsules loaded with thymoquinone in a reserpine-induced model of depression in Wistar rats

Amena Sayed El-Feky[1], Heba Mohamed Fahmy[1], Taiseer Mohamed Abd El-Daim[1], Amera Abdelkrem Abd Rabo[1], Amira Bahaa El-Din Mostafa[1], Eslam Tarek Mostafa[1], Yasser Ashry Khadrawy[2]

[2]Medical Physiology, Department, Medical, Division, National Research, Centre, Egypt

The aim of the present study was to test the antidepressant efficacy of Thymoquinone loaded on Chitosan nanoparticles prepared by the ionic gelation method and modified by the addition of Polysorbate 80 as a non-ionic surfactant. Drug treatment modality was performed on a reserpine-induced animal model of depression based on the depletion of monoamines level; Serotonin, Norepinephrine and Dopamine. The prepared formulations were characterized for size and morphology, encapsulation efficiency, in vitro drug release besides measurements of oxidative stress parameters: malondialdehyde (MDA), glutathione reduced (GSH) and gluatathione-s-transferase (GST). The levels of monoamines: Serotonin, Norepinephrine and Dopamine were also evaluated. In addition, behavioral tests: open field test and forced swimming tests were performed before and after receiving treatments. Transmission electron microscope confirmed that TQ-loaded Chitosan and TQ-loaded Chitosan coated with Polysorbate 80 were nearly spherical, having sizes of 44±1.9 and 74.66±5.6, respectively. The entrapment efficiency of TQ-loaded Chitosan and TQ-loaded Chitosan coated with Polysorbate 80 was found to be 75.67%±17.03% and 85.61%±1.02%, respectively. The loading capacity was found to be 14.093±4.61 and 16.26±1.2 nm for TQ-loaded Chitosan and TQ-loaded Chitosan coated with Polysorbate 80, respectively. The presented study confirmed the effect of reserpine (0.2 mg/Kg) as depleting agent for monoamines neurotransmitters resulting in induced depression animal model. This effect appeared in anxiety-like behavior in the open field and forced swimming tests. Treatment with TQ (20 mg/Kg) enhanced swimming as well as reducing immobility in rats in FST, where struggling appeared to be increased in TQ-loaded TPP-Chitosan coated with polysorbate 80.

Malondialdehyde (MDA) showed significant increases in the spleen, heart and kidney upon treatment with free thymoquinone (20 mg/Kg), whereas, the brain striatum, hippocampus and cortex showed significant increase in the MDA upon treatment with TQ-loaded Chitosan and TQ-loaded Chitosan coated with Polysorbate 80. Glutathione reduced (GSH) and Glutathione s-transferase (GST) showed non-significant differences in different organs, in contrary to different brain areas where significant increases in GSH levels and GST activity were evident when treated rats with TQ-loaded Chitosan and TQ-loaded Chitosan coated with Polysorbate 80. Reserpine induced depression model showed significant decreases in monoamine levels in the positive control group with respect to the negative control group. On the other hand, TQ treatment resulted in significant increases of serotonin and norepinephrine in the cortex, hippocampus and striatum, besides affecting dopamine levels in the hippocampus and striatum. The results of the present study emphasize the role of TQ either free or loaded on Cs as an antidepressant agent. Further studies are still needed to adjust the optimal dose of TQ that leads to minimum toxic effects.

FMRP modulates activity-dependent spine plasticity by binding cofilin1 mRNA and regulating localization and local translation

Jonas Feuge[1], Franziska Scharkowski[1], Martin Korte[1], Kristin Michaelsen-Preusse[1]

[1]TU Braunschweig, Division of Cellular Neurobiology, Spielmannstraße 7, 38106 Braunschweig, Germany

Long-lasting modifications of synaptic efficacy (e.g. L-LTP) of single synapses depend on global and local translation of pre-existing mRNAs. These local translation events allow synapses to autonomously and specifically change their structural and functional properties on a rapid time scale, a phenomenon that crucially depends on a fast remodeling of the actin cytoskeleton mediated by actin-binding proteins (ABPs). In the Fragile X Syndrome (FXS), where the Fragile X mental retardation protein 1 (FMRP) as a regulator of mRNA transport and local translation is absent, alterations in synapse structure and function point towards dysregulated actin dynamics. Therefore, this study aims to analyze the role of dynamic actin and ABPs as an underlying cause of synapse pathology in FXS

Using electrophysiological measurements and spine structural analysis in murine hippocampal neurons, we can show that activity-dependent functional and structural plasticity is impaired in an FXS mouse model (Fmr1 KO). Additionally, fluorescence recovery after photobleaching of eGFP-actin reveals impairments in activity-dependent cytoskeletal remodeling following NMDAR-dependent LTP induction (glycine-mediated chemical LTP). As a potential mechanism we can show that the mRNA of the ABP cofilin 1 (CFL) is a target of FMRP and that CFL expression is altered in Fmr1 KO mice. We offer evidence that an activity-dependent regulation of CFL mRNA is absent and that the local translation of CFL is dysregulated in Fmr1 KO neurons. Finally, we are able to rescue activity-dependent structural plasticity in Fmr1 KO neurons after induction of NMDAR-dependent LTP by mimicking the regulation of CFL that we saw in the WT and that was originally absent in the Fmr1 KO.

In summary, this work provides evidence that spine phenotypes in FXS involve a dysregulation of the dynamic actin network as cofilin expression is altered and an activity-dependent regulation of cofilin mRNA is missing.

The effect of deep-brain stimulation of the medial forebrain bundle on sleep in the FSL rat model of depression

Wilf John Jago Gardner[1,2,3], Laura Durieux[3], Fanny Fuchs[4], Chantal Mathis[3], Volker A Coenen[2], Máté Döbrössy[2], Lucas Lecourtier[3]

[1]Fakultät für Biologie, Albert-Ludwigs-Universität Freiburg, Schänzlestr. 1, 79104 Freiburg, Germany

[2]Department of Stereotactic and Functional Neurosurgery, Universitätsklinikum Freiburg, Breisacherstr. 64, 79106 Freiburg, Germany

[3]Laboratoire de Neurosciences Cognitives et Adaptatives, Université de Strasbourg, 12 Rue Goethe, 67000 Strasbourg, France

[4]Institut des Neurosciences Cellulaires et Intégratives, Université de Strasbourg, 5 Allée du Général Rouvillois, 67000 Strasbourg, France

Major Depressive Disorder (MDD) is a common and complex mental disorder, affecting 300 million people worldwide, with poorly understood biological mechanisms. Depressive symptoms vary between patients, and commonly include disrupted sleep. The Flinders Sensitive Line (FSL) rat is a model for depressive symptoms, and has been reported to show sleep deficits including increased overall duration of REM sleep and increased sleep fragmentation. Principle treatments for MDD include pharmaceuticals and psychotherapy. However, a sizeable subset of patients remain resistant to conventional treatments. Pre-clinical and clinical studies on deep-brain stimulation (DBS) of the medial forebrain bundle (MFB) to treat MDD are currently ongoing. In the current study, the effects of MFB-DBS on the sleep deficits present in the FSL rat are investigated.

FSLs were implanted with electrodes to record oscillatory activity at the prefrontal cortex, nucleus accumbens and dorsal CA1 region of the hippocampus. ECoG was recorded from a screw placed at the dura to assess sleep quality. Recordings were conducted immediately preceding 24-hour MFB-DBS, and at 1 and 7 days post-stimulation. A depressive-like behavioural phenotype was assessed using the forced swim test.

After MFB-DBS, a significant reduction in the depressive-like behavioural phenotype in the forced swim test was observed, compared to non-stimulated controls. There were no significant changes to sleep architecture measures including overall REM time and sleep fragmentation. Preliminary analysis of oscillatory activity suggests changes to the electrophysiological profile during REM sleep after MFB-DBS including increases in hippocampal theta power.

These results suggest an antidepressant-like effect of MFB-DBS on a behavioural phenotype, but not on sleep architecture. Despite the lack of effect on sleep architecture, electrophysiological changes during REM suggest a mechanism by which MFB-DBS may influence sleep. Future work must fully investigate the anti-depressant effects of MFB-DBS, including the relationship between oscillatory activity and sleep architecture.

A functional role for the Neuropeptide-S receptor polymorphism NPSR1 I107N in fear and anxiety

Lena Goedecke[1], Jasmin Remmes[1], Xabier Bengoetxea[1], Sion Park[1], Hans-Christian Pape[1], Kay Jüngling[1]

[1]Westfälische Wilhems-Universität Münster, Institute of Physiology I, Robert-Koch-Str. 27a, 48149 Münster, Germany

The Neuropeptide-S system, consisting of the Neuropeptide-S (NPS) and its G-protein coupled receptor (NPSR1), is implicated in mediating arousal, wakefulness, memory consolidation, and anxiolysis. While the ligand NPS is only expressed in three restricted cell clusters in the brain stem, the NPSR is found in various forebrain regions, among them the basolateral amygdala (BLA). In principal neurons of the BLA (BLA PNs), activation of the NPSR1 induces a membrane depolarization and increased spiking activity. In order to characterize the underlying ionic mechanism, we performed patch-clamp recordings in acute brain slices of mice. There is further evidence from human studies that a polymorphism in the NPSR1 (NPSR1 N107I) correlates with panic disorder and increased anxiety sensitivity. Here, we hypothesize that the alterations in fear-related behavior are based on a different functionality of these NPSR1 variants. Using a novel mouse model with an isoleucine to asparagine substitution in the NPSR1 at position 107 (I107N) – mimicking the human polymorphism – in combination with patch-clamp electrophysiology, we show at the cellular level that the NPSR-induced current and membrane depolarization in BLA PNs is significantly smaller in the N107 variant as compared to the I107 variant. This may result in a hypoactivation of the amygdalar excitatory network. Furthermore, we find that mice expressing the N107 variant exhibit facilitated extinction learning in a standard Pavlovian fear conditioning paradigm. Interestingly, these animals display an altered balance of excitatory and inhibitory activity in brain regions that are relevant to fear expression.
Together, we show for the first time that a human-relevant polymorphism in the NPSR1 (I107N) alters receptor signaling and fear-related behavior in a new mouse model.

Blockade of endogenous opioids enhances threat learning by social observation

Jan Haaker[1,2], Jonatan Yi[2], Predrag Petrovic[2], Andreas Olsson[2]

[1]University Medical Center Hamburg-Eppendorf, Department of Systems Neuroscience, Martinistr. 52, 20255 Hamburg, Germany

[2]Karolinska Institutet, Department of Clinical Neuroscience, Stockholm, Sweden

Many of our fearful expectations are shaped by observation of aversive outcomes to others. Witnessing others' traumatic experiences can thereby impact physioloigcal and emotional well-being of bystanders. Yet, the neurochemistry regulating social learning of threats is unknown.
Here, we examined if an opioidergic circuitry regulates social threat learning by observation in humans. Previous research in animals has shown that endogenous opioids are released during threat learning from directly painful experiences, via activity within the amygdala and periaqueductal gray (PAG).
We employed an observational fear conditioning paradigm in humans including a pharmacological challenge of the opioid receptor system (50mg Naltrexone vs Placebo) while acquiring heamodynamic response in the brain using fMRI.
We found that the blockade of opioid receptors (Naltrexone) enhanced observational learning through activity within the amygdala, midline thalamus and the PAG as compared to Placebo controls. In particular, temporal dynamics of PAG responses to the observed pain in others were affected by opioid receptors function. These temporal dynamics in PAG coding the observed aversive outcomes during the observational US predicted anticipatory responses to learned threat cues (CS+). Moreover, the blockade of opioid receptors enhanced amygdala responses towards the observational US, which predcited the enhanced expression of threat responses 72 hours after learning in the Naltrexone group, but not in Placebo controls. A supervised machine-learning algorithm successfully classified individual endogenous opioid receptor function during the expression of conditioned threats with a kernel restricted to brain regions that were responsive to the observational US.
In sum, our study shows that endogenous opioids, which serve as potent analgesics against the direct experience of pain also code social threat learning from pain that is transmitted solely through observation. These insights are clinical relevant, given our complex social environment in which learning from others is ubiquitous. Reflecting the importance of indirect/vicarious experiences, the most recent DSM-5 has now added vicarious experiences as a diagnostic criterion for PTSD.
Our results mark a starting point for neuropharmacological research on social mechanisms in threat learning.

5-HTT deficient mice after experiencing prenatal stress: Gene expression study focusing on genes related to the vasopressin and oxytocin brain systems

Catharina Sophia Hamann[1], Karla-Gerlinde Schraut[2,3], Gabriela Ortega[3], Lisa Seeberger[1], Klaus-Peter Lesch[3], Angelika Schmitt-Böhrer[1]

[1] Center of Mental Health, Dep. of Psychiatry, Psychosomatics and Psychotherapy, University of Würzburg, Würzburg, Germany

[2] Max-Planck-Institute for Psychiatry, Munich, Germany

[3] Center of Mental Health, Dep. of Psychiatry, Psychosomatics and Psychotherapy, Division of Molecular Psychiatry, University of Würzburg, Würzburg, Germany

The serotonergic system within the brain has important functions regulating mood, anxiety and a variety of cognitive functions. Research focusing on the serotonin transporter (5-HTT) delivered insights into its involvement in the development of anxiety-related traits. In humans, a length polymorphism in the *5-HTT* promotor is known to influence susceptibility to anxiety disorders and depression. Mice with reduced levels of 5-HTT display elevated levels of anxiety and are an established model to investigate the development of anxiety disorders. Different forms of stress, especially early life stress, can also impact the susceptibility and development of these psychiatric disorders. Moreover, the serotonergic system has been linked to a variety of social responses and behaviours and deficits in its regulation might result in abnormal social behaviour.

The vasopressin (AVP) and oxytocin (OXT) systems within the brain have been shown to have different and often opposing effects on various areas of behaviour. For instance, elevated levels of vasopressin have been found in patients suffering from depression. Furthermore, polymorphisms of the vasopressin receptor might alter the susceptibility to panic disorders. Oxytocin plays a major role in social bonding, especially for the bonding between mothers and their newborn children. The vasopressin and oxytocin systems in the brain might be in balance, with high vasopressin levels corresponding to increased anxiety-like behaviour, whereas high levels of oxytocin might correspond to states of hypo-anxiety.

Investigating the relationship between brain AVP/OXT systems and the serotonergic system can give insights into possible interactions between these systems regarding the development of anxiety-related disorders. Important brain areas to consider are the hypothalamus where both, vasopressin- and oxytocin-producing neurons are localized, and the amygdala, which plays an important role in the regulation of fear and anxiety-related behaviour. Raphe nuclei are important regarding serotonin functions, because serotonin is produced in this area and released to other brain regions from there.

To investigate the influence of prenatal stress on 5-HTT wildtype (WT) and heterozygous offspring, WT dams were stressed during late stages of pregnancy. The female offspring underwent behavioural testing regarding social and anxiety-related behaviour, as well as depressive symptoms. Sociability was assessed using a 3 chamber sociability test. Based on the results of this test, mice that had experienced prenatal stress were classified into two different groups, “social” and “unsocial”, depending on the time they spent with an unfamiliar other mouse during the experimental setup. For further analysis, these mice were therefore divided into six different groups, “social”, “unsocial” and a control group for both genotypes. RNA extracted from brain regions mentioned above was used to perform a quantitative real time PCR study. To investigate possible effects of different *5-Htt* genotypes on the AVP/OXT systems, gene expression of vasopressin, its receptors 1A (AVPR1A), 1B (AVPR1B), as well as oxytocin and its receptor (OXTR) was evaluated. Differences detected in these systems might be interrelated with the social/unsocial behaviour investigated mice displayed during the sociability test.

Contribution of CRF and 5-HT in the anterodorsal BNST to phasic and sustained fear in freely behaving mice

Thomas Seidenbecher[1], Margarita Hessel[1]

[1]Westfälische Wilhelms-University Münster, Institute of Physiology I, Robert-Koch-Str. 27a, 48149 Münster, Germany

Sustained fear paradigms in rodents have been developed to model clinical situations in patients suffering from long-lasting anxiety disorders. Recent data suggest that short-lasting (phasic) fear responses rely on the central amygdala, whereas more long-lasting (sustained) fear responses critically depend on the bed nucleus of the stria terminalis (BNST). Conditioned fear can be mediated by the amygdala via corticotropin-releasing factor (CRF), a stress hormone that acts on receptors in the BNST. CRF-containing cell bodies and CRF receptors were found in high concentrations in the BNST and CRF neurons co-localize with 5-HT (Serotonin) terminals in this brain region. Therefore, in this study we used a pharmacological approach combined with fear behavioral protocols in an established phasic/sustained fear mouse model to reveal the critical involvement of CRF and 5-HT in the anterodorsal (ad)BNST to modulate phasic and sustained fear.
Local application of a CRF1-receptor agonist (Stressin I) before fear memory retrieval, 24h after predictable CS-US training, induced sustained fear (maintained freezing) indicating the critical contribution of the CRF1-receptor during sustained states of conditioned fear. Application of saline as control revealed only phasic fear 24 hours after predictable CS-US pairing as expected. Bilateral local application of a 5-HT2A-receptor antagonist (R-96544) either before unpredictable CS-US training or before fear memory retrieval, 24 hours after unpredictable conditioning, blocked the sustained component of fear while phasic fear component was not affected, indicating the critical contribution of serotonergic transmission mediated by the 5-HT2A-receptor during sustained states of conditioned fear. Saline application revealed sustained fear 24 hours after unpredictable CS-US pairing. Further, data also revealed that 5-HT1A– and 5-HT1B-receptor in the adBNST critically contribute to the modulation of phasic and sustained fear.
In summary, here we show the critical contribution of CRF and 5-HT in the adBNST to phasic and sustained fear in freely behaving mice. This study will advance the understanding of clinical anxiety and its treatment strategies and will thus provide a putative perspective for pharmacological treatments that specifically target the BNST.

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Methylphenidate (MPH) produces conditioned place preference (CPP) in marmoset monkeys and cannabidiol exposure during extinction do not inhibit the reinstatement of MPH-induced CPP

Adel Kashefi[1], Renata B Duarte[1], Fernando M Jesus[1], Abbas Haghparast[2], Carlos Tomaz[1]

[1]University of Brasilia, Department of Physiological Sciences, Universidade de Brasilia, Campus Universitário Darcy Ribeiro-IB, Bloco G, Departamento de Ciências Fisiológicas (CFS), G1-10/08 CEP 70910-900 Brasilia- DF, Brazil., 70910-900 Brasilia, Brazil

[2]2. Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Methylphenidate (MPH) is a central nervous system stimulant used as a pharmacotherapy to treat Attention-Deficit/Hyperactivity Disorder and narcolepsy. Little work has been made on the addictive potential of MPH in non-human primates (NHP). At the present study we intend to evaluate whether the MPH is able to produce a conditioned response and if the exposure to cannabidiol (CBD) during the extinction trial of the conditioned preference place (CPP) paradigm can inhibit the reinstatement of the response in male marmoset monkeys. Animals received intraperitoneal (i.p.) alternated injections of either MPH (5 mg/kg) or saline (SAL) to a daily 15 min conditioning trial in the CPP box during 10 consecutive days. After a place preference test the animals were submitted to daily CBD injection in a 15 min extinction trial, until extinction. Then, 24h after the last extinction trial, animals received a prime dose of MPH (1mg/kg) and were submitted to a 15min retest trial. We found that MPH induced strong and long-lasting reinforcing properties during the conditioning period even after extinction training and reinstatement test. Therefore, MPH induced a CPP response in a NHP model and that the CBD administration could not inhibit the reinstatement of the MPH-induced CPP response.

Proteasomal degradation of KCa2.2 channels is involved in emergence of acute epileptiform activity

Rüdiger Köhling[1], Steffen Müller[1], Xiati Guli[1], Victor Sudmann[1], Timo Kirschstein[1]

[1]Rostock University Medical Center, Oscar-Langendorff-Institute of Physiology, Gertrudenstrasse 9, 18057 Rostock, Germany

Voltage-independent, Ca^{2+}-activated K^++ channels ($K_{Ca2.2}$) are powerful regulators of cellular excitability by generating an afterhyperpolarizing potential (AHP) following prolonged excitation. Superfusion hippocampal brain slice preparations with the GABAA receptor blocker gabazine (GZ) induces epileptiform activity acutely. In this epilepsy model, the AHP has previously been shown to be significantly decreased. Here, we asked the question whether $K_{Ca2.2}$ protein degradation occurs in this model, and which pathways are involved. To test this, we applied either GZ alone or GZ together with inhibitors of proteasomal and lysosomal protein degradation pathways, Z-Leu-Leu-Leu-CHO (MG132) and chloroquine (CQ), respectively. Using western blot analysis, we showed a significant decrease of total $K_{Ca2.2}$ protein content in GZ-treated slices which could be rescued by concomitant incubation with MG132 and CQ. In HEK293 cells transfected with a green fluorescent protein-tagged $K_{Ca2.2}$ construct, we demonstrated that proteasomal rather than lysosomal degradation was involved in $K_{Ca2.2}$ reduction. To test for functional significance, we recorded epileptiform afterdischarges at hippocampal Schaffer collateral-CA1 synapses and confirmed that the GZ-induced increase was significantly attenuated by both MG132 and CQ, with MG132 being significantly more effective than CQ. Epileptiform afterdischarges were nearly completely prevented by co-application of protein degradation inhibitors. Furthermore, epileptiform afterdischarges could be rescued by using the $K_{Ca2.2}$ blocker UCL 1684, demonstrating involvement of $K_{Ca2.2}$. We conclude that in GZ-induced acute epilepsy, $K_{Ca2.2}$ degradation by proteasomal rather than lysosomal pathways plays a major role in the generation of epileptiform afterdischarges.

Spatial memory Impairments Following Imunotoxic Lesion of GABAergic Neurons of The basal Forebrain*A*

Lali Kruashvili[1]

[1]I.Beritashvili Center of Experimental Biomedicine, Behavior and Cognition Function, Gotua14 , 0160 Tbilisi, Georgia

Basal forebrain (BF) afferents represent one of the largest cortical-projecting neuromodulatory systems. This brain region has been implicated in cortical activation, attention, motivation, memory, and neuropsychiatric disorders Traditionally, most BF functions have been attributed to its cholinergic neurons. The BF cholinergic system has at the extremes 2 relatively distinct subcomponents, the nucleus basalis magnocellularis (NBM) and the medial septal area (MS), which have projections to the frontal cortex and hippocampus, respectively. Degeneration of cholinergic BF neurons is one of the common features of AD. It has been reported that degeneration of BF cholinergic neurons and the decrease of cholinergic projections could be an important factor characterizing the cognitive decline and functional impairment that characterizes this disorder.The BF contains: cortically projecting cholinergic and noncholinergic neurons as well as various interneurons.The most prominent noncholinergic component of the BF corticopetal projection system are the GABAergic corticopetal projections. Substantial evidence suggests that the NBM and MS plays an important role in learning and memory. In contrast to research on the cortical cholinergic input system, little is known about the functions corticopetal GABAergic neurons.

The central aim was to investigate the modulation of spatial memory function by the GABAergic cells of the NBM and MS using a new more selective toxin for GABAergic neurons - immunotoxin GAT1-SAP.

A total of 36 male rats were used in the present study. The animals were randomly assigned to MS (n = 12) and NBM (n = 12) GAT1-SAP lesioned and MS (n = 6) and NBM (n = 6) shamlesioned groups. All injections of GAT1-SAP (325ng/µl) for immunolesion surgeries or mouse, for control surgeries (Advanced Targetting System, San Diego, USA) were performed stereotaxically. All experiments were approved by the Animal Care and Use Committee of the Center and were in accordance with the principles of laboratory animal care. Animals were tested in a standard Morris water maze,taxing different strutegy (i.e., cue or place) of spatial memory function. The immunotoxic GAT1-SAP lesions of NBM and MS were verified by observing decreased Acetylcholintransferase and parvalbumine staining of the NBM and MS. All statistical analyses were conducted with a significance level of $P < 0.05$.

Immunohistochemical studies showed that injection of GAT1-SAP preferentially reduced GABAergic neurons as compared to cholinergic neurons in the NBM and MS. Behavioral data of the present study suggest that the control and lesioned rats, exhibited corresponding differences in performance during training trials. The control rats, identified as place responders, had significantly more accurate searches on hidden platform days, providing an additional evidence of their effective use of a place learning strategy rather than the NBM and MS-lesioned rats exhibiting a cue strategy in competition trials. The lesioned rats acquired the visible platform version of the water maze task but failed to learn the platform location in space. Present results suggest involvement of the NBM and MS GABAergic neurons in organization of the spatial map-driven behavior and this structures should be viewed as a constituent of the functional system responsible for the cognitive types of spatial memory.

The recognition memory in mice: standardization of behavioural tests and application of the method to study effects of a mycotherapy substance.

Veralice Lanaia[1], Paola Rossi[2]

[1] Universitätsklinikum Erlangen, HNO-Klinik, Experimental Otolaryngology, Waldstrasse 1, 91054 Erlangen

[2]University of Pavia, department of biology and biotechnology "Lazzaro Spallanzani", via Ferrata 9, 27100, Pavia, Italy

Over the last ten years, the process of object recognition has been investigated by the scientific community in order to study its cognitive and neuropsychological characteristics in rodents.
The Novel Object Recognition (NOR) and the Object Localization (OL) are spontaneous tests performed on rodents to study, respectively, the recognition and spatial memory. Both tests consist of three sessions on consecutive days in which the first two are dedicated to the habituation phase where each mouse is free to explore an arena for ten minutes. The third session is divided in two phases. In phase one, familiarization phase, two identical objects are placed in the arena and the animal is free to explore both of them. After that, the animal is removed from the arena and for at least 10 min is left in its homecage. In the second phase of the test, a new object is placed in the arena for the NOR test, and one of the two familiar objects is moved in a different position of the arena in case of the OL test.
The goal of this work is to standardize the experimental conditions for the two tests in mice in order to make them effective, reliable and repeatable. For both tests, several parameters were measured, including the frequency of approach, latency of first approach, total duration of approaches and average duration of an approach. In the NOR test, the most sensitive indicator to evaluate the recognition memory, among the recorded parameters, is the total duration of the approaches. It showed an increase of the attention from mice on the novel object in order to identify its characteristics. In the OL test, the most sensitive indicator to evaluate the recognition memory, among the recorded parameters, is the frequency of approach and the latency of first approach is lower for the relocated object.
Once the techniques have been standardized, we in particular investigated the effect of a mushroom, Hericium erinaceus (Hr), that has numerous mycotherapeutic effects and, in particular, it has been shown to have an effect on some cognitive functions related to memory phenomena. The fruiting body and the mycelium of Hr contains, respectively, erinacines and hericenones, which are composites with low molecular weight able to penetrate the blood brain barrier and induce the NGF synthesis. Despite an extensive investigation of the preventing action of Hr in cognitive pathological conditions to date, no studies have investigated the effects of dietary supplementation with Hr in healthy mice. To address this point a group of wild-type mice were fed with a placebo and another one with Hr dietary supplementation and the effects on cognitive skills related to memory were compared. The NOR test in mice treated with Hr highlighted how these subjects show a higher curiosity towards the new object. The total duration of approaches and average duration of an approach were strongly increased. When comparing the Hr groups with the placebo group we noticed that they dedicate the same attention to the familiar object, but the Hr group was more curiosity than placebo group for the novel object.

Impact of the ASM/ceramide system on hippocampal neuronal excitability

Chih-hung Lin[1,2], Fang Zheng[1], Maria J. Valero[1], Johannes Kornhuber[2], Christian Alzheimer[1]

[1]Institute of Physiology and Pathophysiology, [2]Department of Psychiatry, University of Erlangen-Nürnberg, Germany

The prevalence of major depressive disorder (MDD) is more than 10% over the world population. The acid sphingomyelinase (ASM)/ceramide system has been advanced as a major player in the pathogenesis of MDD. Indeed, the activity of ASM is enhanced in MDD patients, with higher concentrations of plasma ceramide than in healthy individuals. Here, we used wild type (WT) and ASM knockout (koASM) mice to explore the impact of the ASM/ceramide system on hippocampal neuronal excitability. Whole-cell patch-clamp recordings were performed on hippocampal cells (CA1 pyramidal cells and dentate gyrus granule cells) in brain slices from adult WT and koASM mice. Our data show no significant differences in neuronal excitability between WT and koASM hippocampal cells. However, the specific ASM inhibitor ARC39 (1 µM) strongly reduced the excitability of neurons from ventral hippocampus of WT mice. No such inhibitory effect of ARC39 was observed in koASM pyramidal cells, demonstrating the drug´s specific action on ASM. Application of C2-ceramide produced mixed effects on cell excitability along the hippocampal longitudinal axis. The C2-ceramide-induced excitation in dorsal CA1 pyramidal cells (71%) was shifted towards inhibition in ventral CA1 pyramidal cells (only 22% excitation). In dorsal granule cells, the uniformly excitatory effect of ceramide (100%) in WT mice was altered in koASM mice (69%), suggesting that the reduced ceramide level in koASM mice might alter the responsiveness to ceramide. Furthermore, voltage-clamp recordings from granule cells revealed that the ceramide-induced apparent inward currents reversed around -90 mV, close to the reversal potential of potassium channels. Our data suggest that the ASM/ceramide system exerts a complex pattern of acute electrophysiological effects in the hippocampus, depending on which subregion along its longitudinal axis is under study.

Immediate and persisting effect of toluene chronic exposure in adult and adolescent rats: the structure of the hippocampus and learning and memory

Nino Pochkhidze[1,2], Mzia Zhania[1,2], Manana Dashniani[2], Nadezhda Japaridze[2], Nino Chkhikvishvili[2], Lia Gelazonia[2]

[1]Ilia State University,Molecular Neuroscience;

[2]I. Beritashvili Center of Experimental Biomedicine, ; Lab of Brain of Ultractructure and Nanoarchitecture, Gotua 14, 0160 Tbilisi, Georgia

Toluene and toluene-containing volatile substances are the most widely abused solvents with demonstrative addictive potential in humans. Clinical and experimental studies have demonstrated that the exposure to toluene vapor leads to diverse consequences at the level ranging from the cell to the whole organism. The present study has been undertaken to determine whether toluene chronic exposure provokes immediate and/or persistent effect on the structure of hippocampus, learning and memory in adolescent and adult rats. We exposed male Wistar rats at ages P 28-32 (adolescents) and P 150-160 (adults) to 2000 ppm inhaled toluene for 40 days. The immediate and persisting effects of toluene misuse (immediately after the end of toluene chronic inhalation and 90-day after the end of toluene chronic inhalation, correspondingly) on pyramidal cell loss in the CA1 and CA3 of the hippocampus and exploratory behavior and recognition memory in the open field were evaluated. The results reveal that t oluene chronic exposure affects the structure of the hippocampus, exploratory activity and recognition memory in the open field in adolescent and adult rats. In all cases the effect is age-dependent. In particular: in adolescent rats the more significant structural and behavioral alterations were observed immediately after toluene chronic exposure, while in adult rats the most considerable was persisting effect (90 days after withdrawal). Such data indicate that character of alterations depends upon the postnatal age of testing of the animals.

The characterisation of ultrasonic communication in rats lacking brain serotonin.

Agnieszka Potasiewicz[1], Agnieszka Nikiforuk[1], Joanna Golebiowska[1], Malgorzata Holuj[1], Natalia Alenina[2], Michael Bader[2], Piotr Popik[1]

[1]Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland, Department of Behavioral Neuroscience & Drug Development, Krakow, Poland

[2]Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany

Social communication deficits are a hallmark symptom of autism spectrum disorders (ASD). The evaluation of communication in animal models is also possible, since rodents communicate in the ultrasonic range by means of ultrasonic vocalizations (USVs). In adult laboratory rats, two main types of USVs have been described: low (22-kHz) and high (50-kHz) frequency calls. The 22-kHz call type, termed an “alarm” vocalization, have been associated with negative social experiences (e.g., presentation of a predator or intermale aggression). The 50-kHz USVs may be detected in appetitive contexts, including social interactions. Spectrographic analysis has identified multiple call categories within the rich repertoire of rat 50-kHz USV that appear to differ in their behavioural significance. Therefore, measuring USVs emission in rodent models may provide further information about the autistic-like abnormalities in social behaviour and communication. It has been recently suggested that brain hyposerotonemia during critical neurodevelopmental periods can lead to the autistic-like phenotype. Thus, the aim of the study was to measure USVs during reciprocal social interactions in a genetic model of central serotonin depletion.
As such a model we used rats lacking tryptophan hydroxylase 2 (TPH2), a rate-limiting enzyme of serotonin synthesis in the brain.
This study showed that male TPH2-/- rats spent less time in social interactions and demonstrated less episodes of social behaviours. Additionally, TPH2-/- animals demonstrated sexual, copulatory-like behaviours. The number of 22-kHz and 50-kHz USVs emitted during social interaction did not differ between TPH2-/- and TPH2+/+ animals. There was also no effect of genotype on the bandwidth and peak frequency of emitted calls. However, average duration of USVs was affected by TPH2 deletion, as TPH2-/- rats vocalized with longer USVs. Serotonin depletion also affected the distribution of sound categories, as the increased percentage of flat and complex calls and the decreased percentage of short calls were noted in TPH2-/- rats.
The present study further confirms the role of serotonin in the regulation of social behaviour.

This study was supported by the grant ERA-NET Neuron II JTC 2015 Respond and by the Statutory Activity of the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

Effects of an acute decrease of central serotonin on decision making, impulsivity and social abilities of a novel line of rats with inducible serotonin depletion in the brain.

Marion Rivalan[1], Natalia Alenina[2], Michael Bader[2], York Winter[1], Lucille Alonso[1]

[1]Humboldt University, Institut of Biologie, Kognitive Neurobiologie, Philippstr. 13, 10099 Belrin, Germany
[2]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

Although many studies have established that serotonin can play a critical role in the modulation of several human's and animal's socio-cognitive abilities, we surprisingly observed no cognitive deficits (only social deficits) in a genetically modified line of rats which were born with a total lack of central 5-HT in the brain (genetic deletion of the tryptophan hydroxylase 2 (TPH2) gene).

The aim of this project was thus to investigate if a mild acute decrease in central serotonin would affect the performances in decision-making, cognitive flexibility and social recognition memory of another rat model of inducible serotonin depletion, the TetO-shTph2 rats. In these transgenic animals, which grew up with normal rates of 5-HT in the brain, the oral administration of doxycycline induced the expression of shRNAs against rat Tph2 gene which resulted in a 25% decreases in brain serotonin levels.

The TetO-shTph2 rats were tested in the Rat Gambling task (RGT), the reversed-RGT, the social recognition test and the probability discounting task. Post-mortem measures of serotonin (HPLC) were done in the dorsal raphe, prefrontal and orbitofrontal cortices and the enzymatic activity of the TPH2 was assessed in the hypothalamus and the hippocampus.

Our preliminary results indicated that an acute and mild decrease of serotonin function in these rats altered the ability to make advantageous decision and that the induced-poor decision makers (only) were risk-averse in the probability-based decision making task compared to the control animals. These rats were also slower in the recognition of the social partner but not different in cognitive flexibility. More animals are currently tested and if these results are to be confirmed, we will discuss how the 5-HT contributes to the expression of different cognitive abilities in some rats but not in all of them and how the spontaneous individual behavioural profile of an individual is critical to identify if we want to understand the diversity of profiles also existing in humans and be able to eventually circumvent the lack of effect of some pharmacological therapies in subgroups of patients.

Sign- versus Goal-Tracking Behavior in Haploinsufficient Cacna1c Rats

Nivethini Sangarapillai[123], Markus Woehr[123], Rainer K. W. Schwarting[123]

[1] Behavioral Neuroscience, Experimental and Biological Psychology, Philipps University Marburg, Germany
[2]RTG 2271, Breaking Expectations, Philipps University Marburg, Germany
[3]Center of Mind Brain and Behavior (CMBB), Philipps University Marburg, Germany

Interactions between genetic and environmental risk factors play a major role in the onset of neuropsychiatric disorders. The Cacna1c gene encoding for the α1C subunit of the L-type voltage-gated calcium channel $Ca_V1.2$ is considered as one of the vulnerability genes for several disorders, especially autism spectrum disorder, bipolar disorder and schizophrenia. This channel mediates cell membrane depolarization, by increasing membrane permeability for calcium ion influx. Our previous work indicates that behavioral alterations displayed by haploinsufficient $Cacna1c^{+/-}$ rats are at least partly driven by changes in the dopaminergic system.

Here, we used an established rat model for sign- and goal-tracking, introduced by Robinson and Berridge . In this Pavlovian task, rats show individual differences in the sensitivity to the conditional stimulus. So-called sign- and goal-trackers not only respond differently to cue vs. reward presentation, but also behave differently to expectation violations, i.e. concerning extinction. The classification is based on their approach behavior, either to the sign (lever) predicting the reward or the goal (food cup) itself. Previous studies have shown that the dopaminergic system is differentially involved in sign- and goal-tracking behavior.

Specifically, we applied the sign- and goal-tracking paradigm in our genetic Cacna1c rat model and compared haploinsufficient $Cacna1c^{+/-}$ females with their $Cacna1c^{+/+}$ littermate controls. Interestingly, most $Cacna1c^{+/+}$ littermates showed a clear tendency towards goal-tracking behavior. $Cacna1c^{+/-}$ females displayed such a phenotype as well. Also, there was no apparent genotype difference during subsequent extinction. In ongoing neurochemical work, we analyze possible neurochemical differences between these genotypes, focusing, among others, on frontal and striatal dopamine.

This work was supported by grants from Deutsche Forschungsgesellschaft.

Keywords: Cacna1c, Sign- versus Goal-Tracking, Pavlovian Conditioning, Extinction, Rats, Dopamine System

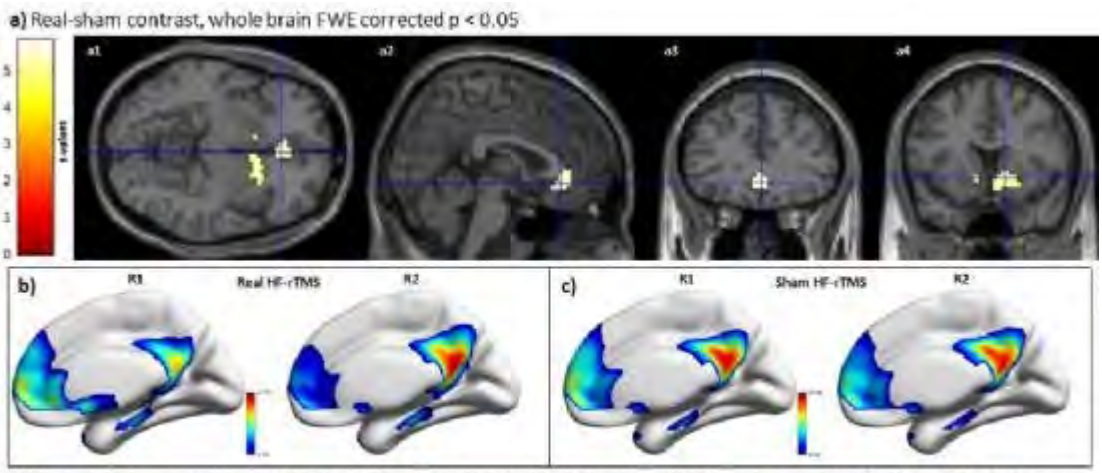
Resting state fMRI based target selection for personalized brain stimulation temporarily alters the default mode network in healthy subjects

Aditya Singh[1], Tracy Erwin-Grabner[1], Grant Sutcliffe[1], Andrea Antal[2], Walter Paulus[2], Roberto Goya-Maldonado[1]

[1]Department of Psychiatry and Psychotherapy of the University Medical Center Göttingen.

[2]Department of Clinical Neurophysiology of the University Medical Center Göttingen.

Although high frequency repetitive transcranial magnetic stimulation (HF-rTMS) is a very good option for the treatment of depression, there is much variability in the resulting antidepressant response most probably through inaccurate brain targeting. Previous work has highlighted that stimulation sites in the left dorsolateral prefrontal cortex (DLPFC) that feature a stronger negative connectivity to the subgenual anterior cingulate cortex (sgACC) can achieve stronger antidepressant effects. We present a new target selection method that incorporates this feature based on individual resting state fMRI (RS-fMRI) data for a more precise and personalized delivery of HF-rTMS. Moreover, our study addressed for the first time the mechanism of action of an entire session (3000 pulses, 10 Hz) HF-rTMS with a double blind, sham-controlled, crossover design. We show that the new target selection method and online neuronavigation monitoring for stimulation is reproducible for clinical utility. The personalized target selection allows targeting regions in left DLPFC that are most highly anticorrelated to the sgACC, which was not possible when using only anatomical information to guide target selection. In response to a single complete FDA approved session of HF-rTMS, we show changes in the functional connectivity of the default mode network with the sgACC and the ventral striatum (vStr), core brain regions implicated in the pathophysiology and treatment response of depression. Our study shows that it is in fact possible to specifically influence the functional connectivity of both these deeper regions by precisely stimulating at cortical targets. It further shows that the reported influences on the functional connectivity might underlie the fundamental mechanism of HF-rTMS, and strongly suggests that its antidepressant effects arise from cumulative effect of HF-rTMS in these regions over multiple sessions. Next, we aim to clinically validate the personalized target selection protocol, as well as predict and improve rTMS antidepressant effects using connectomic, epi/genomic and phenotypic data from patients with depression.



a) Decreased functional connectivity in sgACC (a1-a3) and vStr (a4) during R2 when compared to R1. Default Mode Network after **b)** real HF-rTMS and **c)** sham HF-rTMS during R1 (left) and R2 (right) RSfMRI. Decrease is seen only after real HF-rTMS. [n = 23]

Cocaine preference in *Drosophila melanogaster.*

Raquel Suárez-Grimalt[1], David Owald[1]

[1]Institute of Neurophysiology, Charité – Universtätsmedizin Berlin, Einstein Center for Neurosciences Berlin

The identification of the main neural mechanisms underlying drug addiction is a major challenge in neuroscience research. Recent evidence indicates that the fruit fly *Drosophila melanogaster* is able to develop preference for substances of abuse such as ethanol and drug-associated cues. Therefore, it is of great interest to study how preference for other drugs such as cocaine is maintained in the brain at single cell resolution and to which extent preference for different drugs of abuse share common neuronal pathways and neurotransmitter systems in *Drosophila*. Here, we investigate whether fruit flies show preference for cocaine at the single-fly level and study the dynamics of this preference over time, aiming at identifying the main neuronal elements of the cocaine reward circuitry. Taken together, our results show that fruit flies preferentially self-administrate cocaine-containing food and that this preference is dependent on drug dose and genotype. These results confirm that *Drosophila* is a promising model system to study cocaine reward circuitry and the neuronal mechanisms underlying drug addiction.

Psychophysiological and Epigenetic Markers of Fear Generalization in Anxious and Non-Anxious Depression

Catherina Wurst[1], Carolin Leistner[1], Felix Nitzschke[1], Saskia Stonawski[1], Martin Herrmann[1], Paul Pauli[2], Jürgen Deckert[1], Andreas Menke[1]

[1]Dept. of Psychiatry, Psychosomatics and Psychotherapy, University Hospital of Würzburg
[2]Dept. of Biological Psychology, Clinical Psychology and Psychotherapy, University of Würzburg

Major Depressive Disorder (MDD) is one of the most prevailing psychiatric disorders with a 1-year-prevalence of about 10%. With about 50% anxious depression is a common subtype of MDD, it is associated with high levels of anxiety and commonly defined using the anxiety/somatization factor of the Hamilton Depression Rating Scale (HAMD).
Anxious depression, compared to non-anxious depression, is associated with a poorer prognosis, worse response to antidepressants, more severe symptoms, stronger functional impairments as well as higher rates of chronification.
Variations in fear acquisition and extinction are shown to be important in the etiology of anxiety disorders. Fear generalization is a more subtle learning process, in which the stimuli, that are perceptually similar to the conditioned stimulus, also cause a certain conditioned fear reaction. However, it is not understood if fear acquisition and extinction is altered in anxious depression.
Clinical and preclinical studies have observed epigenetic alterations in depression and anxiety. In fact, fear was associated with differences in methylation patterns of stress-related genes, e.g. demethylation of the FK506 binding protein 5 (FKBP5) gene.
The aim of this study is to elicit underlying mechanisms of fear generalization in anxious and non-anxious depression.
In this case-control study we compared patients with anxious depression, non-anxious depression and healthy controls in a fear generalization paradigm. Every subject underwent a detailed psychopathologic and neuropsychological examination, using different questionnaires (e.g. CTQ, ASI, LTE, Brief Cope). We collected blood samples from every subject for the determination of RNA-expression and DNA-methylation patterns of the co-chaperone FK506 binding protein 5 (FKBP5) and the glucocorticoid receptor, nuclear receptor subfamily 3 group C member 1 (NR3C1). Afterwards, the participants completed the fear generalization paradigm. Six pictures of faces served as conditioned stimuli and generalized stimuli. The unconditioned stimuli (US) was a scream presented together with the picture of a screaming face. During the paradigm, behavioral ratings of valence and arousal of the pictures and the probability of appearance of the US were determined. Heart rate, electrodermal activity and electromyography were recorded simultaneously.
We expected, that patients with anxious depression show a stronger fear conditioning than patients with non-anxious depression. In addition, we hypothesized a stronger generalization in the anxious-depressed sample compared to the other samples. RNA-expression and DNA methylation of the stress-related genes FKBP5 and NR3C1 are analyzed to identify biological mechanisms underlying the fear extinction and generalization processes.
Preliminary data of this study will be presented and discussed.

Involvement of D1/D2 dopamine receptors within the nucleus accumbens and ventral tegmental area in the development of sensitization to antinociceptive effect of morphine [A]

Leila Zarepour [1]

[1]Iran university of medical science , Medical physiology , 26, 1476764661 Tehran, Iran

BThe nucleus accumbens (NAc) and the ventral tegmental area (VTA) are two major areas for the mesolimbic dopaminergic system which are strongly involved in the development of behavioral sensitization. In the present study, we investigated the role of D1/D2 dopaminergic receptors within the NAc or VTA in response nsensitization to morphine by the tail-flick test as a model of acute pain. Sensitization was induced by subcutaneous (SC) injection of morphine (5 mg/kg), once daily for three days followed by 5 days free of drug. After the sensitization period, antinociceptive responses induced by an ineffective dose of morphine (1 mg/kg; SC) were obtained by the tail-flick test, and represented as maximal possible effect (%MPE). In experimental groups, D1 and D2 receptor antagonists, SCH-23390 and sulpiride (0.25, 1 and 4 µg/rat), were separately microinjected into the NAc or VTA, 10 min before morphine administration during the sensitization period, respectively. Results showed that injection of morphine during the sensitization period (development of sensitization) increased %MPE of the ineffective dose of morphine from 2.43±1.4% in naive to 47.75±4.01% in sensitized animals ($P<0.001$). Unilateral microinjections of different doses of the D1/D2 receptor antagonists, SCH-23390 and sulpiride, into the NAc dose-dependently decreased %MPEs in morphine-sensitized animals. Nonetheless, %MPEs were only affected by intra-VTA administration of SCH-23390 in morphine-sensitized animals ($P<0.05$). Our findings suggest that both the D1/D2 dopamine receptors in the NAc and the D1 receptors in the VTA may be of more important in the development of sensitization to in rats.

Human stem cell-based model of alcohol use disorders

Annika Zink[1,2], Gizem Inak[2], Pawel Lisowski[2,3], Erich Wanker[2], Josef Priller[1], Alessandro Prigione[2]

[1]Charité– Universitätsmedizin, Berlin, Germany
[2]Max Delbrueck Center for Molecular Medicine (MDC), Berlin, Germany;
[3]Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Magdalenka, Poland

Alcohol use disorders (AUDs) can lead to serious health problems and represent an increasing health-care burden, as there are no efficient treatments available. Alcohol rapidly crosses the blood-brain barrier and increases the release of dopamine in the ventral striatum in humans (Boileau et al., 2003). However, the molecular mechanisms underlying this effect remain to be fully clarified, given the difficulty to study human live neurons. Recent GWAS studies identified a polymorphism in the gene KLB (ß-Klotho) associated with alcohol consumption (G. Schumann et al., 2016). KLB encodes for a transmembrane protein that functions as co-receptor for the hormone FGF21, which is secreted by the liver and regulates sweet and alcohol preference (Talukdar et al., 2016). KLB-knockout experiments in mice demonstrated that KLB expression in the brain significantly influence the FGF21 effect on alcohol drinking (G. Schumann et al., 2016).
In this project, we developed an imaging-based high content screening method to evaluate neuronal toxicity in live cells. We will apply this method to investigate the consequences of alcohol exposure on human dopaminergic (DA) neurons that we generate from human pluripotent stem cells (hPSCs) including five induced pluripotent stem cell lines (iPSCs) derived from individuals with AUD. With the use of CRISPR/Cas9 technology, we are currently knocking-out the KLB gene in hPSCs to investigate the potential contributory role of the KLB-FGF21 signaling pathway on the activity of DA neurons upon alcohol exposure. Overall, we aim to develop an in vitro model system to unveil the molecular mechanisms involved in the neuronal toxicity associated with AUD.

Brain-computer interface (BCI) based communication with the completely paralysed

Niels Birbaumer, Andres Jaramillo Gonzalez[1], Ujwal Chaudhary[1,2]

[1]University of Tuebingen, Institute of Medical Psychology, Silcherstrasse 5, 72076 Tuebingen, Germany
[2]WYSS Center for Bio and Neuroengineering, Geneva[2]

On the basis of a meta- analysis of all completely paralysed patients with Amyotrophic Lateral Sclerosis (ALS) it was concluded (Kübler and Birbaumer 2008)that brain based communication allowing flexible selection of letters and words is not possible in the completely locked in state (CLIS). We (Chaudhary et al 2017, Gallegos-Ayala et al 2014)) presented evidence in 4 patients in CLIS that non-invasive Near Infrared Spectroscopy- BCI based communication of" yes "and "no" answers to auditorily presented questions with an average precision of 70% is possible over extended time periods.
Here we present the results of several new patients some of them in CLIS and some of them between LIS (locked in state) and CLIS using a BCI measuring EEG-frequecies in these patients: all of them learned to communicate yes-no answers with brain wave features with more than 70% accuracy. Selection of letters and words was possible in LIS patients even if they are recorded during the tranfer phase from LIS to CLIS. However, fluent and reliable brain communication profits from implantation of electrodes in the cortex using spike trains and far field postentials as the data base for online feature selection and classification.
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Poster Topic

T14: Vision: Invertebrates

T14-1A Feature detection and action selection in neuronal circuits for escape and landing in *Drosophila*
Jan Marek Ache, Shigehiro Namiki, Catherine R. von Reyn, Gwyneth M Card

T14-2A Neuronal processing of polarized light presented from ventral direction in the brain of the desert locust
Marius Johannes Beck, Vanessa Althaus, Uwe Homberg, Uta Pegel

T14-3A Tetrode recordings from visual neurons in flying monarch butterflies
M. Jerome Beetz, Martin Strube-Bloss, Basil el Jundi

T14-4A Courting and Walking in the dark - Sensory depression shapes behaviour
Kristina Corthals, Miriam Berger, Bart R.H. Geurten

T14-5A The use of spectral cues for orientation in the monarch butterfly *Danaus plexippus*
Myriam Franzke, David Dreyer, Eric Warrant, Basil el Jundi

T14-1B Calcium imaging in tethered behaving honeybees
Martina Held, Hannah Haberkern, Claire Deo, Luke Lavis, Vivek Jayaraman, Keram Pfeiffer

T14-2B GABAergic and glutamatergic inhibition shape visual motion processing in *Drosophila*
Miriam Henning, Prof. Dr. Marion Silies

T14-3B Organization of the lateral complex in the brain of the desert locust *Schistocerca gregaria* – single-cell analyses and neuropil structure
Ronja Hensgen, Stefanie Jahn, Kim Schneider, Uwe Homberg

T14-4B Intracellular recordings from a time-compensated sun-compass in monarch butterflies *(Danaus plexippus)*
Tu Anh Nguyen Thi, Basil el Jundi

T14-1C Optic flow supports the representation of heading direction in the desert locust central complex
Uta Pegel, Ronny Rosner, Keram Pfeiffer, Uwe Homberg

T14-2C Dynamic properties of central complex neurons in the bumblebee
Keram Pfeiffer, Lisa Rother

T14-3C Natural stimuli for mice
Yongrong Qiu, Zhijian Zhao, Magdalena Kautzky, Frank Schaeffel, Katharina Rifai, Siegfried Wahl, Laura Busse, Thomas Euler

T14-4C A neuron type with variable receptive field properties is required for robust motion processing
Luis Giordano Ramos Traslosheros Lopez, Marion Silies

T14-1D Modality-specific circuits in the fly visual system
Gizem Sancer, Emil Kind, Juliane Uhlhorn, Thomas Mathejczyk, Mathias F Wernet

T14-2D Parallel spatial channels in hawkmoth vision?
Anna Stöckl, Keram Pfeiffer

T14-3D Preferred polarization angle of neurons at the input stage of the locust central complex
Naomi Takahashi, Frederick Zittrell, Uwe Homberg

T14-4D Receptive-Field Tuning to Celestial Polarization Patterns is Topographically Organized in the Locust Central Complex
Frederick Zittrell, Keram Pfeiffer, Uwe Homberg

Feature detection and action selection in neuronal circuits for escape and landing in *Drosophila*

Jan Marek Ache[1], Shigehiro Namiki[1,2], Catherine R. von Reyn[3], Gwyneth M Card[1]

[1]HHMI/Janelia Research Campus, 19700 Helix Drive, 20147 Ashburn, VA, USA

[2]Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

[3]School of Biomedical Engineering, Drexel University, 3141 Chestnut Street, 19129 Philadelphia, PA, USA

To survive, animals must detect and respond to sensory cues in a context-specific manner. Even innate sensorimotor responses are flexible, such that an identical cue can elicit different actions in different situations. How the brain achieves this context-dependent flexibility is unclear. In *Drosophila*, visual looming stimuli elicit an escape takeoff when the fly is standing, but a landing response when the fly is flying. To unravel the mechanisms underlying this flexibility, we dissect the neuronal circuits for escape and landing by combining in-vivo electrophysiology, behavioral genetics, and EM circuit reconstruction. We show that specific looming visual features are detected by distinct populations of visual projection neurons, and that neuronal activity is channeled into separate descending pathways for escape or landing depending on the fly's behavioral state.

The fly's takeoff sequence when escaping a looming predator is determined by the timing of a single spike in the Giant Fiber (GF) descending neuron (DN). GF Spike timing was proposed to result from summation of two visual features whose detection is highly conserved across animals: an object's angular size, and its angular velocity. Velocity encoding was previously attributed to input from LC4 visual projection neurons. Here, we show that a second population of visual projection neurons anatomically specialized to detect looming (LPLC2) provide the size component. We establish LPLC2 as necessary for GF-mediated escape, and find LPLC2 and LC4 directly presynaptic to the GF via EM reconstruction. Critically, LPLC2 silencing eliminates the size component of the GF looming response, leaving only the velocity component. A model summing a linear function of angular velocity (provided by LC4) and a Gaussian function of angular size (provided by LPLC2) fully replicated GF looming responses. Selection and timing of the escape response is therefore determined by the summation of inputs from two populations of visual feature-detecting neurons in the GF descending neuron.

To understand how the selection of escape or landing was achieved, we needed to determine at which stage of sensorimotor processing commands for the two behaviors are distinguished. To this end, we conducted an optogenetic activation screen of a collection of 130 DN lines and identified two DN types whose activation drove landing responses. Silencing either DN significantly reduced visually-evoked landing responses, suggesting they are intrinsically important to the control of landing. In-vivo patch-clamp recordings revealed that the landing DNs integrate visual and mechanosensory cues and control leg extensions in a graded fashion while the fly is flying. Critically, their visual responses are eliminated or severely attenuated when the fly is not flying. This gating occurs by separate mechanisms (neuromodulation and efference copy) in the two landing neuron types.

Descending neurons controlling landing are thus gated out when the fly is not flying, such that landing responses cannot be elicited by a looming stimulus. Giant Fiber, in contrast, is not gated by flight, enabling escape takeoff in standing flies. Our findings show that flexible sensorimotor coupling is achieved by behavioral state-dependent gating of action-specific descending pathways.

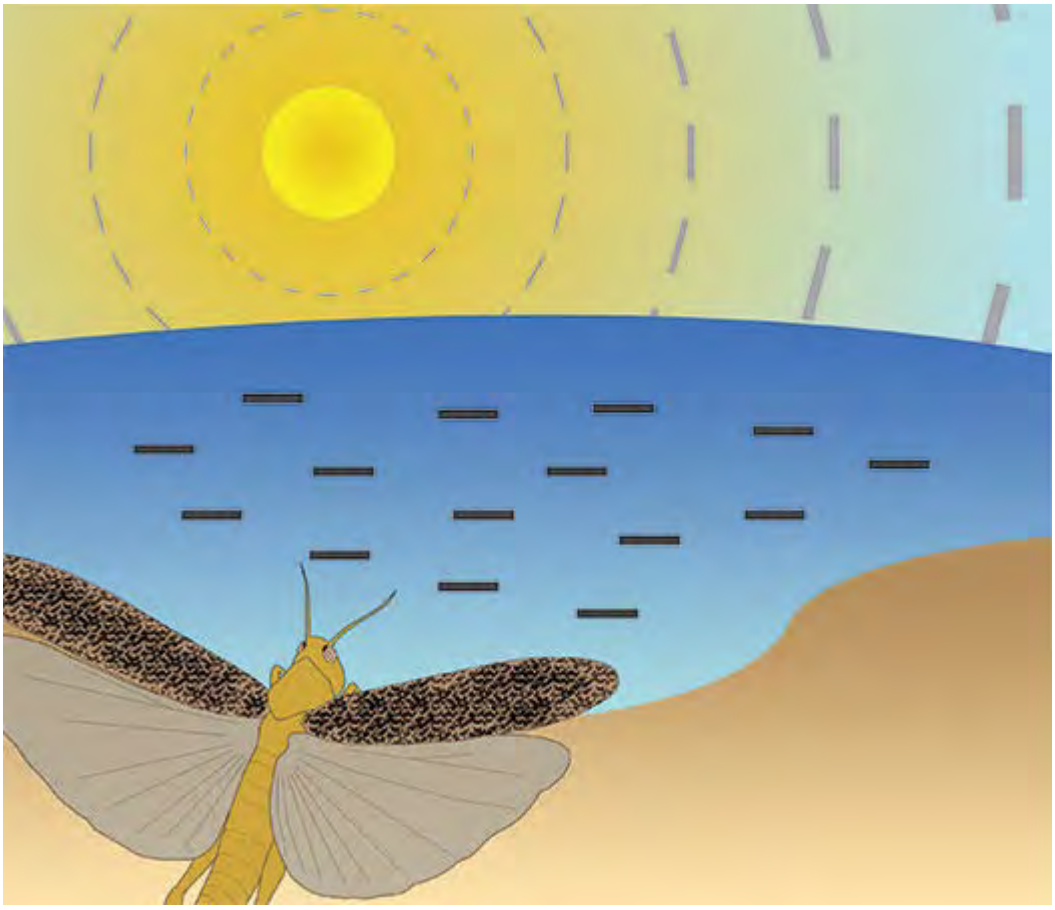
Neuronal processing of polarized light presented from ventral direction in the brain of the desert locust

Marius Johannes Beck[1], Vanessa Althaus[1], Uwe Homberg[1], Uta Pegel[2]

[1]Philipps-Universität Marburg, Animal Physiology, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany

[2]Case Western Reserve University, Department of Biology, 2080 Adelbert Road, 44106 Cleveland, USA

Insects are able to perceive and exploit polarization signals to perform a multitude of tasks. The most prominent one is spatial orientation with the aid of polarized skylight. In the sky, electric field vectors (*E*-vectors) of polarized light (grey bars in the figure) build a pattern that provides reference for solar position. The desert locust (*Schistocerca gregaria*) seasonally performs long distance migrations, likely using this pattern as a compass cue. They perceive polarized light through highly specialized photoreceptors located in the dorsal rim of their compound eyes. Several neuropils of the optic lobes and the central brain are involved in processing polarized light, including the central complex. While sky polarization is well suited for spatial orientation, polarized light generated by reflection of light on smooth surfaces like water bodies, vegetation or horsebacks is mainly exploited by insects to find back to their natural habitat (backswimmer), to find oviposition sites (dragonfly), or their host for a blood meal (tabanid flies). Insects using polarized light coming from ventral directions typically have photoreceptors suited for polarized light detection located in ventral regions of the compound eye (Heinloth et al., 2018, Front. Cell. Neurosci. 12:50). Desert locusts are less well studied in this respect. They perform course changes when flying across reflected and thus polarized light, suggesting that they use the horizontal polarization of water surfaces (horizontal black bars in the figure) to avoid flying over the sea (Shashar et al. 2005, Biol Lett 1:472). No specialized ventral eye region has been detected so far, however, neurons of the optic lobe have been shown to process polarized light that is not perceived by the dorsal rim area (Beetz et al. 2016, J Comp Physiol A 202:759). We, therefore, studied the responses of locust brain neurons to polarized light presented from ventral direction. We performed intracellular recordings from locust brain neurons, while stimulating the animal with polarized blue light presented from ventral direction. To avoid perception of polarized light by photoreceptors of the dorsal rim area, the dorsal part of the eye was covered with black paint. Neurons of the central complex involved in processing polarization from dorsal direction were insensitive to polarized light coming from ventral direction. In contrast, other cell types of the central complex responded to the stimulus. Additionally, certain neurons of the optic lobe as well as some descending neurons were sensitive to the ventral *E*-vector. Responsiveness of descending neurons suggests that polarized light perceived from ventral direction is involved in modulating motor actions that might lead to a course change whenever the animal flies across water bodies.



Tetrode recordings from visual neurons in flying monarch butterflies

M. Jerome Beetz[1], Martin Strube-Bloss[2], Basil el Jundi[1]

[1]University of Wuerzburg, Zoology II Emmy-Noether Animal Navigation Group, Am Hubland, 97074 Wuerzburg, Germany

[2]University of Wuerzburg, Zoology II, Am Hubland, 97074 Wuerzburg, Germany

Each fall, millions of monarch butterflies migrate from North America to their overwintering habitat in Central Mexico. To maintain their southerly direction, these butterflies use celestial cues, such as the sun or polarization pattern of the sky, for orientation. In the butterfly brain, a neuronal network, that is highly conserved in many insects, processes celestial compass information, with the central complex being the key processing brain region that acts as the butterfly's internal compass. As the sun changes its position over the course of a day, the butterflies need to time-compensate these changes in their compass network to ensure that they keep a constant geographical migratory direction. This raises the question of how time information is integrated in the butterfly's sky compass network.
To answer this question, we are performing long-term tetrode recordings from compass neurons in the butterfly's brain while the animal is flying. During recording, the butterflies are dorsally tethered to a holder and can freely rotate around their body axis. By simulating an ersatz sun stimulus, represented by a green light spot, we are testing how the animals' steering behavior changes over the course of a day and how this is encoded in the brain while the animal is actively navigating.

Courting and Walking in the dark - Sensory depression shapes behaviour

Kristina Corthals[1], Miriam Berger[1], Bart R.H. Geurten[1]

[1]Georg-August-Universität Göttingen, Cellular Neurobiology, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

In 1957 several *OregonR* strains were placed into darkness. We now analyse the behaviour of these flies with regard to adaptations to a dark environment. The three main behavioural adaptations that we could easily identify in exploratory experiments were changes in locomotion, courtship and female choice. Each of these behavioural adaptations could be quantified and implies adaptations in the neuronal substrate of the quantified behaviour.

Many organisms segregate their trajectories (flying, swimming or walking) in long translational bouts which are interspersed by fast rotations. Pilot studies revealed that dark-flies cease the separation of translation and rotation, while walking. This indicated a rather swift behavioural adaptation and loss of a behavioural trait depending on the ability to see. Further pilot studies showed that *Drosophila* that are deprived of light, start to adapt their locomotion within less than 10 generations. In case of courtship a gender specific evolutionary change of the sensory organs manifested, as well as a behavioural change during courtship. The presented song pattern changed in comparison with wild type con-specifics.

A pigmentation phenotype allowed us to determine a first candidate gene, which might be causal to this micro-evolution: The relative activity of the yellow enzyme has been shown to determine the occurrence of black abdominal stripes in *Drosophila*. The *abd-b* gene has been discussed as having a regulatory role on *yellow* and thus pigmentation and was shown to be mutated in dark-flies. This would explain the observed pigmentation phenotype and strikingly null mutations of the *yellow* and *abd-b* genes show reduced courtship success.

The use of spectral cues for orientation in the monarch butterfly *Danaus plexippus*

Myriam Franzke[1], David Dreyer[2], Eric Warrant[2], Basil el Jundi[1]

[1]Zoology II, Biocenter, Würzburg University, 97074 Germany
[2]Lund Vision Group, Department of Biology, Lund University, 22362 Sweden

Each fall millions of monarch butterflies (*Danaus plexippus*) migrate over thousands of kilometers from North America southwards to their overwintering habitat in Central Mexico. To maintain their migratory direction over this enormous distance, these butterflies rely on celestial cues, such as the sun and polarized skylight, as orientation references [Mouritsen and Frost, PNAS (2002); Reppert et al., Curr Biol (2004); Stalleicken et al., JEB (2005)]. In addition, a non-uniform distribution of longer and shorter wavelengths of light generates a skylight spectral gradient that could potentially be used as a compass cue by butterflies. Here, we asked whether we can test non-migrating butterflies under laboratory conditions and if they can use spectral cues for orientation. The headings of butterflies were tested individually while the animals were tethered at the center of a flight simulator. We presented spectral cues (green and/or UV light spots) to the animals while they were able to freely change their bearing with respect to the light stimuli. Even though the tested butterflies were in a non-migrating stage, they used the lights (green or UV) to keep a constant heading. When we changed the position of the visual stimuli by 180°, the butterflies changed their flight direction accordingly, suggesting that they use the presented cues for course maintenance. Further investigations will reveal if the butterflies use spectral or intensity information to keep their constant heading. Taken together, our data show that non-migrating monarch butterflies maintain a stable direction under laboratory conditions with respect to different spectral cues.

Calcium imaging in tethered behaving honeybees

Martina Held[1], Hannah Haberkern[2], Claire Deo[2], Luke Lavis[2], Vivek Jayaraman[2], Keram Pfeiffer[1]

[1]University of Wuerzburg, Biocenter Zoology II, Germany
[2]Howard Hughes Medical Institute, Janelia Research Campus, USA

Honeybees are well-known “central place foragers”, a lifestyle deriving from adaptive navigation. This remarkable sensory-guided behavior is achieved by terrestrial landmark orientation, usage of sky-compass cues and distance measurement via optic flow information. These abilities have been demonstrated in a number of behavioral studies, and the neural circuits implicated in navigation in other insects — pathways leading to and including a region called the central complex — have also been traced and identified anatomically in bees (Zeller et al. 2015, Held et al. 2016). The physiological properties of the involved neurons on the other hand are vastly unknown in this model organism. Calcium imaging has previously been used to investigate visual responses in the upper unit of the anterior optic tubercle of fixed bees to stationary light (Mota et al. 2013). In addition, multi-site local field potential recordings have been performed in the optic lobes of tethered bees that walked on an air-supported ball in an LED arena (Paulk et al. 2014).

We seek to combine the strengths of these approaches and establish targeted two-photon calcium imaging in the brain of tethered honeybees, walking on an air-supported ball in an LED arena. To this end, we label populations of neurons using bulk loading of calcium sensors. We are specifically targeting neurons branching in the lower unit complex of the anterior optic tubercle in each brain hemisphere. Anatomical studies showed that these neuropiles are segregated into five subcompartments, which could indicate a functional subdivision of spatial stimuli representation (Zeller et al. 2015). As indicator we test a new generation of synthetic calcium sensors that are masked with acetoxymethyl esters to minimize the damage of the neurons. We will discuss our ongoing efforts to record the physiological responses of neurons in this region during different tasks like single or multiple stripe fixation and optic flow presentation. Ultimately, we hope to use the paradigm to probe neural functions of honeybees during navigational behavior.

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GABAergic and glutamatergic inhibition shape visual motion processing in *Drosophila*

Miriam Henning[1], Prof. Dr. Marion Silies[1]

[1]Johannes Gutenberg-Universität Mainz, Institute for Developmental and Neurobiology, Hanns-Dieter-Hüsch-Weg 15, 55128 Mainz, Germany

Many animals use visual information and especially motion cues to navigate their environment. The underlying circuits need to compare luminance changes over space and time to compute direction selective (DS) signals. Different models have proposed how this can be achieved, relying either on a non-linear amplification of motion in the preferred direction or supression of signals that move in the non-preferred, or null direction, or combination of those models. Recent studies showed that indeed a combination of these two mechanisms is implemented in the *Drosophila* brain (Fisher et al., 2015, Leong et al., 2016, Haag et al., 2016). Using *in vivo* calcium imaging of the first DS cells of the fly visual system, T4 and T5, it was additionally shown that direction selectivity and orientation tuning require inhibitory GABAergic circuits (Fisher et al. 2015). However, loss of the $GABA_A$ receptor in DS neurons themselves does not affect DS responses, arguing that GABAergic signalling is required in upstream circuitry. Furthermore, glutamatergic inhibitory synapses might add to the mechanism of null direction inhibition. Therefore, we aimed to identify upstream inhibitory cells that are required for DS responses.
Reasoning that a loss of DS responses would lead to a loss of motion guided behaviors, we used data from a behavioural forward genetic screen in which we identified InSITE Gal4 driver lines that lead to a deficit in behavioural responses to moving stimuli, when synaptic activity is blocked in the Gal4 pattern (Gohl et al., 2011; Silies et al., 2013). Using immunohistochemistry and an intersectional strategy, we identified four GABA-ergic cell types that repeatedly occurred in the Gal4 expression patterns. We are now combining specific genetic silencing of these GABAergic neurons in combination with *in vivo* calcium imaging of motion responses in the downstream DS neurons. We are characterizing the response properties of T4 and T5 DS neurons to a series of visual stimuli, including moving bars and gratings, and spatiotemporal white noise stimuli, with or without the GABAergic neurons being active. To study the role of glutamatergic inhibition, we recorded DS neurons in a mutant of the glutamate gated chloride channel GluClα, that we recently generated. Together, we aim to describe how and where specifically inhibitory inputs shape motion responses and space-time receptive fields of T4 and T5 direction-selective cells. Our ultimate goal is to identify the inhibitory circuitry of motion computation and understand how the relative contribution of excitatory and inhibitory mechanisms establishes robust motion responses.

Organization of the lateral complex in the brain of the desert locust *Schistocerca gregaria* – single-cell analyses and neuropil structure

Ronja Hensgen[1], Stefanie Jahn[1], Kim Schneider[1], Uwe Homberg[1]

[1]Philipps-University Marburg, Animal Physiology, Karl-von-Frisch Straße 8, 35032 Marburg, Germany

The lateral complex (LX) is an assembly of bilateral neuropils in the insect protocerebrum, which plays a role in motor control related to spatial orientation. In each brain hemisphere, the LX comprises the lateral accessory lobe (LAL), the bulb (BU) and the gall (GA) (Ito et al. 2014, Neuron 81:755-65). Different types of neuron connect the LX to the central complex (CX), a group of midline-spanning neuropils, involved in sensorimotor processing (Pfeiffer and Homberg 2014, Annu Rev Entomol 59:165-84). Especially, information gathered by the visual system is highly represented by the activity of neurons that constitute the CX. A variety of these neurons send their input or output terminals to the LX, where they form connections with neurons coming from - or projecting into - other brain regions. Additionally, the complexity of computations performed by the CX, likely the transformation of multimodal sensory input into appropriate steering commands, suggests an exchange of information between different CX neuropils, which could be promoted by recurrent CX-LX connections.

To investigate the possibility of communication among polarization- and azimuth-sensitive CX-neurons (Pegel et al. 2018, J Exp Biol 221:jeb171207) in the LX, we characterized their arborization domains in the LX in detail. We focused on specific types of tangential input neurons (TL1) and columnar outputs (CP1, CP2) of the CX, whose ramification areas within the LX are largely unidentified and on CL1 columnar neurons that are known to arborize in the GA in other insect species (e.g. Wolff et al. 2015, J Comp Neurol 523:997-1037).

We performed 3D reconstructions of neurons and neuropils based on single-cell Neurobiotin injections and anti-synapsin stainings, respectively. We found that CP1 neurons arborize in a distinct region posterior to the medial bulb and that TL1 and CP2 neurons ramify in a region surrounding the lateral bulb (LBU). Arborizations of CL1 neurons were restricted mainly to a region anterior to the LBU, presumably corresponding to the GA.

Location and polarity of arborizations suggest that TL1 neurons might receive input signaling head direction both from collaterals of columnar inputs (CL1) as well as from CP2 columnar outputs and might thus serve to compare heading signals from different levels of the compass network.

Intracellular recordings from a time-compensated sun-compass in monarch butterflies *(Danaus plexippus)*

Tu Anh Nguyen Thi[1], Basil el Jundi[1]

[1]University of Würzburg, Emmy-Noether Animal Navigation Group, Zoology II, Biocenter, Am Hubland, 97074 Würzburg, Germany

The monarch butterfly *(Danaus plexippus)* is famous for its annual migration from North America to Central Mexico using a sun compass and the celestial polarization pattern as reference to maintain its southerly direction. In the butterfly's brain, these cues are processed in distinct brain areas, with the central complex acting as the internal compass for migration. How exactly the sun- and polarization-information are combined in the central complex is still unknown. In addition to these orientation cues, time of day information from the antennae is essential for the monarch butterfly to maintain its migratory direction. How celestial compass information, detected by the eyes, and time of day information from the antennae is combined in the monarch butterfly's brain is still a matter of speculation.

To test this, we are performing intracellular recordings from central-complex neurons while stimulating the animal with different combinations of simulated skylight cues in natural (polarization angle perpendicular to the ersatz sun) and non-natural (in line with the ersatz sun) constellations. Our first data suggest that the combination of different skylight cues sharpens the neural tuning of central-complex cells. This generates a robust orientation compass that allows the monarch butterfly to maintain its southerly migratory direction with high precision. In addition, we are currently performing antennal backfills and brain injections to trace the time signals into the brain and to investigate where time of day information and skylight cues converge that allows the butterfly to compensate for the daily changes of the sun position.

Optic flow supports the representation of heading direction in the desert locust central complex

Uta Pegel[1], Ronny Rosner[2], Keram Pfeiffer[3], Uwe Homberg[4]

[1]Case Western Reserve University, Department of Biology, 2080 Adelbert Road, 44106 Cleveland, USA

[2]Newcastle University, Institute of Neuroscience, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK

[3]Behavioral Physiology and Sociobiology (Zoology II), Biozentrum, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[4]Philipps-University of Marburg, Department of Biology, Animal Physiology, Karl-von-Frisch-Str. 8, 35043 Marburg, Germany

Desert locusts (Schistocerca gregaria) are well known for building large swarms and flying across long distances during seasonal migrations. To successfully perform this task these animals likely use a sky compass for spatial orientation. The most prominent compass cue in the sky is the azimuth of the sun. Whenever the sun is obscured by clouds another sky compass cue is highly relevant for orientation, the sky polarization pattern. Sky polarization is generated by scattering of sunlight in the atmosphere of the earth, building a pattern of electric field vectors (*E*-vectors) that provides reference for solar position. Both cues, polarized skylight and solar azimuth based on direct unpolarized sunlight are topographically encoded in the locust central complex (Heinze and Homberg 2007, Science 315:995; Pegel et al., 2018, J Exp Biol 221, jeb171207), an insect brain structure involved in many tasks including spatial orientation. While the processing of allothetic sky compass cues is well studied in the locust brain, the relevance of idiothetic signals like optic flow is unknown yet. We therefore studied the responses of central complex neurons to optic flow simulating either progressive motion or a rotation around the yaw axis. Five out of 12 tested neurons were responsive to progressive motion and likewise to polarized light. CX neurons adapt to a stationary *E*-vector. When the preferred stationary *E*-vector was presented together with progressive optic flow, the adapted *E*-vector response disadapted in most neurons. In contrast, optic flow was largely ignored when presented in combination with the neuron's anti-preferred *E*-vector orientation. The data suggest that optic flow as experienced during flight strengthens the animal's internal representation of head direction relative to the zenithal *E*-vector. One type of CX neuron was responsive to optic flow simulating clockwise or counterclockwise turns of the animal. The neurons responded to rotational optic flow with excitation or inhibition, dependent on rotation direction and innervated hemisphere of the CX. These neurons are suited to update the animal's internal head direction relative to a compass signal during clockwise and counterclockwise turns.

Dynamic properties of central complex neurons in the bumblebee

Keram Pfeiffer[1], Lisa Rother[1]

[1]Julius-Maximilians-Universität Würzburg, Biozentrum, Zoologie II, Am Hubland, 97074 Würzburg, Germany

Contrary to common belief, bumblebees are excellent fliers that perform highly dynamic flight maneuvers. Like many flying insect species, bumblebees stabilize the retinal image by limiting the yaw rotation of their head and body to short saccades that interrupt segments of purely translational motion. Boedekker et al. (2015, PLoS One 10 (9), e0135020) have shown that saccadic turns are initiated by a head yaw rotation, which reaches peak angular velocities of several hundreds of degrees per second. These saccadic rotations last between 30 and 130 ms and cover an angular range of up to 60°. Saccades are executed multiple times per second. The visual input resulting from this behavior creates a highly dynamic visual input into the sky-compass system in the central complex.

In electrophysiological experiments targeting sky-compass neurons in various species of insects, celestial polarization is usually mimicked using a dorsally presented polarizer that rotates at a constant velocity of 30 to 60 °/s. This kind of stimulus, however, is fundamentally different from the polarized light information that a bumblebee perceives during its highly dynamic flight. The angular velocities experienced during flight are never constant, except during straight flight, and are much higher than the ones that have been used in experimental conditions. Many experiments in different species and targeting a variety of sensory modalities have shown that naturalistic stimulation often yields neuronal responses that are markedly different from those to more artificial stimuli.

In this study, we used intracellular recordings combined with tracer injections to study the responses of sky-compass neurons in the central complex of bumblebees *(Bombus terrestris)* to highly dynamic, naturalistic stimuli. Dorsal polarized-light stimuli were generated by passing the light from a UV-LED (Nichia NVSU233A-D1, peak wavelength: 365 nm) through a polarizer that could be rotated by a stepper motor. The angular extent of the stimulus was 10 degrees and the intensity was 10^{13} photons/cm^2*s. The stepper motor was either rotated at constant velocities between 30 and 480 °/s or using the head yaw rotation angles recorded during bumblebee flight by Boedekker et al. (2015) as an input. Naturalistic stimulus sequences were repeated ten times for each recording.

We found that the preferred angle of polarization (Φ_{max}) differed between clockwise and counterclockwise rotations and was dependent on rotation velocity. During stimulation at low velocities (<100°/s) rotation from 0-180° yielded smaller Φ_{max} values than rotation from 180°-0°, i.e. the neurons showed peak firing for each rotation direction earlier compared to the opposite rotation direction (phase advance). At higher velocities, peak firing was always later in the phase (phase delay). The latter observation might be a latency effect that consumes the phase advance observed at lower velocities. Naturalistic stimulation showed that central complex neurons are well capable of coding for stimulus velocities of up to 600°/s. Particularly peak responses after an inhibitory phase showed very reliable spike timing in subsequent trials. Our data also demonstrate a strong dependence of spiking activity from stimulus history. In particular, yaw rotations away from inhibitory angles of polarization lead to strong peaks in firing, with a strong phase advance even at the highest rotation velocities. This could allow for anticipation of future spatial orientations during yaw rotation. Our new stimulus method shows for the first time the dynamics of central complex neurons to polarized light stimuli and suggests that naturalistic stimulus patterns change the tuning of the neurons based on stimulus history.

Natural stimuli for mice

Yongrong Qiu[1,2], Zhijian Zhao[1,2], Magdalena Kautzky[3], Frank Schaeffel[2], Katharina Rifai[2,4], Siegfried Wahl[2,4], Laura Busse[3], Thomas Euler[1,2]

[1]Centre for Integrative Neuroscience, University of Tübingen
[2]Institute for Ophthalmic Research, University of Tübingen
[3]Division of Neurobiology, Department Biology II, LMU Munich
[4]Zeiss Vision Science Lab, University of Tübingen

To ensure that an animal survives and procreates, it needs to interact with its environment in an optimal manner. As a consequence, each species' visual system is well-adapted to the species' natural habitat and hence to process efficiently and robustly the natural scenes it encounters. Therefore, to understand how the visual system works, it is crucial to probe it with natural scenes taken from the species habitat and to analyze the neural representations of these scenes statistically.
In the past decade, mice have become an important model in vision research, but the fact that they live in a different environment compared to primates and, thus, have different visual needs, is still rarely considered. For example, unlike primates, mice are dichromatic and perceive UV light. Specifically, their two cone photoreceptor opsins are differentially distributed along the retina's dorso-ventral axis, leading to an upper visual field (viewed by the ventral retina) sensitive to UV around 360 nm, and a lower visual field (viewed by the dorsal retina) sensitive to green light around 510 nm. Therefore, to optimally study mouse vision, not only stimuli containing UV signals are needed, also the retinal regions that are studied need to be taken into account.
To generate natural stimuli for mice and analyze the statistics of their visual environment, we set out to record spectrally correct movies from the mouse perspective outside in the field. Under the assumption that a substantial fraction of mouse eye movements serve to stabilize the retinal image (Meyer et al. bioRxiv 10.1101/294397, 2018), we started towards this goal by building a gimbal-stabilized hand-held camera that is moved close to the ground along mouse tracks to capture natural movies of the mouse habitat. The camera consists of two synchronized camera chips, each equipped with a different spectral bandpass filter matching one of the mouse cone types. One camera chip was modified to increase the chip's UV sensitivity (Wikes et at., Sensors 2016). A fisheye lens mimics the large field of view of the mouse eye. We intensity-calibrated the cameras using LEDs of defined wavelengths and brightness. Ground-truth spectra of the filmed natural environment were acquired using a spectrometric scanner, which generates a static spectral "image" with a resolution of ~10° visual angle by scanning the scene using two servos (Baden et al., Neuron 2013). After this calibration, mean camera image intensities of representative scenes (e.g. grass, sky, trees) in both channels closely matched those in images gained using the spectrometer, confirming our calibration method.
Not unexpectedly, our preliminary analysis of the captured movies revealed differential intensity distributions in the mouse's UV and green channel as a function of elevation, i.e. the position along the vertical axis in the visual field. Consistent with Baden et al. (2013), we found that the UV/green ratio increased towards the sky. Difference between the upper and the lower visual field were also observed in the contrast distribution of each channel. In addition, we analyzed other statistical properties, such as optical flow and spatial frequency distribution. Taken together, mouse-perspective natural movies reveal several anisotropies, which may have differentially shaped visual processing in the dorsal and ventral retina.

A neuron type with variable receptive field properties is required for robust motion processing

Luis Giordano Ramos Traslosheros Lopez[1,2,3], Marion Silies[2,3]

[1]International Max Planck Research School for Neurosciences, Goettingen
[2]European Neuroscience Institute - Goettingen, Goettingen
[3]Johannes Gutenberg-Universität Mainz, Institute for Developmental and Neurobiology, Mainz

Moving objects are part of the world we experience, and the ability to perceive visual motion is crucial for the survival of many organisms. Research using the fruit fly *Drosophila M.*, a powerful genetic model organism, has led to an extensive mapping of the neural substrate for motion computation. The fruit fly uses two pathways to detect moving bright (ON) and dark (OFF) edges. The ON and OFF pathways emerge from the lamina (one synapse downstream of photoreceptors) and connect through interneurons in the medulla to the first direction-selective neurons: T4 (ON) and T5 (OFF) neurons. Classical models for elementary motion detection rely in local correlations of two restricted points in space. The T4 and T5 neurons are considered the neural substrate of these correlation-type motion detectors.

In this project, we study motion detection circuits in *Drosophila*. In particular, we dissect components of a pathway with a neuron exhibiting wide-field responses under some stimulus conditions. While this neuron's responses contrast with the local correlation models, its silencing abolishes behavioral responses of the flies to dark moving edges.
We aim to understand this circuit at the level of single cell visual response properties, and to map the algorithmic steps leading to motion detection. To this end, we investigate the receptive field properties of behaviorally critical neurons for motion detection using visual stimuli and *in vivo* two-photon calcium imaging with the genetically encoded GCaMP6f sensor. We also measure downstream direction-selective neurons or optomotor responses to understand how subcomponents of a receptive field can shape neural computations and animal behavior. We will ultimately explain how behaviorally critical, complex receptive fields are shaped by presynaptic inputs. This explanation will be summarized in computational models of the receptive field organization of direction selective neurons.

Modality-specific circuits in the fly visual system

Gizem Sancer[1], Emil Kind[1], Juliane Uhlhorn[1], Thomas Mathejczyk[1], Mathias F Wernet[1]

[1]Freie Universitaet Berlin, Biologie, Koenigin-Luise Strasse 1-3, 14195 Berlin, Germany

The ~800 unit eyes (ommatidia) in the Drosophila compound eye connect to an equal number of retinotopic microcircuits in the optic lobes. Little is known about differences in cellular composition or synaptic architecture between these repetitive units. Importantly, the adult fly eye contains different ommatidial subtypes, as defined by Rhodopsin expression in their inner photoreceptors (R7 and R8). While stochastically distributed ‘pale’ and ‘yellow’ subtypes mediate colour vision, morphologically and molecularly distinct ommatidia in the ‘dorsal rim area (DRA)’ detect linearly polarized skylight and are necessary for mediating orientation responses. We study how local differences in neural circuit architecture specific to the DRA region of the optic lobes can change the computational logic of the repetitive proto-circuit, thereby shaping its modality-specific function.

Using the Drosophila neuroanatomical toolkit (MultipleColour FlpOut, GRASP, TransTango, etc), we quantify the modality-specific differences in morphology and synaptic distribution of DRA R7 and R8 cells, as well as their post-synaptic targets in the adult Drosophila medulla neuropil (e.g. Dm8 cells). We describe cellular and synaptic differences for both DRA R7 and R8 that appear to be an ideal adaptation for the detection of celestial polarization versus color. The importance of these modality-specific circuit features are now further being characterized using different behavioural assays, like virtual flight arenas for tethered flies, as well as activity imaging. Taken together, our model system serves as a platform for understanding how circuit modules are reorganized in order to adapt a given circuit to a specific function.

Parallel spatial channels in hawkmoth vision?

Anna Stöckl[1], Keram Pfeiffer[1]

[1]Universität Würzburg, Lehrstuhl für Zoologie II[1], Biozentrum, Am Hubland, 0, 97074 Würzburg, Germany

Sensory systems – both biological and artificial ones – process highly multidimensional information. In order to extract relevant features for specific tasks, they filter the complex sensory input into parallel channels and break it down into manageable portions. An important example for this in the visual system are parallel spatial channels. They help to resolve the trade-off between the spatial acuity and contrast sensitivity in the visual system in a task-specific manner. Behavioural and physiological evidence for this strategy is found in a number of vertebrate species, including humans. Their motion vision pathways sacrifice spatial resolution for high contrast sensitivity, while pattern detection pathways retain high spatial acuity, at the cost of contrast sensitivity. In insects, however, it is not clear whether such parallel spatial filters exist. We therefore investigate the spatial acuity of the hummingbird hawkmoth *Macroglossum stellatarum* in a pattern recognition and a motion vision task. Here we present preliminary results from these experiments, and compare the resulting spatial resolution to that of several stages in their visual system: the photoreceptors, lamina monopolar cells and wide-field motion neurons. This will not only show whether there is behavioural evidence for parallel spatial channels in the hawkmoth visual system, but also can provide hints for which visual pathways are responsible for these.

Preferred polarization angle of neurons at the input stage of the locust central complex

Naomi Takahashi[1], Frederick Zittrell[1], Uwe Homberg[1]

[1]Philipps-University of Marburg, Animal Physiology, Department of Biology, 35043 Marburg, Germany

Many insects rely on a celestial compass for spatial orientation. The polarization pattern of the sky is a reliable cue for skylight orientation because scattering of sunlight in the atmosphere creates a pattern of polarization in the sky that directly depends on the solar position. In the desert locust *Schistocerca gregaria*, many neurons of the central complex are sensitive to the oscillation plane of polarized light (angle of polarization). The central complex consists of four subunits: the protocerebral bridge, the upper and lower divisions of the central body and a pair of ventral noduli. The lower division of the central body (CBL) is subdivided into 6 layers from dorsal to ventral, and several types of polarization-sensitive tangential neurons provide sensory input to distinct layers of the CBL. The functional significance of this layer organization is, however, unknown. One hypothesis is that each layer receives input from a subset of tangential neurons that encode angles of polarization in a specific range. To test this hypothesis, we recorded intracellularly from individual tangential neurons during stimulation with blue light passed through a rotating polarizer from various positions in the animal's dorsal visual field and stained the neurons to reconstruct their morphology. We successfully recorded from tangential neurons arborizing in layer 2 of the CBL in several individuals. Each neuron was most sensitive to a particular angle of polarization regardless of the position of the polarized light stimulus in the dorsal visual field. However, angular preference differed between individual neurons ranging from 0° to 90° relative to the animal's body axis. The data show that at least neurons targeting layer 2 of the CBL do not share a similar preference for polarization angle, suggesting that their synaptic partners in layer 2 of the CBL receive input encoding a wide range of polarization angles.

Receptive-Field Tuning to Celestial Polarization Patterns is Topographically Organized in the Locust Central Complex

Frederick Zittrell[1], Keram Pfeiffer[2], Uwe Homberg[1]

[1]Philipps-University Marburg, Department of Animal Physiology, Karl-von-Frisch-Str. 8, 35043 Marburg, Germany
[2]University of Würzburg, Biocenter, Behavioral Physiology and Sociobiology

The desert locust *Schistocerca gregaria* is able to perceive the polarization pattern of the sky, which depends directly on the solar position and may be used to determine geographic directions.
Specialized photoreceptors in the dorsal rim area of the compound eyes are sensitive to the relative orientation of the plane of oscillation (polarization angle) of light. The central complex plays a key role in the integration of this information: Parallel polarization-vision pathways innervate the protocerebral bridge as well as the lower and upper divisions of the central body in the locust brain. Within the central complex, columnar and tangential neurons establish connections between and within the neuropils. The protocerebral bridge is innervated such that the polarization angle preferred by single neurons is represented topographically, mapping a range of 360° across the slices of the bridge. While the tuning of the involved neuron types to polarization angles presented from the zenith is relatively well known, their receptive fields regarding the whole visual field have not been determined yet.
In order to map these receptive fields, we recorded intracellularly from polarized-light sensitive neurons of the central complex while presenting blue light stimuli polarized by a rotating polarizer from different positions in the dorsal visual field of the locust. Neurobiotin tracer injection allowed for identification of neuron types and reconstruction of anatomical relationships. We found that different types of central-complex neuron show polarization-angle tunings arranged in concentric circles, matching the sky-polarization pattern of a distinct solar position. This matched-filter property theoretically allows the cells to unambiguously determine the solar azimuth from the sky-polarization pattern alone. The solar azimuth that corresponds to the best matching polarization pattern is topographically encoded by neurons of the protocerebral bridge, covering the full range of azimuthal directions. These data support the hypothesis that the central complex acts as a hub for spatial navigation that incorporates a sun compass, and provides further insight into the role of polarization vision in constituting this compass. Funded by DFG grant HO 950/24-1.

Poster Topic

T15: Vision: Retina and Subcortical Pathways

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T15-2A Mouse dLGN receives functional input from a diverse population of retinal ganglion cells with limited convergence
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An Electroretinographic Study Using Silent Substitution Stimuli
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T15-4C *In vivo* excitotoxic insult to the mouse retina causes retinal ganglion cell degeneration, optic nerve injury and vascular damage.
Annabelle Schlüter, Bahar Aksan, Eva Bindewald, Daniela Mauceri

T15-5C Binocular processing and receptive fields of motion-sensitive neurons in the zebrafish pretectum and tectum
Kun Wang, Julian Hinz, Väinö Haikala, Dierk F Reiff, Aristides B Arrenberg

T15-1D Chromatic processing in mouse retinal ganglion cells
Klaudia Szatko, Thomas Euler, Katrin Franke

T15-2D An intrinsic electroretinogram response in isolated mouse retina
Motoharu Takao, Yumi Fukuda, Takeshi Morita

T15-3D Spontaneous oscillatory networks in the outer and inner *rd10* retina
Prerna Srivastava, Luke E. Rogerson, Meng-Jung Lee, Deniz Dalkara, Günther Zeck, Philipp Berens, Thomas Euler, Timm Schubert

T15-4D Fast Axial Imaging of Glutamate Dynamics in The Mouse Inner Plexiform Layer
Zhijian Zhao, Dario Protti, Katrin Franke, Luke Rogerson, Deniz Dalkara, Timm Schubert, Thomas Euler

T15-5D A Retinal Origin of nystagmus in nyx-/- mice
Maj-Britt Hölzel, Beerend H.J. Winkelman, Marcus H. Howlett, Coen Joling, Gobinda Pangeni, Hiraki Sakuta, Masaharu Noda, Huib J. Simonsz, Maureen A. McCall, Chris de Zeeuw, Maarten Kamermans

A NOVEL TYPE OF VISUAL RESPONSES IN THE RAT SUPERIOR COLLICULUS NEURONS IS ABLE TO TRACK SLOW CHANGES IN THE ILLUMINATION LEVELS

Gytis Baranauskas[1]

[1]Lithuanian University of Health Sciences, Neuroscience Institute, Eiveniu g. 4, 50161 Kaunas, Lithuania

The superior colliculus (SC) is responsible for covert visual attention and a number of visually driven behaviors such as avoidance of dangerous objects. These functions require rapid recognition of visual objects. Integration of light fluxes over both narrow and wide areas of visual field and over brief periods of time is required for such an object recognition task. However, it is usually believed that collicular neurons have very limited spatial and temporal integration capacity. Single-unit recordings in the superficial SC layers of urethane anesthetized rats were used to characterize novel slow OFF visual responses that extend both the spatial and the temporal integration capacity of collicular neurons well beyond the limits of the previously described collicular visual responses.

It is well known that in SC neurons a bright stimulus offset triggers a transient OFF response that lasts less than 1 s. However, when the stimulus diameter exceeded 10 degrees, in many neurons the duration of OFF responses increased beyond 1 s. These long lasting OFF responses will be termed ‘slow’ to distinguish them from the fast OFF responses, which lasted much less than 1 s. A simple test permitted to clearly discriminate between these two responses: when a stimulus disappeared not abruptly but gradually, the fast but not the slow OFF response vanished completely. Moreover, the peak firing frequency of the slow OFF responses depended little on the speed of the stimulus disappearance. These data indicated that the slow OFF responses displayed unusual capacity to integrate light fluxes both over wide visual field areas and long periods of time. In fact, both the peak firing frequency and the number of evoked action potentials during the slow OFF responses correlated with the total light flux of the stimuli lasting from 50 ms to 1.5 s and varying in size from few degrees to 70 degrees in diameter. Similarly to the fast OFF responses but in contrast to the slow OFF responses, ON responses, induced by the stimulus onset, were almost unaffected by increases of the stimulus duration beyond 250 ms. The slow OFF responses persisted in the rats that had their visual cortex lesioned, indicating that the collicular slow OFF responses were independent of the cortical circuitry.

These unusual properties of the slow OFF responses indicate that they can provide information on the time-averaged illumination levels while ON and the fast OFF responses communicate the details of visual stimuli. This ability of the slow OFF responses to integrate the light flux over 1 s can be considered the simplest form of a short term memory present at very early stages in the visual pathway.

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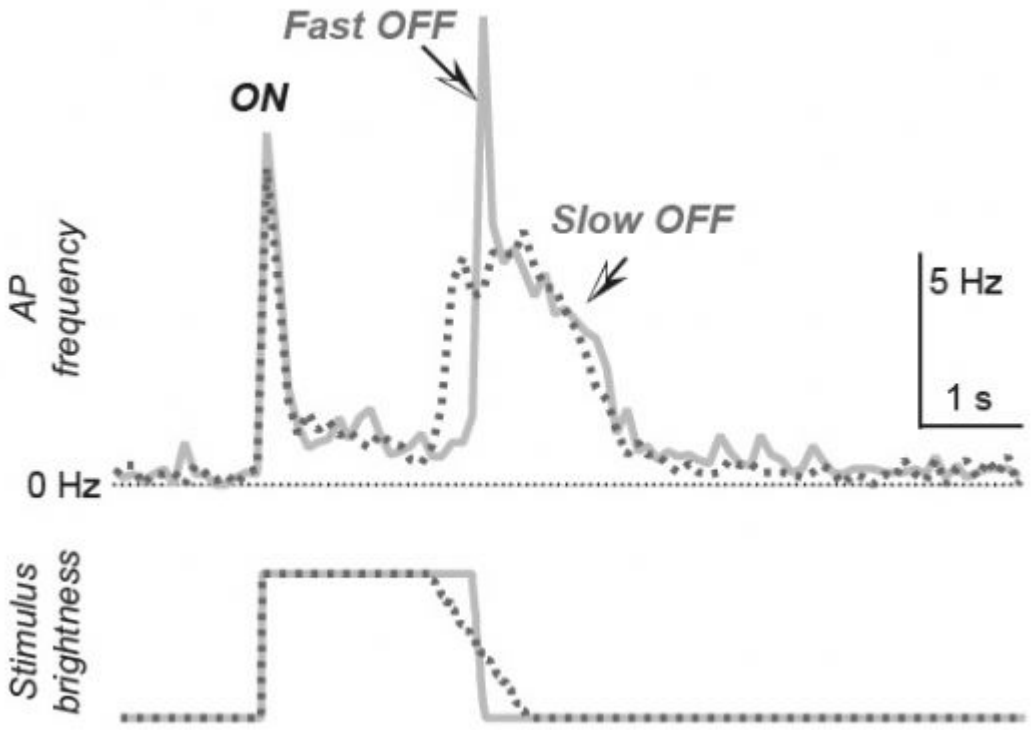
Fast OFF
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Slow OFF
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1 s
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Stimulus brightness

Mouse dLGN receives functional input from a diverse population of retinal ganglion cells with limited convergence

Yannik Bauer[1,3,5], Miroslav Roman-Roson[1,2,3,4], Ann H Kotkat[3,6], Philipp Berens[1,2,7], Thomas Euler[1,2,7], Laura Busse[1,3,7,8]

[1]Centre for Integrative Neuroscience, University of Tübingen, 72076 Tübingen, Germany
[2]Institute for Ophthalmic Research, University Hospital Tübingen, 72076 Tübingen, Germany
[3]Division of Neurobiology, Department Biology II, LMU Munich, 82151 Munich, Germany
[4]Graduate School of Neural & Behavioural Sciences | International Max Planck Research School, University of Tübingen, 72074 Tübingen, Germany
[5]Graduate School of Systemic Neuroscience (GSN), LMU Munich, 82151 Munich, Germany
[6]ENB Elite Master of Science program in Neuroengineering, Technical University of Munich, 80333 Munich, Germany
[7]Bernstein Centre for Computational Neuroscience, 72076 Tübingen, Germany
[8]Bernstein Centre for Computational Neuroscience, 82151 Munich, Germany

In the mouse, the parallel output of more than 30 functional types of retinal ganglion cells (RGCs) serves as the basis for all further visual processing. Little is known about how the representation of visual information changes between the retina and the dorsolateral geniculate nucleus (dLGN) of the thalamus, the main relay station between the retina and cortex. Interest in these questions has been fueled by recent estimates of retinogeniculate convergence obtained by anatomical work, which far exceeded those obtained in electrophysiological recordings.

To get insights into the nature of retinal input to dLGN, we conditionally expressed the genetically encoded Ca2+ indicator GCaMP6f in dLGN-projecting (dLGN-p) RGCs, followed by in vitro retinal two-photon Ca2+ imaging of light-evoked responses. Using the same stimulus set as an earlier RGC classification by Baden et al. (Nature 2016), we compared the responses of each dLGN-p RGC to those of the previously described RGC types and identified the RGC population cluster with the best-matching response properties. We found that most functional RGC types seem to innervate dLGN, with certain types, such as ON- and OFF alpha cells or OFF contrast-suppressed cells, showing clear overrepresentations.

Using in vivo extracellular multi-electrode recordings in awake, head-fixed mice, we then recorded the responses of dLGN neurons to the same visual stimuli. We quantitatively assessed the degree of diversity in the dLGN responses by using cross-validated non-negative matrix factorization (NNMF), which decomposed the dLGN population response into a rich and highly diverse set of ca. 30 response components.

Finally, using a linear model to assess functional connectivity between RGC types and dLGN neurons, we found that the responses of dLGN neurons could be predicted as a linear combination of inputs from on average five RGC types, but only two of those had the strongest functional impact.

In summary, our study reveals that most mouse RGC types project to the dLGN, which yields an unexpectedly diverse representation that can be reconstructed by a feedforward model revealing limited RGC input convergence.

Visual response properties of mouse TRN are consistent with its potential role for feedback-mediated surround suppression

Gregory Born[1,2], Martin A. Spacek[1], Chu Lan Lao[3], Laura Busse[1,4]

[1]Division of Neurobiology, Department Biology II, Ludwig-Maximilians-Universität München, Germany
[2]Graduate School for Systemic Neurosciences, Ludwig-Maximilians-Universität München, Germany
[3]Institute of Physiological Genomics, Viral Vector Facility, Ludwig-Maximilians-Universität München, Germany
[4]Bernstein Center for Computational Neuroscience Munich, Germany

Neurons in the dorsolateral geniculate nucleus (dLGN) of the thalamus are suppressed by stimuli extending beyond the classical receptive field into the surround. Surround suppression increases between retinal ganglion cells and dLGN, where it has been hypothesized that corticothalamic (CT) feedback from layer 6 (L6) contributes to this enhancement (Andolina et al., 2012). Because L6 CT neurons are excitatory they can inhibit thalamic relay cells only indirectly via local geniculate interneurons or inhibitory neurons in the thalamic reticular nucleus (TRN). We hypothesized that if neurons in TRN were responsible for mediating feedback-induced surround suppression in dLGN, they should have large receptive fields (RF) and be suppressed if CT feedback is disrupted.

We tested this hypothesis by head-fixing C57BL/6 mice on a floating Styrofoam ball and recording extracellular single-unit activity using high-density silicon probes in the visual part of TRN (perigeniculate nucleus, PGN). For post-mortem confirmation of our recording site, we injected a retrograde AAV into dLGN leading in connected PGN neurons to the expression of green fluorescent protein. We presented full-field drifting gratings to identify visually responsive neurons and mapped their RFs with sparse noise stimuli. We measured surround suppression by showing drifting gratings of varying sizes.

We observed that activity in PGN was strongly modulated by behavioral state. During locomotion periods, visually evoked responses were higher (mean = 32.74 Hz) than visual responses elicited during stationary periods (mean = 25.4 Hz, $p < 10^{-3}$). Thus, we restricted our analysis to trials, in which the animal was sitting. Numerous PGN neurons showed visually evoked responses at the temporal frequency of the drifting grating. Overall, PGN neurons had large RFs (mean = 514.8 deg²), substantially exceeding the size of those in dLGN (mean = 224.4 deg², $p < 10^{-4}$). The temporal component of PGN RFs peaked later compared to the RFs of dLGN cells (89.6 ms vs. 80.8 ms, $p = 0.05$). PGN RFs exhibited a rough topographic mapping, where units that resided more ventrally in the PGN tended to have lower and more medial RF centers. Examining responses to varying stimulus sizes revealed that the majority of PGN neurons exhibited weak suppression strengths.

Next, we tested how PGN responses are affected by CT feedback. In visual cortex (V1) of PV-Cre mice we used a viral approach to conditionally express Channelrhodopsin-2 in parvalbumin positive interneurons. Activating these neurons allowed us to suppress V1 activity and hence disrupt CT feedback to PGN. Suppression of CT feedback led to a substantial reduction of PGN responses (26.4 Hz vs. 11.07 Hz, $p < 10^{-4}$).

We conclude that PGN, given its large RFs and its suppression during disruptions of CT feedback, might play an important role for feedback-mediated surround suppression in dLGN.

Expression of Sox2 in the Visual System of Fish

Laura deOliveira-Mello[1], Juan Manuel Lara Pradas[1], Almudena Velasco Arranz[1], Rosário Arévalo Arévalo[1], Andreas F. Mack[2]

[1]Institute of Neuroscience of Castilla y León – University of Salamanca. SALAMANCA, Spain
[2]Institute of Clinical Anatomy and Cell Analysis – University of Tübingen. TÜBINGEN, Germany

The visual system of teleost fish is known for its continuous growth and high regeneration capacity but the role of the different cell types involved and the processes occurring to make this possible, are not fully understood. Thus, the retina and optic nerve (ON) of adult fish are important models for studying neurogenesis and regeneration in the vertebrate central nervous system (CNS).
Our study focused on the one hand on glial cells as master candidates involved in both processes, continuous growth and regeneration of the vertebrate CNS. Previous studies have demonstrated the participation of astrocytes and oligodendrocytes, and the glial cells of teleost fish have been postulated as a model to design palliative or regenerative cellular therapies of ON dystrophies of mammals.
On the other hand, we were interested in the expression of Sox2, a transcription factor known for its primary function in stem cell proliferation, which is also established as a regulator of cell fate decision during development, especially in the visual system. The absence of Sox2 in embryonic development causes microphtalmia or anophtalmia. In addition, several reports suggest that the Sox2 plays a role in adult tissue homeostasis and regeneration, not only in the CNS but also in other tissues. Our goal with this study was to identify cell types expressing Sox2 in the visual system of teleost fish: retina, optic nerve head (ONH) and ON. For this, we used three different fish species each with a particular advantage: *Danio rerio*, *Astalotilapia burtoni* and *Carassius auratus*. Staining retina and ON cryosections with antibodies for Sox2, we found many positive cells in retinal layers and in the ON. Surprisingly, and unlike the other tissues, we did not find any Sox2 expression in the ONH. For cell identification, we used co-localization with antibodies for cell proliferation (PCNA), Müller and astroglial cells (GFA and GS), amacrine cells (parvalbumin: PV, ChaT, calretinin: CR) and in addition, Sox10 transgenic fish to identify olgodendroglial cells. Moreover, we used antibodies for doublecortin (DCX) and Neurolin (Zn8) to identify new and/or growing axons.
We showed co-localization of Sox2 and PCNA in the peripheral growth zone and retinal fiber layer, but not in the ON. We identified Müller cells and astrocytes in the ON positive for Sox2 by GFAP and GS immune-detection. We observed some Sox10 positive cells double-labeled with Sox2 in retina and in the ON, but it is not clear whether these cells are oligodendrocytes or precursor cells. For the first time we found that several amacrine cells identified by PV and CR immune detection, were also positive for Sox2. ChaT amacrine cells were negative for Sox2. Analyzing the possible relationship between Sox2 positive cells and new axons stained with DCX and Zn8, we found many cells apparently very closely associated with new axons in the retinal NFL and the ON. Strikingly, in ONH where new axons are topographically rearranged, no Sox2 positive cells were present. We conclude that Sox2 is expressed in a variety of retinal and ON cell types with apparently different functions: First, it has likely a stem cell-maintaining function in proliferating cells in the retina and ON. Second, Sox2 is expressed in glial cells which have a function in facilitating axonal growth and myelination. Finally, subtypes of amacrine cells express Sox2 where it serves likely a function different from cell production and differentiation.

Signal transmission at invaginating cone photoreceptor synaptic contacts following deletion of the presynaptic cytomatrix protein bassoon in mouse retina

Andreas Feigenspan[1], Norbert Babai[1], Kaspar Gierke[1], Hanna Regus-Leidig[1], Johann Helmut Brandstätter[1]

[1]Friedrich-Alexander-Universität Erlangen-Nürnberg, Biology, Staudtstrasse 5, 91058 Erlangen, Germany

Aim

A key feature of the mammalian retina is the segregation of visual information in parallel pathways, starting at the photoreceptor terminals. Cone photoreceptors establish synaptic contacts at two different synaptic specializations: at invaginating, ribbon-containing synaptic sites with On bipolar and horizontal cells and at flat, non-ribbon-containing synaptic sites with Off bipolar cells. The cytomatrix protein bassoon anchors ribbons at the active zone, and its absence induces detachment of ribbons from the active zone. The aim of this study was to investigate the impact of a missing ribbon on synaptic transmission at the first synapse of the visual system.

Methods

Release properties of cones were studied in wild-type and mutant mouse retinae with a genetic disruption of the presynaptic cytomatrix protein Bassoon using whole-cell voltage-clamp recordings. Ultrastructural analysis revealed the number and location of synaptic vesicles in both mouse lines.

Results

Whole-cell recordings from postsynaptic horizontal cells of the two mouse lines showed that the presence of Bassoon and a ribbon enhanced the rate of exocytosis during tonic and evoked release by increasing synaptic vesicle pool size and replenishment rate, while at the same time slowing vesicle release. Furthermore, the number of synaptic vesicles was significantly higher at ribbon-containing than at non-ribbon-containing synaptic sites.

Conclusion

The results of our study demonstrate that glutamate release from cone photoreceptor terminals can occur independent of a synaptic ribbon, but seems restricted to active zones, and they emphasize the importance of a synaptic ribbon for sustained and spatially and temporally synchronized neurotransmitter release.

Functional Binocular Convergence in the Retinogeniculate Pathway

Martin Hans Peter Fernholz[1], Simon Weiler[1], Joel Bauer[1], Juliane Jäpel[1,2], Volker Scheuss[1,3], Mark Hübener[1], Tobias Bonhoeffer[1], Tobias Rose[1]

[1]Max Planck Society, Bonhoeffer, Am Klopferspitz 18, 82152 Martinsried, Germany
[2]Current address: Max Planck Florida Institute for Neuroscience, Jupiter, 1 Max Planck Way, Florida 33458, USA
[3]Current address: Ludwig-Maximilians-Universität München, Großhaderner Str. 9, 82152 Martinsried, Germany

The dorsal lateral geniculate nucleus (dLGN) is believed to forward the activity of specific retinal ganglion cells (RGCs) via parallel, eye-specific, and largely unmodified visual *processing* streams to primary visual cortex (V1). This is supported by experiments showing that only a small number of RGCs contribute to the firing of a thalamocortical (TC) neuron[1]. In addition, *in vitro* evidence suggests that functional binocular convergence is largely eliminated during the first postnatal weeks[2]. In mice, this view has been challenged by recent trans-synaptic tracing experiments showing frequent binocular convergence of RGCs onto individual TC neurons[3]. Similarly, we[4] and others[5,6] recently found that a fraction of neurons in the dLGN of adult mice respond to visual stimulation of both eyes *in vivo*. Moreover, in distinct contrast to prevailing models of dLGN function, we showed that the eye-specific responses of TC neurons undergo prominent experience-dependent plasticity[4,6].

Here we address this apparent mismatch between structural tracing data, functional *in vitro* results, and recent *in vivo* recordings by directly mapping cellular and subcellular functional binocular convergence onto individual TC neurons in dLGN brain slices of adult mice. To this end, we established a novel optogenetic approach for cross-talk free quantification of eye-specific TC neuron responses by dual-color, channelrhodopsin-assisted circuit mapping (2CRACM) using the red- and blue-light excitable opsins ChrimsonR and Chronos after differential viral transduction of RGCs in both retinae. We find, in contrast to previous *in vitro* approaches[2], that a significant fraction of TC neurons in the dLGN of adult mice receive robust binocular RGC input, albeit with a variable contribution of AMPA- and NMDA-receptor mediated currents. Local patterned dual-color stimulation of short RGC axon stretches (subcellular 2CRACM) revealed that binocular TC neurons in the contralateral eye projection zone receive ipsilateral input if their dendrites cross over into the ipsilateral termination patch in the dLGN core, and vice versa. However, in accordance with our *in vivo* data[4], we find that the majority of TC cells in the dLGN segment putatively projecting to binocular V1 receive monocular input. This includes recently contested[3,5,6] purely ipsilateral responses in the ipsilateral RGC projection patch. We conclude that previous recordings have underestimated the degree of functional binocular convergence onto mouse TC neurons, potentially because of the known difficulty to maintain ipsilateral RGC axons in optic-tract dLGN slices[1]. Our data now provide the groundwork to address if functional retinogeniculate binocularity is indeed modified in paradigms of experience-dependent plasticity like ocular dominance plasticity after monocular deprivation[4]. Importantly, our approach allows answering this question in isolation from potentially confounding corticothalamic feedback.

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Adaptation in Mouse Rods and Cones *In Vivo*: An Electroretinographic Study Using Silent Substitution Stimuli

Anneka Joachimsthaler[1,2], Jan Kremers[1]

[1]University Hospital Erlangen, Dept. of Ophthalmology, 91054 Erlangen, Germany
[2]FAU Erlangen-Nürnberg, Dept. of Biology, Animal Physiology, 91058 Erlangen, Germany

Purpose: To study rod- and cone-driven adaptation dynamics separately, we used the silent substitution technique to selectively stimulate rods or cones in the Opn1lw^{LIAIS} (LIAIS) mouse, in which the native M-cone pigment is replaced by a human L-cone pigment (L*).
Method: ERG recordings were performed on anesthetized LIAIS mice. Stimuli were sinusoidal modulations stimulating selectively either the L*-cones or the rods. Additionally, luminance stimuli were employed. Procedure: After 10 minutes adaptation to 0.4 cd/m² ERGs were measured, followed by 11 min adaptation to 8.8 cd/m² background and recordings directly after the luminance increase and subsequently every 2nd minute. Finally during adaptation to 0.4 cd/m² for 32 minutes, ERG responses were recorded directly after the change in background and every 2nd minute. This protocol was repeated with rod-isolating stimuli (8 Hz; 75% rod contrast), L*-cone-isolating stimuli (12 Hz; 55% cone contrast) and with luminance stimuli (8 Hz and 12 Hz; 100% Michelson contrast).
Results: At 8.8 cd/m², responses directly displayed photopic response properties without further changes neither in cone nor in luminance responses. Rod-driven responses were very small. After the return to 0.4 cd/m² both rod-driven and luminance responses increased over a time course of about 30 minutes. Cone-driven responses were very small and showed no changes due to adaptation. Response phases changed directly after a change in background without further substantial alterations.
Conclusion: Rod- and cone-driven signal pathways display strongly different adaptation characteristics: Adaptation of cone-driven responses to photopic conditions is instantaneous, whereas rod-driven responses change with a time course up to 30 minutes during scotopic conditions. Luminance responses are cone-driven at 8.8 cd/m² and rod-driven at 0.4 cd/m².

Combining two photon microscopy and CMOS MEA to uncover the mechanism of aberrant activity in blind retina

Meng-Jung Lee[1,2,3], Prerna Srivastava[3,4,5], Luke Rogerson[3,4,5], Philipp Berens[4,5,6], Thomas Euler[4,5,6], Timm Schubert[4,5], Günther Zeck[1]

[1]NMI, Neurophysics, Reutlingen, Germany
[2]Multi Channel Systems MCS GmbH, Reutlingen, Germany
[3]Graduate Training Centre of Neuroscience, University of Tübingen, Germany
[4]Werner Reichardt Centre for Integrative Neuroscience (CIN), University of Tübingen , Germany
[5]Institute for Ophthalmic Research, University of Tübingen, Germany
[6]Bernstein Center for Computational Neuroscience, University of Tübingen, Germany

Motivation
The photoreceptor-degenerated retina shows intrinsic oscillations along with the increased spontaneous activity in different cell layers. Understanding the origin and underlying cellular mechanisms of these oscillations could help developing strategies to treat diseases such as Retinitis Pigmentosa and age-related macular degeneration. Here, we present the simultaneous recording of synaptic glutamate signals in the inner and outer retina using two-photon imaging and of action potentials from retinal ganglion cells (RGCs) using high-density CMOS based microelectrode arrays (CMOS MEAs).

Material and Methods
Retinas from 3-4 month old photoreceptor-degenerated rd10 mice were used in this study. The AAV-encoded iGluSnFR biosensor was injected intravitreally into the mouse eye 3-4 weeks prior to the dissection of the retina for the glutamate imaging. The retina expressing the biosensor was placed RGCs down onto a poly-lysine coated CMOS-based MEA (CMOS 5000, Multi Channel Systems MCS) with a 1 mm2 sensitive area. The two photon recording was performed in a local area of 71.5 x 26.8 µm for xy-scans and 47.6 x 11.9 µm for imaging at different planes using an excitation laser tuned to 927 nm (Mai Tai DeepSee HP; Spectra Psychics). Data acquired from two photon imaging was analyzed with IGOR Pro, while spikes from RGCs were first sorted with CMOS-MEA-Tools and then further analyzed with Matlab.

Results
First, we solved the main technical challenge of simultaneous MEA recording during two-photon imaging by applying a reset paradigm which removed the electrical artifact caused by the scanning laser beam in the sensitive electronic structures. It allowed us to record action potentials from RGCs simultaneously with the two photon imaging of synaptic glutamate signals in the outer and inner plexiform layers. Inhomogeneous oscillation frequency of the RGCs across the retina was observed, instead they varied within a range of 4-10 Hz. Furthermore, the oscillation frequency of RGCs changed after applying the mGluR6 agonist L-AP4, indicating that the remnant photoreceptors modulate the aberrant activity in the rd10 retina. Similar changes could be observed by adding strychnine, showing that glycine receptors also play a role in the intrinsic oscillation activity. Simultaneously recording from the inner and outer plexiform layers and the RGCs may give us more insights of how the aberrant activity is modulated the in the rd10 retina in both vertical and horizontal direction.

Conclusion
Our results demonstrate that two-photon imaging can be performed together with CMOS-MEA based

extracellular recordings to study vertical signaling in the degenerated retinas. This combination balances strengths and weaknesses of both recording techniques, offering a more complete and comprehensive way to study neuronal networks.

Chromatic Processing at the Mouse Photoreceptor Synapse

Maria Magdalini Korympidou[1,2,4], Deniz Dalkara[5], Thomas Euler[1,2,3], Timm Schubert[1,2], Katrin Franke[1,2,3]

[1]Center for Integrative Neuroscience, Tübingen University
[2]Institute for Ophthalmic Research, Tübingen University
[3]Bernstein Centre for Computational Neuroscience, Tübingen University
[4]Graduate School of Cellular & Molecular Neuroscience, Tübingen University
[5]Institut de la Vision, INSERM, CNRS, Sorbonne Université

The basis of color vision emerges at the very first stage of the visual pathway, the retina. Here, chromatic signals originating from different photoreceptor types sensitive to different wavelengths are locally compared by retinal circuits. Mice, as dichromatic mammals, express the short- (S; “blue”) and the medium- wavelength (M; “green”) sensitive opsins. While “true” S-cones, exclusively expressing S-opsin, are homogeneously distributed across the retina (Haverkamp et al. J Neurosci 2005), M-cones co-express S-opsin with an increasing gradient towards the ventral edge (Röchlich et al. Neuron 1994; Applebury et al. Neuron 2000). This results in a green-sensitive dorsal and a blue-sensitive ventral retina. Due to this uneven opsin distribution (especially in the ventral retina), it was unclear whether mice can extract chromatic information. However, previous behavioral studies demonstrated that mice are able to discriminate colors (Jacobs et al. Vision Research 2004) – at least in the upper visual field (Denman et al. eLife 2017), which corresponds to the ventral retina. In line with this, color-opponency has been reported in distinct types of mouse retinal ganglion cells (Chang et al. Neuron 2013; Stabio et al. Neuron 2018), also in the ventral retina, where green-sensitive rod photoreceptors likely play an important role (e.g. Joesch & Meister Nature 2016). However, it is still not well understood where this color-opponency arises within the retinal circuit.

Here, we studied mouse cone responses to chromatic stimuli by two-photon imaging of glutamate release from their axon terminals in the whole-mounted retina. We found that color-opponency is already present in the cone output, but varies along the dorso-ventral axis: Ventrally, where the S-opsin is predominantly expressed, cones possessed an antagonistic green-ON surround. Dorsally, however, the chromatic tuning of the surround was not as clear cut; instead, many cones possessed surrounds that elicited ON responses both to green and UV stimuli. Blocking horizontal cell function pharmacologically eliminated the surround responses suggesting that lateral inhibition provided by horizontal cells is involved in the generation of cone color-opponent signals. In conclusion, our findings support the existence of rod-cone color-opponency in the ventral mouse retina, suggesting that the green-sensitive rods create the antagonistic surround of UV-sensitive cones via horizontal cells (Joesch & Meister Nature 2016).

Natural stimuli reveal a spectrum of spatial encoding across the output channels of the retina

Dimokratis Karamanlis[1,2,3], Tim Gollisch[1,2]

[1]Department of Ophthalmology, University Medical Center Göttingen, Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience Göttingen, Göttingen, Germany
[3]International Max Planck Research School for Neurosciences, Göttingen, Germany

The visual system routinely encounters natural scenes that contain fine spatial structure. Standard models of sensory processing assume that early visual neurons encode just the average light intensity in their receptive fields, ignoring any spatial detail. Such linear models may capture retinal ganglion cell responses to artificial stimuli, but often fail for natural stimuli. A potential reason is the nonlinear processing of light intensities in the receptive field. However, it has been challenging to link receptive field nonlinearities and linear model failure with natural stimuli, because of the diversity of functional properties between ganglion cell types (>30 in the mouse). To address this problem, we recorded the spiking activity of ganglion cells with multielectrode arrays from isolated mouse retinas and built linear receptive field models for hundreds of cells. We compared the predictions of such models to measured cell responses under natural image flashes. Linear models were able to completely capture responses for some cells (linear), but failed for a significant proportion of cells (nonlinear). When the receptive fields of nonlinear cells sampled image patches with rich spatial structure, the cells responded stronger than expected from a linear model. Additionally, we measured response changes when natural images were systematically altered by blurring. While the responses of linear cells barely changed, the responses of nonlinear cells diminished with a scale on the order of the receptive field size of their presynaptic neurons, bipolar cells. Using an artificial stimulus with two spatially separate light intensities, we showed that nonlinear cells strongly rectified their non-preferred intensities. Finally, we associated model performance with other cell-type specific properties, such as direction selectivity, and identified a known ganglion cell type that is particularly sensitive to spatially homogeneous stimuli. Our investigations establish the link between natural scene processing and receptive field nonlinearities in diverse populations of retinal ganglion cells. Thus, our approach may offer insights towards more complete encoding models and better understanding of cell type diversity in the context of natural stimuli.

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Spherical stimulus arena reveals precise dependence of optokinetic response gain on stimulus location across entire visual field of larval zebrafish

Rebecca Meier[1], Florian A. Dehmelt[1], Julian Hinz[1,2], Clara A. Simacek[1], Takeshi Yoshimatsu[4], Kun Wang[1,2], Väinö Haikala[3], Dierk Reiff[3], Tom Baden[4], Aristides B. Arrenberg[1]

[1] Werner Reichardt Centre for Integrative Neuroscience & Institute for Neurobiology, University of Tübingen, D-72076 Tübingen, Germany;

[2] Graduate Training Centre for Neuroscience, Tübingen, Germany;

[3] Animal Physiology, Institute for Biology I, University of Freiburg, Hauptstr. 1, D-79104 Freiburg, Germany + present address: Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland;

[4] School of Life Sciences, University of Sussex, UK

Experiments on visually evoked behavior often sample only part of the visual field. Whole-field visual stimulation has already been developed for a number of model organisms, but not zebrafish. Zebrafish have large visual fields, spanning about 160° in azimuth for each larval eye. We here present a spherical stimulus arena with 14,848 LEDs covering almost the entire visual field of immobilized zebrafish larvae. LED tiles were assembled using a 3D-printed scaffold, are operated by microcontrollers, and visual stimulation can be temporally interleaved with calcium indicator fluorescence recording using laser scanning microscopes. Mounting fish in a water-filled glass bulb on a rotation mount within the arena, we can stimulate animals with large stimuli covering up to 75% of the spherical surface. This is particularly relevant to studies of whole-field gaze stabilization behavior, such as the optokinetic response (OKR) and the optomotor response (OMR). We are able to measure the precise gain of the OKR at different stimulus positions relative to the fish, and with different stimulus sizes and spatial frequencies. We discovered that zebrafish larvae react most strongly and consistently to stimuli located laterally and slightly above the equator, and also investigate whether responses are asymmetric between the eyes. The OKR gain depends on the logarithm of stimulus area in a sigmoid fashion. We compare behavioral data to a dataset of photoreceptor densities in the larval retina to judge how photoreceptor density correlates with behavioral performance, and we furthermore characterize potential extra-retinal factors. In combination with neurophysiological studies on optic flow processing in visual brain areas, our results will improve our understanding of the sensory neural pathways underlying optomotor and optokinetic responses in zebrafish.

Towards spatio-temporal optogenetic threshold maps in blind retinas

Miriam Reh[1], Meng-Jung Lee[1], Martin Kriebel[1], Günther Zeck[1]

[1]NMI, Neurophysics, Markwiesenstr. 55, 72770 Reutlingen, Germany

Retinitis pigmentosa, an inheredited disorder leading to photoreceptor degeneration, affects nearly 2 million people worldwide. A series of promising attempts for partial vision restoration exist, which comprise retinal implants, gene-replacement therapy, stem cell transplantation and optogenetic stimulation. One important factor in electrical and optogenetic stimulation is the quantitative evaluation of the spatial selectivity when assessing the potential of axonal stimulation. While for electrical stimulation temporally and spatially resolved thresholds have been reported these parameters are still to be investigated for optogenetic stimulation.

Here we explore the evoked activity of optogenetic stimulation of opsin-expressing retinal ganglion cells with CMOS-based high-density microelectrode arrays (CMOS MEAs).
Experiments are performed with ex vivo retina from two transgenic mouse lines, both of them expressing ChR2-EYFP under the control of the endogenous parvalbumine promoter in a subset of retinal ganglion cell types. While in the first line photoreceptors were functional, the second line was obtained by additional intercrossing with rd10.

Customized spatial and temporal light patterns were created using a combination of selectable LEDs and a digital mirror device (DMD). Spike sorting of the recorded activity is performed based on a convolutive ICA approach [Leibig C. et al. 2016].

We first estimated the temporal filters (mean effective stimuli) of retinal ganglion cells upon white-noise light stimulation in the photopic regime and upon optogenetic stimulation. Upon light stimulation temporal filters revealed ON or OFF polarity with a mean time-to-peak of 50 ms. All optogenetically derived temporal filters were much shorter (~10ms) and showed ON-type polarity only. In ongoing work we classify amplitude differences of the temporal filters for ON and OFF RGC classes.

Secondly, we investigated the spatial selectivity of optogenetic stimulation using rectangular stimuli presented at different positions with respect to the cell soma. Preliminary results indicate a better spatial selectivity of optogenetic stimulation as compared to electrical stimulation. In ongoing experiments we increase the irradiance to achieve a full spatial activation map and spatially resolved stimulation thresholds.

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In vivo excitotoxic insult to the mouse retina causes retinal ganglion cell degeneration, optic nerve injury and vascular damage.

Annabelle Schlüter[1], Bahar Aksan[1], Eva Bindewald[1], Daniela Mauceri[1]

[1]Heidelberg University, IZN Neurobiology, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany

Neurodegenerative disorders are marked by loss of neurons within the central nervous system displaying diverse clinical features that depend on the particular CNS region involved. For the visual system, glaucoma has major clinical relevance since it is a group of eye diseases characterized by diverse etiopathologies and one of the most common causes of blindness in the world. Visual processing starts in the retina where retinal ganglion cells (RGCs) receive visual information from photoreceptors for transmission to the visual centers of the brain. During glaucoma, RGCs and their axons forming the optic nerve progressively degenerate resulting in the loss of vision. The underlying mechanisms are diverse and include oxidative stress, glial activation, neurotrophin deficiency, ischemia, vascular injury, and excitotoxicity. Excitotoxicity leads to neuronal death by increased calcium influx triggered by high glutamate or N-methyl-D-aspartate (NMDA) levels. Recent studies also show that the neurovascular interdependence plays a roles not only in physiology but also in several pathologies of the visual system such as glaucoma. For example, it has been shown that vascular occlusion and damage can contribute to the progression of neuronal degeneration in the retina. However, little is known about the reverse direction, thus the impact of RGC degeneration on the retinal microvasculature.

Here, we used in vivo NMDA-induced excitotoxicity as a mouse model for glaucoma to investigate the integrity of RGCs and the retinal capillary network. Moreover, we characterized epigenetic factors such as histone deacetylases (HDACs) and gene transcription. First, we could show that in a dose-dependent manner, a single intravitreal NMDA injection caused rapid RGC degeneration and damage to the retinal microvasculature. During excitotoxic events, the subcellular HDACs localization in RGCs and retinal gene transcription was altered. Further, excitotoxicity-triggered neurodegeneration was followed by injury to RGC axons within the optic nerve. Finally, we successfully dampened the NMDA-induced excitotoxic effects on retinal damage by modulation of the putative protective properties of vascular endothelial growth factors.

In summary, our work characterize the impact of excitotoxicity on the diverse cellular and molecular factors involved in glaucoma and reveals a functional role of key factors in the physiopathology of the neurovascular unit that might be important for future therapeutic studies on degenerative eye diseases.

Binocular processing and receptive fields of motion-sensitive neurons in the zebrafish pretectum and tectum

Kun Wang[1,2], Julian Hinz[1,2,3], Väinö Haikala[4], Dierk F Reiff[4], Aristides B Arrenberg[1]

[1]University of Tübingen, Institute of Neurobiology and Werner Reichardt Centre for Integrative Neuroscience, Germany
[2]Graduate Training Centre for Neuroscience, University of Tübingen, D-72076 Tübingen, Germany
[3]Current address: Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland
[4]Neurobiology and Behavior, Institute Biology 1, Faculty of Biology, University of Freiburg, D-79104 Freiburg, Germany

Optic flow processing by neurons in the diencephalic pretectum is essential for visually guided behaviors in vertebrates, such as the optokinetic and optomotor responses. Animals need to distinguish translational and rotational stimuli to actively stabilize both their gaze and position relative to their surroundings. Recently, pretectal neurons involved in this task have been identified. However, the underlying sensorimotor transformations in zebrafish are still unclear. To elucidate the mechanisms, we investigated the sensory representations in the larval zebrafish brain with calcium imaging. We find that the directional space of both pretectal and tectal neurons is represented by four preferred optic flow directions during monocular stimulation (roughly corresponding to Up, Down, Forward, and Backward motion). Similar numbers of direction-selective neurons are found for each of the four preferred directions. No anatomical segregation of direction-selective tectal neurons was found. Furthermore, we identified neurons responding to specific translational or rotational whole-field patterns by presenting all possible binocular combinations of the four (monocularly) preferred stimulus directions to both eyes of the fish. These binocular selective neurons could – in principle – directly instruct appropriate compensatory eye and tail movements during optokinetic and optomotor behavior respectively. Monocular receptive field mapping shows that the vast majority of motion-sensitive tectal neurons are tuned to motion in a small portion of the visual field, many of them with surrounding inhibition. In contrast, many pretectal neurons have large-sized receptive fields (> 60°x30°, azimuth and elevation). Compared to the receptive field centers of cells with large receptive fields, visual space of the small-sized tectal receptive fields is over-represented in the nasal-dorsal visual field. Our study characterizes fundamental features of optic flow processing in the zebrafish pretectum and tectum. Our results provide the basis for further investigations into the vertebrate sensorimotor circuits of visually guided behaviors and into the adaptations of the larval zebrafish brain to habitat and lifestyle.

Chromatic processing in mouse retinal ganglion cells

Klaudia Szatko[1,2,4], Thomas Euler[1,2,3], Katrin Franke[1,2]

[1]Bernstein Center for Computational Neuroscience Tübingen (BCCN)
[2]Institute for Ophthalmic Research, Tübingen University
[3]Werner Reichardt Centre for Integrative Neuroscience (CIN)
[4]Graduate School for Neural & Behavioural Sciences

The ability to discriminate colors is very important for animals. Most mammals are dichromatic, as are mice. They have two cone photoreceptor types, expressing the short- (S, "blue") and medium-wavelength (M; "green") sensitive opsin. While true S-cones exclusively expressing S-opsin are homogenously distributed across the mouse retina (~5% of all cones; Haverkamp et al. J Neurosci 2005), M-cones co-express S-opsin with increasing co-expression levels towards the ventral retina (Röhlich et al. Neuron 1994; Calderone and Jacobs Vis Neurosci 1995). Basically, this results in a green-sensitive dorsal and a UV-sensitive ventral retina (Baden et al. Neuron 2013; Wang et al. J Neurosci 2011). Color vision requires local comparison of chromatic information from photoreceptors. Due to the uneven opsin distribution (especially in the ventral retina) it was unclear whether mice can extract chromatic information. However, recent behavioral studies demonstrated that mice can discriminate between light spots of different colors in the upper visual field (Denman et al. eLIFE 2018), observed by the ventral retina. Here, we investigate how the retina contributes to that behaviour by analyzing chromatic signal processing in mouse retinal ganglion cells (RGCs).

To systematically examine how the population of RGCs encode chromatic information, we bulk-electroporated the synthetic calcium indicator OGB-1 into RGCs. Then, we used two-photon calcium imaging with visual stimulation to characterize chromatic RGC responses. For that, we employed a center-surround flicker stimulus (center/surround diameter: 250/1400 µm) using an intensity-calibrated UV and green stimulator to analyze chromatic preference of RGC receptive fields.

Our dataset of >5,000 recorded cells confirms that the dorso-ventral opsin expression gradient largely determines the spectral sensitivity of center responses of RGC receptive fields. Interestingly, we found that chromatic preferences of RGC surround responses did not strictly follow the opsin distribution, resulting in colour-opponent center-surround antagonism for ventral and, to a lesser extent, dorsal RGCs: Here, some dorsal cells showed UV-shifted surround response, while most surround responses in the ventral retina were green-dominant. As green-sensitive M-opsin rarely occurs in the ventral retina, green surround responses likely originate from rods, which are green-sensitive. In our dataset, center-opponent RGCs were rare and therefore the existence of chromatic center-opponency in the mouse retina remains to be determined. In conclusion, we identified mouse RGCs with color-opponent responses predominantly located in the ventral retina. Our results are in line with recent behavioural studies demonstrating color discrimination in the upper visual field of mice.

An intrinsic electroretinogram response in isolated mouse retina

Motoharu Takao[1], Yumi Fukuda[2], Takeshi Morita[2]

[1]Tokai University, Department of Human and Information Science, 4-1-1 Kitakaname, 259-1292 Hiratsuka, Japan[2]Fukuoka Women's University, Department of Living Environmental Science, 1-1-1 Higashi-ku Kasumigaoka, 813-8529 Fukuoka, Japan

Since the discovery of intrinsic photosensitive retinal ganglion cell (ipRGC) was reported in 2002, many features specific to this cell type have been described. However, scare information is available on the retinographic components directly reflecting ipRGC activity. In this study, we identified the electroretinogram (microERG) that reflects the photoresponses by ipRGCs in ex vivo preparations of the mouse retina, in which classical photoreceptors (cones and rods) were ablated mechanically and photochemically. MicroERG consisted of three components: a large transient ON response, a small and lazy hump 19s after the onset of the light, and a large transient OFF response. A complete microERG recording required at least 30s of light exposure. MicroERG showed the highest spectral photosensitivity at 478nm. This wavelength corresponds to the peak wavelength in the ipRGCs' photosensitive curve. The psychophysical test using a blue light-emitting diode (LED) light (470nm) revealed that the absolute threshold illuminance for microERG was greater than 12.26 log photons/s/cm2 in both ON and OFF responses, whereas microERG was not adapted for dark. The amplitude of microERG increased linearly with irradiance. The sensitivity of temporal frequency was high in microERG (at least 100Hz), as suggested by the study on melatonin suppression by flickering light in human subjects (Zelter et al., 2014). Melatonin secretion was suppressed by light via ipRGCs and the suprachiasmatic nucleus. These properties of the photoresponse indicate that microERG may reflect the functions of ipRGC as a luminance detector in the mouse retina.

Spontaneous oscillatory networks in the outer and inner *rd10* retina

Prerna Srivastava[1,2,3], Luke E. Rogerson[1,2,3,4], Meng-Jung Lee[3,5,6], Deniz Dalkara[7], Günther Zeck[5], Philipp Berens[1,2,4], Thomas Euler[1,2,4], Timm Schubert[1,2]

[1]Werner Reichardt Centre for Integrative Neuroscience (CIN), University of Tübingen, Germany
[2]Institute for Ophthalmic Research, University of Tübingen, Germany
[3]Graduate Training Centre of Neuroscience, University of Tübingen, Germany
[4]Bernstein Centre for Computational Neuroscience, University of Tübingen, Germany
[5]Neurophysics Research Group, Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany
[6]Multi Channel Systems MCS GmbH, Reutlingen, Germany
[7]Sorbonne Université, INSERM, CNRS, Institut de la Vision, Paris, France

In the majority of retinal diseases, such as Retinitis Pigmentosa, the degeneration and subsequent loss of photoreceptors leads to blindness. The loss of this neuron class and the absence of light-evoked activity generates spontaneous rhythmic signals in the degenerated retina: In the outer retina, synaptic remodelling at the photoreceptor synapse leads to spontaneous oscillations between remnant cones, rod and cone bipolar cells (BCs) and horizontal cells (Haq et al., Front Neural Circuits 2014). In the inner retina, loss of light-evoked synaptic input is sufficient to generate oscillatory activity in the AII amacrine cell (AC)-cone BC network (Choi et al., J Neurophysiol 2014; Trenholm et al., J Physiol 2012) which is then relayed to retinal ganglion cells (RGCs) (Borowska et al., J Neurosci 2011). Both networks have only been studied in isolation, and it is unclear how and where these networks interact with each other. Although activity in both networks can be generated independently (Euler & Schubert, Front Cell Neurosci 2015), they are structurally connected and may modulate each other. Based on their synaptic input and output synapses in the outer and inner retina, respectively, BCs are possible candidates to relay outer retinal activity to the inner retina. Here, we aim to identify the role of BCs as the potential linking neurons between the two oscillatory networks in the degenerated retina. To this end, we recorded somatic and synaptic signals in the retina of the *rd10* mouse, a popular photoreceptor degeneration model.

In the whole-mount *rd10* retina, we first recorded spontaneous somatic Ca^{2+} signals using 2-photon imaging in populations of neurons in the inner nuclear layer (INL) at different retinal eccentricities and degeneration stages. To assess the vertical signal spread from the outer to the inner retina, we recorded neurons at the same retinal location but at different depths in the outer and inner INL, likely representing remnant cones/BCs and ACs, respectively. On the population level, we observed a high degree of heterogeneity. Spontaneous signals consisted of two components: Fast noisy uncorrelated activity and a slower auto-correlated activity. We used the Durbin-Watson test statistic to quantify the slow auto-correlated activity, representing somatic oscillatory activity. First, we did not detect any difference in activity at distinct retinal eccentricities. Second, during progression of degeneration from postnatal day 30 to 90, the signals in neurons located in outer and inner INL layers became more similar, suggesting an interaction between the outer and inner retinal activity that strengthens over time. We then used the glutamate biosensor iGluSnFR in combination with an electrically tunable lens to assess the role of BCs directly, by measuring the synaptic input to BC dendrites and output from BC axon terminals simultaneously. Blocking glutamatergic input pharmacologically reduced the correlation for a subset of outer and inner retinal glutamate signals indicating that outer retinal activity indeed modulates some properties of the inner retinal activity. In complement, to explore the relationship between the vertical BC pathway and lateral GC population activity, we combine 2-photon imaging with high-density CMOS

micro-electrode arrays.

P.S. and L.E.R. contributed equally to this work.

Fast Axial Imaging of Glutamate Dynamics in The Mouse Inner Plexiform Layer

Zhijian Zhao[1,2], Dario Protti[3], Katrin Franke[2,4], Luke Rogerson[1,2,4], Deniz Dalkara[5], Timm Schubert[1,2], Thomas Euler[1,2,4]

[1]Centre for Integrative Neuroscience (CIN), University of Tübingen, Germany
[2]Institute for Ophthalmic Research, University of Tübingen, Germany
[3]Department of Physiology and Bosch Institute, The University of Sydney, Australia
[4]Bernstein Center for Computational Neuroscience, University of Tübingen, Germany
[5]Sorbonne Université, INSERM, CNRS, Institut de la Vision, Paris, France.

Vision starts in the retina, a light-sensitive tissue lining the inner surface of the eye. The retinal network extracts numerous features from the visual scene before these are relayed via approx. 40 parallel channels to higher visual areas in the brain. The photoreceptors of the retina transduce light into electrical signals, which are then distributed to about 15 different types of bipolar cell (BC). These, in turn, relay the signals to the post-synaptic neurons in the inner plexiform layer (IPL). The functional properties of the different BC channels are determined by several factors, including the number and types of photoreceptors they contact (Behrens et al., eLife 2016) and the glutamate receptors they express (e.g. Puller et al., Neurosci 2013), but largely by synaptic interactions at their axon terminals in the IPL (e.g. Franke et al., Nature 2017). There, distinct sets of amacrine cells shape the spatio-temporal properties of the BC output to retinal ganglion cells (RGCs). Thus, BC output provides a *bona fide* readout of how information is partitioned at the first stage of the visual system and reflects the main excitatory “building blocks” that generate functional diversity in RGCs.

In previous studies, BCs were mainly investigated by using single-cell electrical recordings in vertically sliced retina (Wu et al. J Neurosci Meth 1987). However, lateral connections of the retinal network are significantly severed by slicing. An alternative is two-photon (2P) Ca2+ or glutamate imaging in the whole-mounted retina. This preserves the integrity of the retinal network, but typically requires recording horizontal planes at different IPL levels sequentially, which is time-consuming. Here, we introduce a method to image the whole IPL in the intact, whole-mounted retina, allowing us to record from all BC channels virtually simultaneously.

To this end, we equipped a 2P microscope with an electrically tunable lens (ETL, Optotune), which enables rapidly shifting the x-y focal plane along the z-axis (F. Helmchen, personal communication). The axial scan (x-z scan) gives a point spread function (PSF) of 0.5 µm in x-y, 2.0 µm in z and scan rates up to ~11 Hz, which can resolve the complete IPL and capture the dynamics of biosensors such as iGluSnFR. In addition, we modified our signal correlation-based ROI detection analysis (Franke et al., Nature 2017) to address the issue of variations in signal-to-noise due to fluorescence labelling differences across the IPL and to determine the relative position of ROIs according to IPL boundaries or ChAT bands.

The new scan configuration was evaluated by imaging light stimulus-evoked glutamate release using iGluSnFR ubiquitously expressed via AAV transduction. We find that x-z scans resolve (functional signatures of) mouse BC terminals across the IPL at a comparable spatial resolution as “traditional” x-y scans. Eventually, we demonstrate the effectiveness of this approach by studying neuromodulation via the cannabinoid system on BC contrast sensitivity.

A Retinal Origin of nystagmus in nyx-/- mice

Maj-Britt Hölzel[1], Beerend H.J. Winkelman[1,2], Marcus H. Howlett[1], Coen Joling[1], Gobinda Pangeni[3], Hiraki Sakuta[4], Masaharu Noda[4], Huib J. Simonsz[5], Maureen A. McCall[3], Chris de Zeeuw[1,2], Maarten Kamermans[1,6]

[1]Netherlands Institute for Neuroscience, Retinal Signal Processing, Meibergdreef 47, 1105 BA Amsterdam, Netherlands
[2]Department of Neuroscience, Erasmus University, Rotterdam, the Netherlands
[3]Departments of Ophthalmology & Visual Science and Anatomical Sciences and Neurobiology, University of Louisville, Louisville, USA
[4]National Institute for basic Biology, Okazaki, Japan
[5]Department of Ophthalmology, Erasmus University, Rotterdam, the Netherlands
6Department of Biomedical Physics, Academic Medical Center, University of Amsterdam, the Netherlands

The origin of infantile nystagmus, oscillating involuntary small eye-movements, is commonly thought to be located in the brainstem. However, Simonsz et al (2009) found that a specific group of young (3 month – 2 years) nystagmus patients had mutations in proteins implicated in the retinal cone to ON-bipolar cell synapse. These mutations were found both, in pre- (Cacna1f) and post-synaptic (Nyx) proteins and therefore suggest that this form of nystagmus has a retinal origin. We asked the question whether Nyx-/- mice had a similar form of nystagmus and aimed at resolving the mechanism leading to these oscillating eye movements.

Nyx-/- mice have a disturbed optokinetic response and show small amplitude oscillating horizontal eye-movements with a frequency of about 5 Hz. Interestingly, the oscillating eye-movements disappear in the dark. Since Nyx-/- mice have no functional ON-bipolar cells we asked the question whether the retinal output of these mice was affected using optic nerve recordings. We found that the ganglion cells in these mice oscillate with a frequency of about 5 Hz. The ganglion cell oscillations synchronized after light stimulation.

We propose that the synchronized output of ON-dsGCs to the accessory optic system is interpreted as oscillating global image motion inducing compensatory eye-movement. Some retinal ganglion cells detect image motion. The so called ON-directional selective ganglion cells (ON-dsGCs) detect global motion and send this information to the accessory optic system where it is used to control the eye-position, such that we can stabilize images on our retina while moving. We tested whether the ON-dsGCs were affected in Nyx-/- mice. For that purpose we used a SPIG1+ mouse in which all ON-dsGCs coding for upward motion express GFP. We found that in SPIG1+ nob mouse ON-dsGCs are not responsive to light anymore, but oscillate with a frequency of about 4.5 Hz. Blocking the ganglion cell oscillations pharmacologically with DNQX and D-AP5, which block AMPA and NMDA receptors, stopped the oscillation eye-movements indicating a direct link between the ganglion cell oscillations and the oscillatory eye movements.

Here we showed that there is a direct relation between oscillating ON-dsGCs and oscillating eyemovements in nyx-/- mice.

Poster Topic

T16: Vision: Striate and Extrastriate Cortex, Eye Movement and Visuomotor Processing

T16-1A Space Oddity: Observation of visual MMN in mice via in vivo 2-P Ca imaging
Elisabeta Balla, Ivo Vanzetta, Bjoern M. Kampa

T16-2A From Structure to Function: Stability and Plasticity in Mouse Visual Cortex
Joel Bauer, Simon Weiler, Mark Hübener, Tobias Bonhoeffer, Tobias Rose

T16-3A A virtual spatial navigation task for multisensory discrimination
Alexander Bexter, Christina Nothbaum, Florent Haiss, Bjoern M Kampa

T16-4A Postnatal development of electrophysiological properties in Layer 2/3 and Layer 5 pyramidal neurons in the primary visual cortex
Natalja Ciganok, Claas Halfmann, Thomas Rüland, Björn Kampa

T16-5A Binocular integration and matching of neuronal responses in the primary visual cortex of PSD-95 knockout and wildtype mice
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T16-3C Active navigation increases reliability of neuronal responses in primary visual cortex
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T16-4C Eye movements minimize overhead blind area in freely moving rats during both exploratory and stimulus-triggered behavior
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T16-5C A unifying model explaining perceptual stability and motion illusions under to fixational eye movements
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T16-1D Integrated circuit analysis of layer 2/3 principal cells in mouse visual cortex
Simon Weiler, Drago Guggiana Nilo, Mark Hübener, Tobias Bonhoeffer, Tobias Rose, Volker Scheuss

T16-2D Orientation discrimination in mice examined with a novel flexible touchscreen chamber reveals cardinal preference over oblique orientations
Christopher Wiesbrock, Stephanie Eichhorn, Jenice R. M. Linde, Björn M. Kampa

T16-3D Characterization of receptive fields in primate extra-striate area MSTd
Benedict Wild, Amr Maamoun, Stefan Treue

T16-4D Projections of the hyperpallium in the barn owl (*Tyto alba pratincola*)
Marcus Joseph Wirth, Hermann Wagner

T16-5D Ocular dominance plasticity and visual properties of PSD-93/-95 double-knockout mice
Rashad Yusifov, Leon Hosang, Hendrik Heiser, Oliver Schlüter, Siegrid Löwel

Space Oddity: Observation of visual MMN in mice via in vivo 2-P Ca imaging

Elisabeta Balla[1,2], Ivo Vanzetta[1], Bjoern M. Kampa[2,3]

[1]Institut de Neuroscience de la Timone, UMR 7289, CNRS & Aix-Marseille Université, Marseille, France
[2]Department of Neurophysiology, Institute of Biology II, RWTH Aachen University, Aachen, Germany
[3]JARA BRAIN, Institute of Neuroscience and Medicine (INM-2), Forschungszentrum Jülich, Jülich, Germany

Mismatch negativity (MMN) is a component of the event-related potential (ERP) to violations of an abstract rule, inferred from a sequence of sensory stimuli. A way to probe this complex neural phenomenon is to use the oddball paradigm. The experimental subject is exposed to several repetitions of a visual or auditory stimulus (“standard”), followed by a sudden interruption of this series by the presentation of a “deviant” version of the stimulus, i.e., differing from the standard one with respect to one parameter.

The MMN response was originally observed in electroencephalogram (EEG) recordings, where it is seen as a negative displacement of the difference wave obtained by subtraction of the ERP elicited by the deviant stimulus from that elicited by the standard (or repetitive) one, particularly in its late component around 150-250ms. However, due to its low spatial resolution, EEG does not allow to address the question of which neuronal circuits underlie the effect.

In order to address this question at the level of a large population of individual neurons, we used in vivo 2-photon calcium imaging in an experimental mouse model expressing the activity dependent calcium indicator GCamP6 (Thy1-GCaMP6 line 5.17). Despite this approach having been tried previously (1); a comprehensive analysis of the paradigm is still missing.

Here, we present a qualitative parametric meta-analysis of an oddball paradigm for 2-P Ca imaging in mouse visual cortex, and compare the effect in primary visual cortex (V1) and higher regions (LI). Notably, cells had inhomogeneous behavior differing one from another both within and among areas, some responding stronger to the deviant, some to the standard, and some responding comparably to both stimuli. Using gratings drifting in different directions as standard and deviant we furthermore characterized stimulus specific differences in the extent of deviant responses.

Together, these results provide a comprehensive protocol for MNN studies in mouse visual cortex using optogenetic methods.

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Figure 1. Graphical abstract.

a) *The presentation of our experimental configuration (4mm recording window covering both regions of interest of the mouse visual cortex). Right: the three main questions on the odball paradigm adressed here.*

b) *Example of an Odball Stimulation Protocol. (Inter-stimulus-interval 1s; stimulus duration 0.5 s).Presentation of a pseudo-random sequence with one stimulus identity used as standard (p=0.875,blue contour) and another one as deviant. (p=0.125, red contour).*

c) *Example of a Multi-Standard Control Protocol. (same stimulus parameters as in (b)). Sequential presentation of drifting square wave gratings of different orientations and motion directions (purple arrows). "p" is the probability of occurrence of a given stimulus.*

From Structure to Function: Stability and Plasticity in Mouse Visual Cortex

Joel Bauer[1], Simon Weiler[1], Mark Hübener[1], Tobias Bonhoeffer[1], Tobias Rose[1]

[1]Max Planck Institute of Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

Persistent modifications of synaptic connection strengths are often considered to be the basis of learning and stable long-term memory. This is consistent with the observation that both turnover and size of dendritic spines undergo prominent experience-dependent changes throughout the cortex[1]. However, the theory of a stable 'synaptic memory trace' is challenged by the high levels of spine turnover and morphological changes in the absence of experimental intervention. How information is retained in the face of constant synaptic rewiring is therefore unclear.

On average, the stimulus selectivity of individual excitatory neurons in primary visual cortex (V1) is surprisingly stable in spite of synaptic turnover during normal experience. Moreover, while brief perturbations of sensory input (like monocular deprivation) enhance synaptic turnover[1], individual neurons' response properties are only temporarily modified, and their original tuning profile is reinstated after the end of the intervention[2]. Here, we take advantage of small differences in the long-term functional stability of individual neurons in mouse V1 to assess the extent to which (i) spontaneous spine turnover and (ii) the balance of excitatory and inhibitory inputs[3] relate to the stability of long-term feature selectivity.

To this end, we have developed a novel approach that allows us to assess the stability of neuronal responses to a wide array of visual stimuli, observe structural synaptic changes, and obtain a quantitative snapshot of the laminar profile of excitatory and inhibitory connectivity of the same neurons. We perform chronic *in vivo* 2-photon imaging of excitatory layer 2/3 neurons that were sparsely transduced with a structural (mRuby2) and a functional marker (GCaMP6s) using adeno-associated viruses. We follow both the changes in functional properties and spine turnover of the very same cells for an extended period of time at short intervals. To investigate the correlation between cell stability and laminar input pattern we later re-identify the functionally and structurally characterized cells *in vitro* and perform laser-scanning photo-stimulation by glutamate uncaging to map the excitatory and inhibitory input to these cells across cortical layers[4].

To date, we have generated an extensive dataset of chronic recordings of cellular function and structure at session intervals ranging from 0.5 to 4 days for several weeks, and were able to re-identify the majority of labeled neurons *in vitro* for subsequent mapping of the excitatory and inhibitory connectivity. We are confident that this dataset will provide the groundwork for a much better understanding of how the apparent volatility of the cortical connectome relates to the stability and robustness of cortical information processing and storage.

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2. Rose, T., Jäpel, J., Hübener, M. & Bonhoeffer, T. Science 352, 1319–22 (2016).
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A virtual spatial navigation task for multisensory discrimination

Alexander Bexter[1], Christina Nothbaum[1], Florent Haiss[2], Bjoern M Kampa[1,3]

[1]Department of Neurophysiology, Institute of Biology II, RWTH Aachen University, Aachen, Germany
[2]Institute Pasteur, Unit of Neural Circuits Dynamics and Decision Making, Paris, France
[3]JARA BRAIN, Institute of Neuroscience and Medicine (INM-2), Forschungszentrum Jülich, Jülich, Germany

The ability to process and make use of sensory information is crucial for animals. To test the ability of mice to detect and discriminate between distinct visual and tactile stimuli, we designed a 2-AFC task in combination with running through a simplified virtual corridor. Sinusoidal gratings of different spatial frequencies were projected onto the corridor walls on both sides of the animal and were moved at constant speed (open-loop) or according to the running speed of the mouse on the treadmill (closed-loop). Mice had to discriminate between the different textures by licking on the water spout on the side corresponding to the higher target spatial frequency. All animals were able to discriminate less than two-fold differences in spatial frequency of the two gratings. We found no difference in performance between open-loop and closed-loop conditions, but when the movement of the animal was blocked, the performance dropped to chance level.

In a next step, we now extended this task including tactile stimulation. Here, mice hadve to discriminate bilateral whisker stimulation, applied at different frequencies. Tactile and visual stimulation frequencies were matched on both sides to evoke the impression of a multisensory virtual tunnel with different textures on both side walls. Mice showed similar performance in tactile task with discrimination of down to two-fold differences in whisker stimulation frequency. Running improved the performance similarly to the visual task.

We show a novel multisensory virtual environment for combined visual and tactile discrimination tasks in mice. The task allows testing of different combinations of the sensory modalities. Head-fixation of the tested mice further enables parallel high-resolution imaging and electrophysiological recordings.

Postnatal development of electrophysiological properties in Layer 2/3 and Layer 5 pyramidal neurons in the primary visual cortex

Natalja Ciganok[1], Claas Halfmann[1], Thomas Rüland[1,2], Björn Kampa[1,3]

[1]Department of Neurophysiology, Institute of Biology II, RWTH Aachen University, Aachen, Germany

[2]Institute of Complex Systems (ICS-4), Forschungszentrum Jülich, Jülich, Germany

[3]JARA BRAIN, Institute of Neuroscience and Medicine (INM-2), Forschungszentrum Jülich, Jülich, Germany

In the past years our knowledge about the brain has grown with the help of different methods, especially with the help of electrophysiological recordings. But understanding the whole brain means also understanding the smaller building blocks on the cellular level. To address this, we studied the development of electrophysiological and morphological properties in the primary visual cortex of C57Bl/6J mice of both sexes. Ocular dominance and functional retinotopic maps are formed in mice already before eye opening (around postnatal day 14) and refinement occurs afterwards during postnatal development. To investigate differences in intrinsic properties we characterized the electrophysiological properties of pyramidal neurons in layers 2/3 and 5 in acute slices of mouse primary visual cortex. Separating our results into postnatal days before (p10-14) and after (p25-p29) eye opening we found significant differences in passive membrane properties. In older mice the resting membrane potential was significantly more negative in both layers with stronger differences in layer 2/3 than in layer 5 cells. The membrane resistance also decreases in older mice with a larger drop in layer 2/3 cells than in L5 cells. This also resulted in faster time constants in cortical neurons of older mice. These changes lead to a reduced excitability of cortical neurons likely as homeostatic compensation for the increased sensory input after eye opening. In addition, we also observed a cell growth during development which could also explain the reduction in input resistance. To investigate indirectly the dramatic change of dendritic morphology and electrogenesis during postnatal development, we measured the action potential afterdepolarization (ADP) at the soma as indicator for dendritic spikes. Indeed, we found more prominent ADPs in neurons from older mice. Together, we have characterized electrophysiological properties of neurons from supra- and infragranular layers of mouse visual cortex before and after eye opening. The observed changes are in line with a homeostatic compensation in response to the increased sensory input as well as the maturation of passive and active cellular properties.

Binocular integration and matching of neuronal responses in the primary visual cortex of PSD-95 knockout and wildtype mice

Susanne Dehmel[1], Kanishka Waghmare[2], Michael Weick[3], Kalina Makowiecki[1], Christina Stoldt[1], Lisa Stamme[1], Xiaojie Huang[4], Man Ho Wong[5], Oliver M. Schlüter[4,6,7], Siegrid Löwel[1,7]

[1]Department of Systems Neuroscience, Johann-Friedrich-Blumenbach Institute for Zoology and Anthropology, Universität Göttingen, Germany

[2]current address: Department of Vision & Cognition, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands

[3]Department of Ophthalmology, University Medical Center Göttingen, Germany

[4]current address: Department of Neuroscience, University of Pittsburgh, USA

[5]current address: Picower Institute for Learning and Memory, Massachusetts Institute of Technology, USA

[6]Department of Psychiatry and Psychotherapy c/o European Neuroscience Institute Göttingen, Germany

[7]Sensory Collaborative Research Center 889, Universität Göttingen, Germany

Our labs have recently shown that adult PSD-95 knockout (KO) mice have 9x more AMPA-silent synapses in the primary visual cortex (V1) than wildtype littermates (WT), retain a lifelong juvenile-like ocular dominance plasticity (Huang et al., 2015), and display reduced orientation discrimination (Favaro et al., 2018). Together with the impaired synapse maturation of layer 4 inputs to excitatory pyramidal cells in layers 2/3 these data raised the question how V1-neurons respond to visual stimuli *in vivo*.
To this end, we recorded extracellular responses of neurons in the binocular part of V1 of isoflurane-anesthetized PSD-95 KO and WT mice with silicon micro-electrodes. The template matching spike sorting algorithm OPTICS (Prentice et al. 2000) was used for spike sorting. Visual stimuli consisted of moving sine wave gratings (8 different orientations, 7 spatial frequencies 0.05-0.32 cycles/degree, 2 Hz temporal frequency, full contrast) which were presented monocularly to either eye and binocularly. In addition, we performed 2-photon Ca2+-imaging using GCaMP6s in awake PSD-95 KO and WT-mice. Multielectrode recordings and 2-photon Ca2+-imaging revealed rather preserved neuronal tuning, like unchanged orientation selectivity (high OSI) in the PSD-95 KOs compared to WT-animals. In addition, KO-mice showed elevated response rates to monocularly and binocularly presented gratings of preferred orientation and spatial frequency. In contrast, binocular matching of neuronal responses was severely impaired, i.e. in KOs, inputs from the two eyes onto single binocular neurons, did not match (differences in peak rate and OSI), suggesting modified binocular interactions. We could substantiate this hypothesis with behavioral tests (visual water task) showing indeed improved orientation discrimination of KOs - even to WT- values - upon monocular visual experience. In contrast, WT-mice were worse with monocular visual experience.

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Evoking and tracking zebrafish eye movement in multiple larvae with *ZebEyeTrack*, an open-source application

Florian Alexander Dehmelt[1], Adam von Daranyi[2], Claire Leyden[1,3], Aristides B. Arrenberg[1]

[1]University of Tübingen, Werner Reichardt Centre for Integrative Neuroscience and Institute of Neurobiology, Otfried-Müller-Str. 25, 72076 Tübingen, Germany

[2]University of Tübingen, Werner Reichardt Centre for Integrative Neuroscience, Central Office System Administration, Otfried-Müller-Str. 25, 72076 Tübingen

[3]University of Tübingen, Graduate Training Centre of Neuroscience, Österbergstr. 3, 72074 Tübingen

Reliable measurements of spontaneous and evoked eye movement are crucial to behavioural vision research. Zebrafish are increasingly used as a model organism for visual neural circuits, but ready-to-use eye tracking solutions are scarce. We thus developed a custom-built software solution, *ZebEyeTrack*, for the automated real-time measurement of angular horizontal eye position in up to six immobilized larval fish, and share it online. Detailed manuals and experimental protocols are also available. On-site at the conference, we demonstrate the ability of *ZebEyeTrack* to perform eye tracking analysis on pre-recorded videos, and highlight key processing steps.

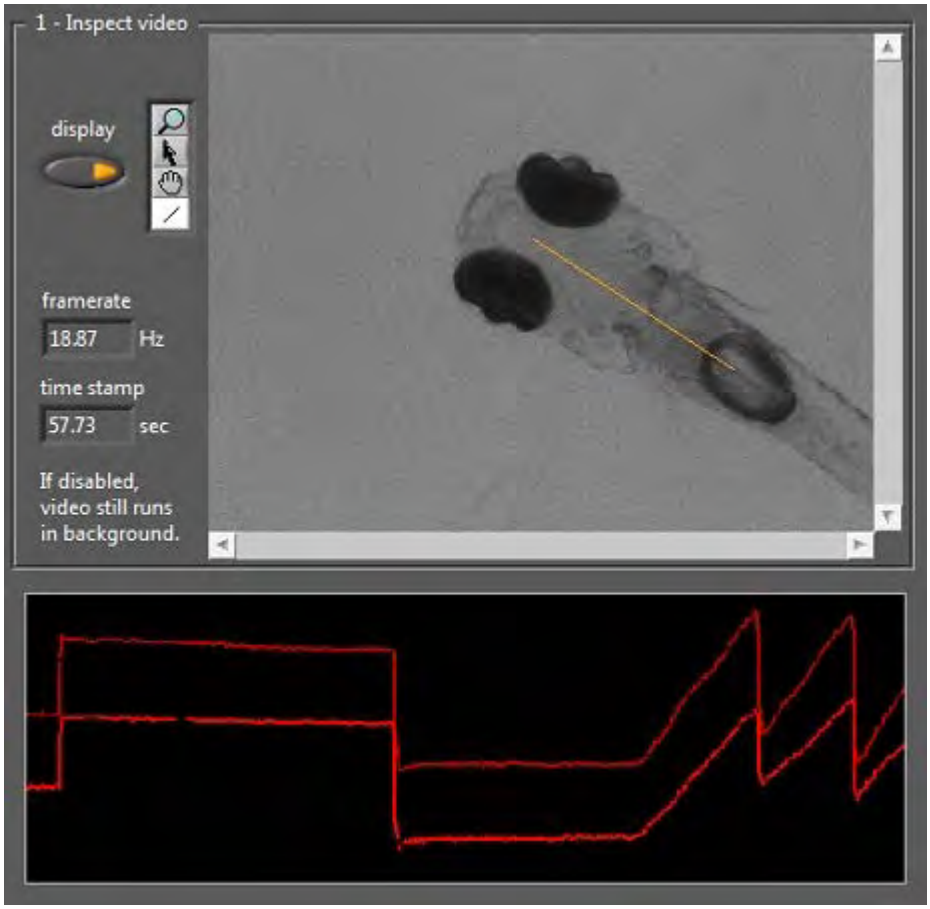
The full software has additional features, controlling all required hardware and synchronizing six aspects of the experiment:

1. Stimulus design
2. Visual stimulation with moving bars
3. Eye detection and tracking, as well as general motion detection
4. Real-time data analysis
5. Eye-position-dependent closed-loop event control
6. Recording of external event times

This includes optional integration with external hardware such as lasers and scanning microscopes.

The software, its customizable source code, as well as a simpler, compiled version of the software, are all available under a Creative Commons license. Experiments, including stimulus design, can be completed in a few minutes, and recordings can last anywhere between seconds and many hours. Results such as angular eye positions and hardware status can be used to compute tuning curves, optokinetic gain, and other custom data analysis. After the experiment, or based on existing videos, optokinetic response performance can be analyzed semi-automatically via the graphical user interface, and results can be exported.

Precursor software to *ZebEyeTrack* was used successfully in published studies including psychophysics experiments, optogenetic stimulation, and combinations with calcium imaging.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Orientation selectivity in cortical neurons ex-vivo from acute, tangential slices of mouse primary visual cortex

Jonas Franz[1,2,3,4], Ricarco M. Merino[3,4,5], Manuel Schottdorf[3,4,5], Julian Vogel[1,3,4,5], Andreas Neef[1,3,4,5], Walter Stühmer[3,4], Fred Wolf[1,3,4,5]

[1]Campus Institute for Dynamics of Biological Networks
[2] Institute of Neuropathology, UMG
[3] Max-Planck-Institute for Experimental Medicine
[4] Neurophysics, Max-Planck-Institute for Dynamics and Self-Organization
[5] Bernstein Center for Computational Neuroscience, Göttingen

Holographic stimulation of acute slices cut tangentially through the primary visual cortex was performed on ChR2 expressing mice while the activity of the neurons was recorded by a multielectrode array.

In mice, orientation tuned input is known to exist in thalamocortical projections to neurons of the cortical layer IV of the primary visual cortex. Recent findings by Schottdorf showed that already in dissociated primary neuronal cultures orientation selectivity can be measured. Optogenetics provided direct input to neurons and the visual pathway was simulated in-silico. Interestingly another mechanism additionally to the Hubel and Wiesel connectome is able to generate orientation selectivity in cortical neurons.
Here the same holographic stimulation with pure moving gratings was performed not only on the dissociated neurons but also on acute slices. Since we cut the slices tangentially, the horizontal connectome of the primary visual cortex is assumed to be preserved. The optogenetic stimulation was possible in those mice through a thy1 Promotor dependent expression of ChR2. After stimulation, the positioning of the electrode array was confirmed via immunostaining of the thalamocortical projection marker ROR-β.

In this setting, we were able to record orientation selectivity ex-vivo in cortical neurons only.

Phase-coupling in the superior colliculus of the feline brain

Zsófia Giricz[1], András Puszta[1], Diána Nyujtó[1], Ákos Pertich[1], Viktória Balikó[1], Nándor Görög[1], Balázs Bodosi[1], Attila Nagy[1]

[1]University of Szeged, Faculty of Medicine, Department of Physiology, Dóm square 10., H-6720 Szeged, Hungary

The superior colliculus (SC), which is the homologue of the optic tectum of other vertebrate groups, is the main retino-recipient nucleus of the mammalian mesencephalon and is regarded as the origin of the tectofugal multisensory system. Although several studies addressed the electrophysiological properties of the collicular neurons, the connection between the low frequency oscillations (LFP) and the spiking activity of the single neurons is still poorly understood. We have therefore aimed to investigate in a background condition, without any sensory stimulus and without the influence of motor activity, the phase coupling of the SC neurons to different frequency bands of the LFPs.

Extracellular, high-density electrophysiological recordings were performed in the SC of two halothane-anaesthetised, paralyzed, artificially ventilated cats. The broad-band signals were recorded with 64-channel platina-iridium microelectrodes and were analyzed offline. The spikes of the different SC neurons were separated with Klusta software package. The direction of the phase coupling of the single neurons to different frequency bands (theta (4-7 Hz), alpha (8-12 Hz), beta (13-30 Hz), gamma (31-50 Hz)) was statistically analyzed with Rayleigh uniformity test.

Altogether 956 neurons were recorded from the SC of the two cats. The large majority of them (888/956; 93%) possessed phase coupling in at least one of the investigated frequency bands. The phase coupling analysis to alpha- and gamma-band oscillations revealed the similar preferred phase in both cats. The phase coupling to beta-band oscillation was also similar in the two cats but without any preferred phase of the local oscillations. On the other hand, the direction of the phase coupling to theta oscillations was not uniform in the two animals. We have found no connection between the direction of the phase coupling and the depth of the neurons in the SC.

Our results demonstrated that the background activity of the SC neurons is strongly phase locked in both cats. Similarly, high number of phase locked neurons were found in both animals. Excluding theta band, the distribution of preferred directions of the phase coupling was similar in the two cats. However further investigation is necessary to clarify the influence of the sensory signals on the phase coupling of the SC neurons.

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Mapping overhead binocular field on identified visual cortical neurons in rats

Takashi Handa[1], Kay-Michael Voit[1], Alexandr Klioutchnikov[1], Verena Pawlak[1], Damian J Wallace[1], Jason ND Kerr[1]

[1]research center caesar, Dept. Behavior and Brain Organization, Ludwig-Erhard-Allee 2, 53175 Bonn, Germany

Rats have laterally facing eyes which, combined with the large field of view of each eye, provide a panoramic visual field covering not only frontal and lateral field of view but also above and behind the animal's head. Eye movements in freely moving rats maintain the visual fields of both eyes above the animal independent of the position and orientation of the head, which has the effect of minimizing 'blind spots' (locations not in the visual field of either eye) as the animal moves around. Visual stimuli presented overhead, but not laterally, induced a shelter-seeking response, suggesting a role of overhead field of view in visual detection, presumably a response to predation from birds [Wallace et al. 2013]. Both left and right visual fields overlap above the animals head, however, how the binocular overhead fields are retinotopically represented in visual cortex remains unclear. In this project we are quantifying the characteristics of binocular cortical neurons' receptive fields in the overhead field of view, the extent to which they cover this region and the anatomical organization in the retinotopic map in primary visual cortex.

We performed multi-photon imaging in Lister hooded rats of cortical neurons expressing genetically encoded calcium indicator GCaMP6s at various retinotopical locations. Receptive fields were determined using noise movie stimuli, and visual responses to grating stimuli characterized. The locations of the receptive fields relative to the animal's head were reconstructed in an epipolar coordinate frame and the locations of the neuronal recordings were post hoc confirmed in histological sections. We present preliminary findings showing neurons with orientation selective responses in regions up to 80 degrees above the horizon together with their anatomical organization.

Reference:
Wallace, D. J., Greenberg, D. S., Sawinski, J., Rulla, S., Notaro, G., & Kerr, J. N. (2013). Rats maintain an overhead binocular field at the expense of constant fusion. Nature, 498(7452), 65.

Pathway-specific optogenetic inhibition reveals that prefrontal area FEF has a direct influence on the attentional modulation of firing rates in visual area MT in the non-human primate

J. Hüer[1], P. Saxena[1], M. G. Fortuna[1], H. Guo[1], L. T. Schiller[1], A. Gail[1,3,4,5], J. Gruber[1], E. Gruber-Dujardin[1], H. Scherberger[1,3,4,5], J. Staiger[2,6], S. Treue[1,3,4,5]

[1]German Primate Center - Leibniz Institute for Primate Research, Göttingen, Germany
[2]Institute for Neuroanatomy, University Medical Center Göttingen, Germany
[3]Faculty of Biology and Psychology, University of Göttingen, Göttingen, Germany
[4]Bernstein Center for Computational Neuroscience, Göttingen, Germany
[5]Leibniz-ScienceCampus Primate Cognition, Göttingen, Germany
[6]DFG Center for Nanoscale Microscopy and Molecular Physiology of the Brain, Göttingen, Germany

Higher cognitive processes, like attention, have a modulatory effect on sensory-evoked responses in visual areas of the non-human primate. For example, firing rates in visual area MT are modulated dependent on the spatial attentional focus of an animal even with an unchanging sensory input. Several studies suggest that the frontal eye field (FEF) located in the prefrontal cortex has a major impact on this modulation. However, previous studies mainly investigated the influence of FEF on visual area V4. In addition, they manipulated area FEF in a comprehensive manner by electrical microstimulation or neuropharmacological interventions, which both cannot differentiate between direct influences of FEF or effects mediated via indirect pathways emanating from FEF.

We investigated whether FEF plays a role in modulating firing rates in visual area MT during attention via direct projections. In contrast to previous studies, we directly targeted the axonal projections from area FEF to area MT with optogenetics. More specifically, we used optogenetics to only inhibit the direct mono-synaptic input from area FEF to visual area MT.

We injected a viral vector (AAV5-αCaMKII-eNpHR3.0-mCherry) into FEF of four rhesus macaques. In three of the animals we conducted a histological assessment of opsin expression in area FEF and projecting axons in area MT at different time points after viral vector injection. Two of the animals were trained in a spatial attention task prior to viral vector injection. The purpose of the behavioral task was to activate MT neurons with an external sensory input, and compare different attentional conditions while keeping the sensory input constant. Starting several months after viral vector injection we conducted single-cell recordings in area MT and simultaneously inhibited the input from area FEF with a continuous laser pulse.

Our histological examination revealed that opsins were incorporated in the cell bodies of FEF neurons as well as in axons projecting to area MT, and were still present two years after viral vector injection. Laser stimulation of FEF axons in area MT reduced the attentional modulation of firing rates in area MT. More specifically, blocking FEF input decreased firing rates when a target stimulus was presented in the receptive field of an MT neuron and increased firing rates when a distractor stimulus was presented in the receptive field.

Our results support the hypothesis that FEF impacts area MT via its direct mono-synaptic axonal projections that have a causal influence on attentional modulation of firing rates in area MT, causing both, a target enhancement and a distractor suppression.

Recruitment orders underlying binocular coordination of eye position and velocity in the larval zebrafish hindbrain

Claire Leyden[1,2], Christian Brysch[1,2], Konstantin Willeke[1,2], Aristides B. Arrenberg[1]

[1]Werner Reichardt Centre for Integrative Neuroscience & Institute for Neurobiology, 72076 Tübingen, Germany

[2]Graduate Training Centre for Neuroscience, University of Tübingen, 72074 Tübingen, Germany

Oculomotor circuits in the vertebrate hindbrain are responsible for generating eye movements. They need to integrate sensory information and produce coordinated motor output. Vertebrates possess several oculomotor brain nuclei subserving different functions, including the velocity-to-position neural integrator for horizontal eye movements and the velocity storage mechanism, which form short-term memories of eye position and velocity respectively. These nuclei connect to the abducens nucleus, which generates motor signals for the ipsilateral and contralateral eye via motoneurons and internuclear neurons respectively. Several studies have recently advanced our understanding of the encoding of horizontal eye position in the teleost brain. However, the precise tuning of zebrafish oculomotor neurons to motor variables has not yet been determined. Using broadly expressed nuclear GCaMP6f, we recorded cellular calcium signals in the active oculomotor circuits of larval zebrafish. Concurrently, we applied optic flow stimuli to evoke monocular and binocular optokinetic eye movements and disambiguate the eye position and velocity components of the recorded neural activity. Our investigation of binocular coordination reveals four major neuronal response types: (I) neurons monocularly encoding the right eye or (II) the left eye, (III) neurons exclusively active during conjugate binocular eye movements, or (IV) active during all monocular and binocular eye movements. Within rhombomeres 5/6 (corresponding to the nucleus abducens), putative motor and internuclear neurons could be functionally distinguished and were observed to form distinct anatomical clusters. Response type III (binocular exclusive) is frequently encountered in rhombomeres 5/6, which suggests the absence of a final common motor pathway within the zebrafish abducens. We generated two-dimensional tuning maps for eye position and optokinetic slow-phase eye velocity for each responding neuron. Neurons in the nucleus abducens were mostly coding for eye position, while a velocity-to-position coding gradient exists along the rostro-caudal axis in rhombomeres 7/8. The velocity encoding neurons within rhombomere 7 putatively form the velocity storage mechanism and smoothly transition into the anatomical regions of the neural integrator for horizontal eye movements and the inferior olive, which are located caudally. Our analysis of eye position and velocity firing rate thresholds confirms the expected existence of a recruitment order for eye position neurons, which has also been reported in other species. Furthermore, we identify and characterize two additional recruitment orders for velocity neurons within the oculomotor system, one for firing thresholds of eye velocity and the other one for firing rate saturation of eye velocity. Our functional and anatomical cell maps illustrate the binocular coordination and encoding of slow-phase eye velocity and eye position in the larval zebrafish hindbrain. Our report of motor variable tuning will hopefully facilitate further investigation into the mechanisms underlying binocular coordination and persistent activity (short-term memory) in the zebrafish oculomotor system.

Electrophysiological properties of superior colliculus neurons: high resolution recordings

Diána Nyujtó[1], Zsófia Giricz[1], András Puszta[1], Ákos Pertich[1], Nándor Görög[1], Viktória Balikó[1], Balázs Bodosi[1], Attila nagy[1]

[1]University of Szeged, Faculty of Medicine, Department of Physiology, Dóm square 10., H-6720 Szeged, Hungary

The superior colliculus (SC) of the mammalian mesencephalon is a laminar structure, where the functions of the different layers are strongly diverse. The superficial layers are strictly visual, the intermediate layers are multisensory and the deep layers are involved in sensorimotor processes. Although these functional differences are obvious, little is known about the laminar organization of the neurons based on their electrophysiological properties. The main goal of this study is to investigate if the background discharge properties of the SC neurons, without any sensory stimulus and without the influence of motor activity, correlate to the depth of the neurons in the tectal region.
Extracellular, high-density electrophysiological recordings were performed in the whole dorsoventral extension of the SC of two halothane-anaesthetised, paralyzed, artificially ventilated cats. Broad-band signals were recorded by means of 64-channel platina-iridium microelectrodes, which were band-pass filtered for the further analysis. The spikes of the different SC neurons were separated with Klusta software package. We have investigated the connection between the depth of the SC neurons and the background discharge rates, the interspike interval ratios longer than 2 s (propISI>2sec), the shape of the autocorrelograms, the strength and the direction of phase-coupling in alpha (8-12 Hz), beta (13-30 Hz) and gamma (31-50 Hz) frequency bands.
Altogether 956 neurons were recorded from different layers of the SC. The firing rates, the shape of the autocorrelograms as well as the direction and strength of the phase-coupling did not correlate with the depth in the SC. None of these eight properties was different among the three layers of the SC. Comparing the electrophysiological properties the propISI>2sec was the exemption, which showed a weak correlation with the depth. Similarly, propISI>2sec was different among the three layers of the SC. It was significantly higher in the deep than in the superficial and intermediate layers of the SC.
Concerning the background activity of the SC neurons it can be concluded that merely the propISI>2sec values were different among the three different layers. On the other hand, the other eight investigated electrophysiological properties did not show this tendency. Our results indicated that the background firing properties of the SC neurons are not strongly connected to the functional laminar organization of the SC. However, further investigation is necessary to clarify whether the sensory stimulation can modify this.

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Active navigation increases reliability of neuronal responses in primary visual cortex

Magdalena Robacha[1,2], Thomas Rüland[1,3], Gerion Nabbefeld[1], Alexander Bexter[1], Lyuba Zehl[1], Björn M. Kampa[1,3]

[1]Department of Neurophysiology, Institute of Biology II, RWTH Aachen University, Aachen, Germany
[2]JARA BRAIN, Institute of Neuroscience and Medicine (INM-2), Forschungszentrum Jülich, Jülich, Germany
[3]Institute of Complex Systems (ICS-4), Forschungszentrum Jülich, Jülich, Germany

Recent studies made it clear that the role of primary visual cortex is not only that of a passive receiver of retinal input. Previous research provides compelling evidence for the existence of modulatory effects of locomotion (Keller et al., 2012; Leinweber et al., 2017), location in a virtual corridor (Fiser et al., 2016) as well as prediction of a visual sequence (Gavornik and Bear, 2014) in mouse primary visual cortex. We tested whether this modulation also affects the variability of evoked activity in primary visual cortex. For this, a virtual reality experiment was designed in which mice traversed an over-trained virtual corridor in three experimental conditions of different predictability: RUN, MOVIE and REPLAY. During RUN, mice traversed the virtual corridor while running on a wheel (closed loop condition), whereas in MOVIE and REPLAY mice were placed inside a tube and passively watched the presentation of a consistent- (MOVIE) or variable-speed (REPLAY) run through the corridor (open loop condition). Activity of neurons in mouse primary visual cortex was recorded using two-photon calcium imaging.

Variability of evoked activity was measured across traversals of the corridor and confirmed by accuracy of a classifier predicting the location in the corridor. The acquired data show that integration of locomotion and variable visual flow during RUN formed reliable visual cortex activity which was also observed in the MOVIE condition where visual flow was constant. However, during REPLAY activity in the primary visual cortex was highly variable, presumably because visual flow was neither constant nor predictable from locomotion. In summary, the results of our research show, that the measured variability in primary visual cortex is modulated by the animal's locomotion. When the visual flow is constant or predictable from own motion responses become more robust. This finding aligns with the theory of predictive coding and further confirms the hypothesis that the activity of the primary visual cortex might be top-down modulated and shaped by previous experience.

Eye movements minimize overhead blind area in freely moving rats during both exploratory and stimulus-triggered behavior

Federica Bianca Rosselli[1], Kay M. Voit[1], Damian J. Wallace[1], Jürgen Sawinski[1], David S. Greenberg[1], Jason N. D. Kerr[1]

[1]Center of Advanced European Studies and Research (Caesar) , Behavior and brain organization, Ludwig-Erhard-Allee 2, 53175 Bonn, Germany

Rats have a large visual field that extends vertically from below their snout, over the top of the head to almost 60 degrees beyond vertical behind their head. This large coverage is due, in part, to the eye optics, which have a large angle of acceptance (>180 degrees), and in part to the semi-lateral position of the two eyes optical axis, (~58 degrees from frontal plane and ~36 degrees above horizontal). When freely moving, rats exhibit large horizontal and vertical disconjugate eye movements that are strongly correlated with changes in head position. We suggested that, in addition to maintaining the large binocular region overhead, a concurrent purpose of eye movements is to reduce the area blind to either eye which is located behind the animals head (blind area) where objects cannot be visually detected. Here, we quantified the relationship between eye movements and the visual coverage surrounding the animals head during free movement, and the role eye movements played in reducing blind area under two different visual conditions. Using our miniaturized ocular videography system with simultaneous overhead pose and position tracking we recorded eye and head positions when animals were presented with a sudden overhead looming dot stimuli during free behavior and secondly when performing a visually based gap crossing task. We then computed a probability map for coverage of visual space around the animals head (n=7 animals) for all head positions during the two visual tasks and during free behavior. To assess the extent to which eye movements minimize the blind area, the probability map was recomputed with the eyes fixed to their mean positions for the same data. This showed that eye movements increase total visual coverage above the horizon by ~10% compared to no eye movements. To quantify the range of head positions where eye movements could show the greatest blind area reduction effect, we computed the probability of visual space coverage as a function of elevation and azimuth. The starkest effect was found during periods of most extreme downward pitch of the head, when eye movements always increased the average coverage of visual space above the horizon (and concomitantly reduced the blind area). These measurements suggest that, in addition to maintaining the overhead binocular field, eye movements also reduce the blind areas above the horizon. We next assessed whether the relationship between eye movements and overhead visual field was maintained during either gap crossing tasks (n=3 animals) or during the presentation of an unexpected overhead looming stimulus (n=4 animals). Although the animals undertook each task with different behavioral strategies, there was no difference in the relationship between head and eye positions, which, like in controls, maintained the overhead binocular region and minimized the blind area. In summary, our study confirms the role of eye movements in counteracting head position for the purpose of not only maintaining a binocular overlap in the overhead field of view, but also in minimizing blind spots, and this relationship is not modified during visually based behavioral tasks. We suggest that rats have developed a distinct vestibulo-ocular reflex that ensures a large coverage of the overhead visual space at all times which provides a significant advantage for the evasion from predators.

A unifying model explaining perceptual stability and motion illusions under to fixational eye movements

Felix Schrader[1], Thomas Wachtler[1]

[1]Ludwig-Maximilans-Universität, Department of Biology II, Ludwig-Maximilians-Universität München, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany

The existence of fixational eye movements (FEM), which can be well above the detection level of the visual system, imposes two major problems for visual perception: how to stabilize the image and how to differentiate between motion in the visual environment and retinal motion due to self-movements of body and eyes. We proposed a model to discern between local retinal motion, as typically elicited by object motion, and global motion, as elicted by eye movements, and to suppress motion signals arising from the latter type at early visual stages (Greene et al. 2016). The model utilizes the properties of linear midget and non-linear parasol retinal ganglion cells in combination with a detection mechanism for global motion. It dramatically reduced the error in position estimates introduced by eye movements, enabling reliable object position tracking. Furthermore, the model also predicted illusory motion percepts in certain static image patterns like the Spillmann-Ouchi illusion.

To further investigate this mechanism of image stabilization, we applied the model to stimuli used in psychophysical studies investigating perceptual stability during and without FEM (Poletti et al. 2010). We simulated the responses to visual input consisting of moving or static dot stimuli with or without visual references, and with or without compensation of retinal motion due to FEM, and derived object motion estimates from the model's responses. Motion responses by the model were high for conditions in which human observers reported perceiving motion, and were low for conditions in which observers did not perceive motion. The correlation between the simulation results and the psychophysical data across all conditions was 0.85.

We further applied the model to flickering random-dot stimuli eliciting illusory motion percepts in human observers (Murakami 2003). The simulations showed that the model produced motion responses to these stimuli, and moreover the dependence of the motion signal strength on the stimulus flicker frequency paralleled the results from human observers in the psychophysical experiments.

Based on the experimental data, we determined the size of a motion detection unit to be comparable to the receptive field size of parasol ganglion cells. This important assumption for the model has recently been corroborated by experimental findings (Manookin et al. 2018). Our model thus provides a simple, biologically plausible, explanation for the perceptual insensitivity of vision to FEM as well as the occurrence of FEM-induced motion illusions.

Integrated circuit analysis of layer 2/3 principal cells in mouse visual cortex

Simon Weiler[1], Drago Guggiana Nilo[1], Mark Hübener[1], Tobias Bonhoeffer[1], Tobias Rose[1], Volker Scheuss[2]

[1]Max Planck Institute of Neurobiology, Synapses-Circuits-Plasticity , Am Klopferspitz 18, 82152 Martinsried , Germany
[2] Ludwig-Maximilians-Universität München, Großhaderner Str. 9, 82152 Martinsried, Germany

Neocortical principal cells (PCs) display functional specializations defined by their connectivity as well as their molecular, anatomical, and electrophysiological properties. PCs in layers 5 (L5) and 6 (L6) have been classified into different subtypes based on their *in vivo* functional response properties, connectivity patterns, as well as genetic and electrophysiological characteristics[1,2]. However, for layer 2/3 (L2/3) PCs little is known about the detailed relationship between their stimulus-response properties and their cellular physiology and connectivity. Here, we address this gap in knowledge by directly correlating the stimulus selectivity of individual L2/3 neurons *in vivo* with their morphological, electrophysiological, and connectivity signatures *in vitro*.

First, we recorded the morphological and electrophysiological properties of L2/3 PCs in mouse primary visual cortex (V1) in acute brain slices to reveal potential L2/3 PC subtypes. Hierarchical clustering revealed six electrophysiological and seven morphological clusters of L2/3 PCs. However, we found no correspondence between electrophysiological and morphological clusters, therefore arguing against morpho-electrophysiological L2/3 PC subtypes in mouse V1.

We next asked whether L2/3 PCs differ in their connectivity patterns. To address this question, we characterized the excitatory and inhibitory cortical inputs to L2/3 PCs using laser-scanning photostimulation (LSPS) by UV glutamate uncaging in brain slices. Cluster analysis showed that L2/3 pyramidal cells can be classified into three subgroups based on their excitatory and inhibitory laminar input patterns and the spatial overlap of these patterns.

In order to explore the functional implications of the different input patterns and morpho-electrophysiological L2/3 PC characteristics we developed an *in vivo* / *in vitro* approach[3]: First, we characterized the visual response properties (orientation/direction selectivity, temporal/spatial frequency preference, ocular dominance) and spontaneous activity profile of individual neurons. To this end, we sparsely expressed a genetically encoded calcium indicator (GCaMP6m) together with a structural marker (mRuby2) and recorded neuronal activity using *in vivo* 2-photon calcium imaging in anesthetized mice. Subsequently, we retrieved the same neurons in brain slices and performed circuit analysis with LSPS. Given the known correlations of local and long-range connectivity and feature selectivity in L5 and L6 PCs[1,2] we expected to reveal similar correlations in our dataset. However, analyzing the relation between functional response properties measured *in vivo* and the laminar connectivity assessed *in vitro* showed that the differences in laminar input connectivity of L2/3 PCs were not directly reflected in the functional response properties of the same neurons.

In conclusion, in contrast to L5 and L6 PCs[1,2], we did not observe any obvious correspondence between any of the clusters across the different property sets, i.e. morphology, electrophysiology, connectivity, and stimulus-response properties. This potentially reflects the different role of L2/3 as an intermediate integration layer compared to the output layers 5 and 6.

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Orientation discrimination in mice examined with a novel flexible touchscreen chamber reveals cardinal preference over oblique orientations

Christopher Wiesbrock[1], Stephanie Eichhorn[1], Jenice R. M. Linde[1], Björn M. Kampa[1,2]

[1]RWTH Aachen, Neurophysiology, Worringerweg 3, 52074 Aachen, Germany

[2]JARA BRAIN, Institute of Neuroscience and Medicine (INM-2), Forschungszentrum Jülich, Jülich, Germany

The perception of our visual environment can be formalized into different parameters like spatial frequency and orientation. Analysis of natural images showed an orientation bias towards cardinal orientations compared to obliques (Girshik et al. 2011). Similarly, humans showed a behavioral bias in the discrimination of orientations towards cardinals, also called oblique-effect. It has been suggested that the observed discrimination bias is caused by an overrepresentation of cardinal tuned neurons in visual cortex with more precise tuning compared to oblique tuned neurons. We have recently shown that neurons in mouse primary visual cortex are tuned for different orientations and spatial frequencies. Importantly, the visual cortex of mice reflects this overrepresentation of cardinal orientations (Roth et al. 2012). To examine if also a behavioral oblique-effect exists in mice, we established an adaptive staircase psychophysical experiment in a custom-built operant touchscreen chamber. Mice were pretrained to discriminate a horizontal orientated sine-wave grating against a vertical grating of same phase and spatial frequency. After reaching the performance criterion, we started the adaptive procedure. To our knowledge this is the first implementation of a staircase method in an orientation discrimination task in mice. By this procedure it is possible to examine a discrimination threshold each session with lower number of trials in total, compared to a constant stimulus strategy. For cardinal targets mice showed a discrimination threshold of 23.13 ± 1.01 degree (mean ± SEM). Mice, which were trained to an oblique target showed significantly higher thresholds of 43.37 ± 1.64 degree (mean ± SEM; H-Test: $p<0.001$). Furthermore, the results also show a broader distribution of thresholds for oblique targets. This is also consistent with studies in mice regarding tuning bandwidth of neuronal populations in the primary visual cortex, showing that cells tuned to a horizontal orientation have an orientation tuning bandwidth of 23° - 28°, while neurons tuned for other orientations have a tuning bandwidth up to 40° for other orientations (Niell & Stryker 2008, Li et al. 2003, Roth et al. 2012).

In this study we were able to show a well-described neuronal finding on the behavioral level and prove the functional relevance of orientation tuned neurons in the mouse visual cortex. Here we report to our knowledge the first findings of the behavioral relevance of the well characterized oblique-effect on the neuronal level.

Characterization of receptive fields in primate extra-striate area MSTd

Benedict Wild[1], Amr Maamoun[1], Stefan Treue[1,2,3]

[1]Cognitive Neuroscience Laboratory, German Primate Center (DPZ), Göttingen, Germany
[2]Faculty of Biology & Psychology, University of Göttingen, Göttingen, Germany
[3]Bernstein Center for Computational Neuroscience, Göttingen, Germany

A visual neuron's receptive field (RF) can be thought of as a filter that describes the relation between an image and the neuron's response. Characterizing these RFs has therefore been one of the central problems within systems neuroscience. We report a series of experiments that aim at determining the RF properties of neurons in the dorsal part of the medial superior temporal area (MSTd) in macaque extra-striate visual cortex. MSTd plays a central role in the processing of complex motion patterns. Neurons in this area are tuned not only for motion along a straight path, but also for 'spiral motion', a continuous circular space of complex motion patterns that includes expansion, contraction and rotation. In addition, MSTd cells have also been reported to be position-invariant in their responses to spiral motion stimuli.

We first mapped the spatial outline of RFs of MSTd neurons, established their basic tuning properties for traditional motion stimuli, and determined their position dependency to spiral motion patterns. To characterize their motion preference profile in more detail, we then measured the neurons' responses to a newly developed stimulus: a large random dot pattern, formed by the smooth variation of local dot motion between a grid of positions in the stimulus where the local direction and speed were chosen randomly every 100ms from all possible directions and a large range of speeds. We used reverse correlation to calculate a spike-triggered average, a linear method which has been successfully used to characterize RFs in earlier cortical areas (e.g., V1 and MT). With this approach, we hoped to gain a more detailed description of the specific motion preferences of individual MSTd neurons, compared to the simple assumption of linear and/or spiral direction tuning.

We recorded from more than 160 MSTd neurons in three awake, fixating rhesus monkeys. The reverse correlation analysis recovered significantly structured spatial motion preference profiles and partial maps of the RF in ~30% of the cells. Recovering RF maps through reverse correlation was more likely to be successful in cells that were strongly tuned for linear and spiral motion compared to cells with weaker tuning. The recovered maps show a preference to linear motion rather than more complex motion patterns, but the neural responses to spiral and linear motion patterns were significantly correlated with their motion similarity to the reverse correlation maps in about 50% of those cells. Nearly all of the cells showed position invariant responses to spiral motion patterns, i.e., their preferred direction did not change across different parts of the RF.

In conclusion, we show that reverse correlation can be used to recover some of the spatial and directional selectivities of MSTd neurons, especially for neurons that are well tuned for linear and spiral motion. However, the spike-triggered average only provided RF maps that were dominated by homogeneous linear motion preferences. Higher-order analyses might be able to generate maps that can explain the selectivities for more complex motion patterns.

Projections of the hyperpallium in the barn owl (*Tyto alba pratincola*)

Marcus Joseph Wirth[1,2], Hermann Wagner[2]

[1]RWTH Aachen University, Institute of Biology 2, Department of Chemosensation, Worringer Weg 3, 52074 Aachen, Germany
[2]RWTH Aachen University, Institute of Biology 2, Department of Zoology and Animal Physiology, Worringer Weg 3, 52074 Aachen, Germany

In vertebrates, there are two main visual pathways to the telencephalon: the thalamofugal and the tectofugal pathway. In mammals, the thalamofugal pathway is the geniculo-cortical pathway whereas the tectofugal pathway corresponds to the colliculo-thalamo-extrastriate pathway. In birds, the hyperpallium contain the telencephalic visual center, the hyperpallium that is homologue to the visual cortex. The last study of such projections was carried out using degeneration techniques (Karten and Hodos, J. comp. Neurol., 1973). We here present new data from the barn owl. The barn owl constitutes an interesting case for birds, because it has frontally oriented eyes and binocular cells in the hyperpallium.
We pressure injected cholera toxin fragment B to the hyperpallium of barn owls *in vivo* to study the connections of the hyperpallium. Labelling of somata and fibers was detected by standard immunohistochemistry.
We observed retrogradely stained somata and anterograde labelling of fibers in a variety of nuclei. Amongst them were intra-hyperpallial projection, ipsi- and contralateral connections with the N. opticus principalis thalami, and the optic tectum. The anterograde projection to the optic tectum is especially interesting, because recent physiological data suggested relations of activity in the optic to visual search. Our data suggest that there may be telencephalic modulation of the activity of the cells in the optic tectum.

Ocular dominance plasticity and visual properties of PSD-93/-95 double-knockout mice

Rashad Yusifov[1,2], Leon Hosang[1,2], Hendrik Heiser[1,2], Oliver Schlüter[3,4,5], Siegrid Löwel[1,4]

[1]Department of Systems Neuroscience, JFB-Institut für Zoologie und Anthropologie, Universität Göttingen, Germany
[2]Göttingen Graduate School for Neurosciences and Molecular Biosciences, Göttingen
[3]Department of Psychiatry and Psychotherapy, Göttingen, Germany
[4]Sensory Collaborative Research Center 889, University of Göttingen, Germany
[5]Department of Neuroscience, University of Pittsburgh, U.S.A.

Our lab has recently shown that two membrane associated guanylate kinases (MAGUKs), postsynaptic density proteins (PSD)- 93 and 95, have opposing roles in regulating the critical period closure for ocular dominance (OD) plasticity (Huang et al., 2015; Favaro et al., 2018). Maturation of AMPA-silent synapses has been shown to be one of the mechanisms mediating the strengthening the neural circuitry, which leads to the closure of critical periods. While both proteins are known to be involved in AMPA receptor trafficking to the excitatory synapses (Malinow and Malenka, 2002), deletion of these genes have reverse effects on AMPA-silent synapse maturation. Adult PSD-95 KO animals have 9 times more AMPA-silent synapses compared to wild type animals, and exhibit lifelong OD-plasticity (Huang et al., 2015). On the other hand, silent synapses were largely absent in PSD-93 KO mice already in the early critical period which resulted in precocious closure of critical period for OD-plasticity.

Motivated by these results we were interested in checking the OD-plasticity and also visual parameters of PSD-93/-95 double knockout (dKO) mice. We have performed optical imaging of intrinsic signals after 7 days of monocular deprivation (MD), a well-established model for studying cortical plasticity. Intrinsic imaging of visual cortex in adult (> postnatal day 110) PSD-93/-95 dKO animals suggests that in mice lacking both MAGUKs, monocularly depriving animals leads to stochastic outcomes—while some animals showed strong OD-plasticity, in similar number of cases there were no OD-shift.

In addition, we tested visual performance in dKO mice. Although they showed severe impairments in visual water task, their innate defensive response to visual looming test was similar to WT mice (Favaro et al., 2018).

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Poster Topic

T17: Auditory Mechanoreceptors, Vestibular, Cochlea, Lateral Line and Active Sensing

T17-1A Physiological characterization of two fast red-shifted channelrhodopsin variants in the mouse auditory system
Burak Bali, David Lopez de la Morena, Antoine Huet, Vladan Rankovic, Tobias Moser

T17-2A Unraveling potential role of the visual scaffolding protein INAD in hearing
Narges Bodaghabadi, Martin C. Göpfert

T17-3A Loss of inner hair cell ribbon synapses and auditory nerve fiber regression in *Cldn14*$^{-/-}$ mice
Maike Claußen, Hans Gerd Nothwang

T17-4A retracted: Cochlea-specific deletion of $Ca_V1.3$ calcium channels before birth excludes causative the efferent feedback system for IHC immaturity in systemic $Ca_V1.3^{-/-}$ mice and unravels pitfalls of conditional mouse models
Stephanie Eckrich, Dietmar Hecker, Katharina Sorg, Kerstin Blum, Kerstin Fischer, Stefan Münkner, Gentiana Wenzel, Bernhard Schick, Jutta Engel

T17-1B Characterization of sensory heterogeneity among P-type electroreceptors in the weakly-electric fish *A. leptorhynchus*
Tim Hladnik, Jan Benda, Jan Grewe

T17-2B Ultrafast Optogenetic Stimulation of the Auditory Pathway by Targeting-optimized Chronos
Antoine Huet, Daniel Keppeler, David Lopez de la Morena, Vladan Rankovic, Tobias Moser

T17-3B Towards optogenetic cochlea implants in a non-human primate, the common marmoset
Marcus Jeschke, Antoine Huet, Burak Bali, Alexander Meyer, Amirouche Sadoun, Lukasz Jablonski, Vladan Rankovic, Tobias Moser

T17-4B *Drosophila* thermosensation is modulated by a mechano-TRP-channel
Robert Kossen, Andrea Adden, Diego Giraldo, Martin C. Göpfert, Bart R. H. Geurten

T17-1C retracted

T17-2C The complexity of electric social flows of freely behaving weakly electric fish tracked in their natural neotropical habitats
Till Raab, Juan Felipe Sehuanes, Jan Benda

T17-3C Interaural time difference sensitivity at higher pulse rates in an early deafened auditory system
Nicole Rosskothen-Kuhl, Alexa N Buck, Jan W Schnupp

T17-1D Sensory Flow in Electrolocation: Characterizing the Responses of Electroreceptors to Moving Gratings
Carolin Sachgau, Jan Benda, Jan Grewe

T17-2D Retreat site quality outweighs compromised sensory perception in the weakly electric fish *Apteronotus leptorhynchus*.
Juan Felipe Sehuanes, Till Raab, Jorge A. Molina, Jan Benda

T17-3D Exploring the neural basis of pattern recognition in the cricket brain
Xinyang Zhang, Berthold Hedwig

Physiological characterization of two fast red-shifted channelrhodopsin variants in the mouse auditory system

Burak Bali[1,2,3], David Lopez de la Morena[1,2,3], Antoine Huet[1,3], Vladan Rankovic[1,3], Tobias Moser[1,3]

[1] Institute for Auditory Neuroscience and InnerEarLab, University Medical Center Göttingen, Germany. [2] Göttingen Graduate School for Neurosciences and Molecular Biosciences, University of Göttingen, Germany. [3] Auditory Neuroscience and Optogenetics laboratory, German Primate Center, Göttingen, Germany.

Optogenetics has paved the way for precise spatiotemporal control of various cellular activities using light. Hence, it also holds a promise for future optogenetic cochlear implants that are expected to orchestrate the tonotopically-organized cochlea in a finer fashion than the electrical ones. Activation of the spiral ganglion neurons (SGNs) of the auditory nerve practically exemplifies the feasibility of light stimulation using the conventional channelrhodopsin-2 (ChR-2). Yet, slow deactivation and activation of ChR2 initiated a quest for a kinetically more compatible and medicinally more translatable rhodopsin. We have recently reported (Mager T. and de la Morena DL et al., 2018) that a red-shifted fast Chrimson (f-Chrimson) variant is able to stimulate SGNs at high spiking rates. Further single unit recordings at the auditory nerve upon increasing light intensities indicates that optogenetic stimulation offers improved dynamic range and intensity resolution compared to previously reported data with electrical stimulation. Finally, preliminary data using even faster Chrimson mutant; i.e. very fast Chrimson (vf-Chrimson), suggests a further improvement of temporal fidelity in optogenetically driven neural spiking in the auditory nerve.

Unraveling potential role of the visual scaffolding protein INAD in hearing

Narges Bodaghabadi[1], Martin C. Göpfert[1]

[1]University of Göttingen, Department of Cellular Neurobiology, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

The fastest known second-messenger cascade is the phototransduction cascade of *Drosophila*, which operates about 10 times faster than that of mammalian rods. One reason for this exquisite speed seems related to the structural organization of all the cascade components, which are compacted into one macromolecular signalplex, also known as the transducisome. Central to this transducisome is INAD, a 674 amino acid scaffolding protein with five PDZ domains that holds together the phototransduction complex. Here, we report on the requirement of INAD for Drosophila hearing, presenting mutant analyses and expression data. We show that mutations in *inaD* cause hearing defects, raising the possibility that, analogous to vision, INAD contributes to organizing the auditory transduction complex.
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Loss of inner hair cell ribbon synapses and auditory nerve fiber regression in *Cldn14*$^{-/-}$ mice

Maike Claußen[1], Hans Gerd Nothwang[1]

[1]University of Oldenburg, Department for Neurosciences, Carl-von-Ossietzky Str 9-11, 26129 Oldenburg, Germany

Hearing depends on the proper transmission of mechanical stimuli into neuronal signals by the inner hair cells and the propagation of these signals along the auditory nerve fibers to the brain. Loss of auditory hair cells often results in auditory nerve degeneration. However, the presence and proper functioning of the auditory nerve is a prerequisite for auditory rehabilitation in human patients. Here we characterized the extent and time course of synapse loss and auditory nerve fiber regression in the *Cldn14*$^{-/-}$ mouse model for human autosomal recessive deafness DFNB29.

retracted

Cochlea-specific deletion of $Ca_V1.3$ calcium channels before birth excludes causative role of the efferent feedback system for IHC immaturity in systemic $Ca_V1.3^{-/-}$ mice and unravels pitfalls of conditional mouse models

Stephanie Eckrich[1], Dietmar Hecker[2], Katharina Sorg[2], Kerstin Blum[1], Kerstin Fischer[1], Stefan Münkner[1], Gentiana Wenzel[2], Bernhard Schick[2], Jutta Engel[1]

[1]Saarland University, Department of Biophysics, Center for Integrative Physiology and Molecular Medicine (CIPMM), 66421 Homburg, Germany

[2]Saarland University, Department of Otorhinolaryngology, 66421 Homburg, Germany

$Ca_V1.3$ voltage-gated Ca^{2+} channels are crucial for the function of inner hair cells (IHCs): before the onset of hearing (postnatal day 12 in mice), IHCs generate Ca^{2+} action potentials that drive terminal maturation of IHCs and the auditory pathway. In mature IHCs, Ca^{2+} currents elicit transmitter release. IHCs of deaf systemic $Ca_V1.3^{-/-}$ mice remain immature with persistent expression of SK2 and lack of BK K^+ channels. SK2 channels mediate transient efferent inhibition of neurons from the superior olivary complex (SOC) onto the spontaneously active immature IHC. $Ca_V1.3$ is expressed in SOC neurons and plays an important role for their survival, health and function. Persistence of SK2 channels and lack of BK channels in IHCs of systemic $Ca_V1.3^{-/-}$ may thus result from malfunctioning SOC neurons [Hirtz (2011) J Neurosci 31:8280]. To separate these multiple roles of $Ca_V1.3$ in IHCs and auditory pathway, we analyzed *Pax2::cre;Cacna1d-eGFP$^{flex/flex}$* and *Pax2::cre;Cacna1d-eGFP$^{flex/-}$* mice with a cochlea-specific $Ca_V1.3$ knockout from embryonal day 9.5 coupled to GFP (flex switch) reporter function [Satheesh (2012) HumMolGen 21:3896; Ohyama (2004) Genesis 38:195].

We analyzed Ba^{2+} currents through $Ca_V1.3$ channels using whole-cell patch clamp recordings of IHCs from 3 week-old mice. Expression of IHC proteins was studied by immunolabeling of whole-mount organs of Corti. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded from 4-6 week-old mice to assess hearing function.

Lack of Ba^{2+} currents, profound hearing loss, persistence of SK2 channels and lack of BK channels in *Pax2::cre;Cacna1d-eGFP$^{flex/-}$* mice recapitulated the phenotype of systemic $Ca_V1.3^{-/-}$ mice. In addition, we noticed GFP toxicity resulting in death of basal coil IHCs of *Pax2::cre;Cacna1d-eGFP$^{flex/flex}$* but not *Pax2::cre;Cacna1d-eGFP$^{flex/-}$* mice, which was most likely caused by high GFP concentration and small repair capacity. Moreover, the success of Pax2-driven cre in inactivating both *Cacna1d-eGFP^{flex}* alleles was limited, leading us to mainly study *Pax2::cre;Cacna1d-eGFP$^{flex/-}$*. Notably, IHCs of control *Cacna1d-eGFP$^{flex/-}$* mice without cre expression showed a significant reduction in $Ca_V1.3$ channel cluster sizes and currents, suggesting that the intronic construct interfered with gene translation or splicing.

The $Ca_V1.3^{-/-}$-like phenotype of *Pax2::cre;Cacna1d-eGFP$^{flex/-}$* mice indicates that during terminal differentiation, K^+ channel expression is intrinsically regulated by the IHC rather than the efferent input.

Additionally, the pitfalls found in our mice are likely to be a general problem of many genetically modified mice with complex or multiple gene-targeting constructs, showing that great caution and appropriate controls are required.

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Characterization of sensory heterogeneity among P-type electroreceptors in the weakly-electric fish *A. leptorhynchus*

Tim Hladnik[1], Jan Benda[1], Jan Grewe[1]

[1]University of Tübingen, Neuroethology, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Neuronal convergence is a common theme in sensory coding. Theoretical approaches predict that integration over a heterogeneous population of input neurons can be beneficial for the encoding of sensory signals. A well studied example of diversely coding sensory neurons are the P-type electroreceptors of the weakly-electric fish's active electrosensory system.

Weakly-electric fish use their self-generated electric field to navigate their environment and to communicate with conspecifics. Tuberous electroreceptors are tuned to frequencies close to the discharge rate of their own electric organ. P-units, a subpopulation of the tuberous receptors, fire phase-locked to the electric oscillations in a probabilistic fashion whereas the spiking probability depends on the amplitude of the electric field. Their firing rate thus encodes amplitude modulations of the electric field which can carry information about objects or conspecifics, as well as a fish's identity and communication signals.

P-units are distributed across the whole body and each projects onto pyramidal cells in three topographically organized maps in the fish's medullary electrosensory lateral line lobe (ELL). Any pyramidal cell integrates the input from 10s to 1000s of P-units. These P-units show marked differences in their baseline response properties and in the way they encode sensory stimuli. Stochastic resonance effects as described in theoretical accounts could be exploited if the inputs to pyramidal neurons were indeed heterogeneous. We here investigate if sensory coding properties are unordered, or if they vary consistently along the surface of the fish's body.

We made axonal electrophysiological recordings of P-units in the lateral line nerve of *Apteronotus leptorhynchus*, characterized their coding properties and related them to the receptor's position on the fish's longitudinal axis.

Ultrafast Optogenetic Stimulation of the Auditory Pathway by Targeting-optimized Chronos

Antoine Huet[1], Daniel Keppeler[1], David Lopez de la Morena[1,3], Vladan Rankovic[1,2], Tobias Moser[1,4,5,6,7,8]

[1]University Medical Center Göttingen, Institute for Auditory Neuroscience, 37075 Göttingen, Germany
[2]Restorative Cochlear Genomics Group, German Primate Center, Göttingen, Germany
[3]Göttingen Graduate School for Neurosciences and Molecular Biosciences, University of Göttingen, Göttingen, Germany
[4]Biophysics of Neural Computation Group, Bernstein Center for Computational Neuroscience Göttingen, Göttingen, Germany
[5]Max Planck Institute for Experimental Medicine, Göttingen, Germany
[6]Auditory Neuroscience and Optogenetics Laboratory, German Primate Center, Göttingen, Germany
[7]Collaborative Research Center 889, University of Göttingen, Göttingen, Germany
[8]Center for Nanoscale Microscopy and Molecular Physiology of the Brain, Göttingen, Germany

Optogenetic stimulation of spiral ganglion neurons (SGNs) in the ear provides a future alternative to electrical stimulation used in cochlear implants. As light can be conveniently confined in space, optical stimulation promises to increase the number of independent stimulation channels. However, most channelrhodopsins do not support the high temporal fidelity pertinent to auditory coding because they require milliseconds to close after light-off. The opsin Chronos could overcome this main limitation by ultrafast closing kinetics (Klapoetke et al., 2014). Using a viral approach for in vivo transduction of SGNs, we successfully expressed Chronos in mice and gerbils. In order to enhance Chronos expression at the plasma membrane, we improved its trafficking to the plasma membrane (Chronos-ES/TS). Following efficient transduction of SGNs using early postnatal injection of the adeno-associated virus AAV-PHP.B into rodent cochlea, fiber-based optical stimulation elicited optical auditory brainstem responses (oABR) with minimal latencies of 1 ms, thresholds of 5 µJ and 100 µs per pulse, and sizable amplitudes even at 1000 Hz of stimulation. Recordings from single SGNs demonstrated high temporal fidelity of light-evoked spiking. To conclude, efficient virus-mediated expression of targeting-optimized Chronos-ES/TS achieves ultrafast optogenetic control of neurons.

Towards optogenetic cochlea implants in a non-human primate, the common marmoset

Marcus Jeschke[1,3,4], Antoine Huet[1], Burak Bali[1,2,3,4], Alexander Meyer[1], Amirouche Sadoun[3,4], Lukasz Jablonski[1,3], Vladan Rankovic[1,5], Tobias Moser[1,3,6,7]

[1]Institute for Auditory Neuroscience and InnerEarLab, University of Göttingen Medical Center, Germany
[2]Göttingen Graduate School for Neurosciences and Molecular Biosciences, University of Göttingen, Germany
[3]Auditory Neuroscience and Optogenetics Group, Deutsches Primatenzentrum, Göttingen, Germany
[4]Cognitive Hearing in Primates Group, German Primate Center, Göttingen, Germany
[5]Restorative Cochlear Genomics Group, German Primate Center, Göttingen, Germany
[6]Max Planck Institute for Experimental Medicine, Göttingen, Germany
[7]Collaborative Research Center 889, University of Göttingen, Göttingen, Germany

Disabling hearing loss affects a large number of people worldwide and is partially restored by electrical cochlear implants which directly stimulate the auditory nerve. Electrical cochlea implants provide the majority of users with open speech comprehension in quiet. Nonetheless, limitations linked to the poor frequency resolution of electrical stimulation caused by the large current spread around each electrode contact remain.

Optogenetic cochlea implants promise greatly enhanced frequency resolution as light can be conveniently confined in space. Work on rodents has provided proof of principle for optogenetic manipulation of neurons via viral gene transfer, activation of the auditory pathway and auditory percepts by optical cochlear implants. However, before optogenetic cochlea implants can be translated to clinical trials, experiments in non-human primates need to be conducted to 1) establish the efficacy and safety of optogenetic cochlea stimulation as well as to 2) compare electric and optical stimulation.

Towards this goal, our lab is currently establishing behavioral and electrophysiological experiments with electrical cochlea implants in common marmosets as well as is investigating gene transfer of optogenetic constructs into the primate cochlea via adeno associated virus. Three animals were deafened and received an electrical cochlea implant on the same side. Intraoperative monitoring of electrode impedances and middle ear reflexes confirmed successful placement. Preliminary results showed the expected electrical activation of the auditory pathway by postoperative audiological assessment as well as behavioral experiments. We adopted a retroauricular approach to inject a viral construct containing one of two channelrhodopsin variants in 4 additional animals and will report on the functional and morphological analysis of the outcome.

Together the presented work lays to groundwork to compare electrical and optical cochlea stimulation in behavioral and electrophysiological experiments.

Drosophila thermosensation is modulated by a mechano-TRP-channel

Robert Kossen[1], Andrea Adden[1,2], Diego Giraldo[1], Martin C. Göpfert[1], Bart R. H. Geurten[1]

[1]Department of Cellular Neurobiology, Georg-August-Universität Göttingen, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

[2]current adress: Lund Vision Group, Lund University, Sölvegatan 35, 223 62 Lund, Sweden

Accurate thermosensation is critical for survival. Animals exhibit temperature preference and distinctive avoidance behaviour, in order to prevent injury/damage and in the case of poikilotherm animals, to maintain optimal metabolic function. These processes are based on specific and highly sensitive thermosensors. A set of thermo-receptive neurons, which show a very distinct response pattern to heat and cold, can be found in the arista of *Drosophila melanogaster*, and we now report that a prime mechanotransduction channel functions in a subset of these receptor cells.

In addition to the aristas role in audition, it harbours 6 thermosensitive neurons consisting of two sub-groups of 3 cells each: One group exhibiting excitatory responses to temperature increments, referred to as hot-cells, while the second group, referred to as cold-cells, reacts to temperature decrements in an excitatory pattern.

In the study presented here, expression analysis revealed that the hot-cells specifically express a mechano-TRP, and we show how this TRP-channel modulates the responses of these thermosensory neurons. Employing *in vivo* calcium imaging, confocal microscopy as well as behavioural essays, we show that a genetic knockout/knockdown of said channel leads to a decrease in neuronal response amplitudes, together with an altered temperature preference behaviour. Furthermore, it is shown that the thermosensory neurons in the arista do not encode absolute temperature, rather functioning as relative temperature sensors.

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The complexity of electric social flows of freely behaving weakly electric fish tracked in their natural neotropical habitats

Till Raab[1], Juan Felipe Sehuanes[1], Jan Benda[1]

[1]Neuroehology and CIN, University of Tübingen, Germany

To create an accurate representation of the world sensory systems need more than a static stimulus. Sensory flow, the spatio-temporal shift across a sensory surface, also contains important information for guiding appropriate sensorimotor and behavioral responses. While optic flows and their processing in visual systems are well understood, sensory flows in other modalities are less well understood. Of particular interest is the electrosensory system of weakly electric fish, since it resembles many aspects of visual systems, but has different physical properties. For example, electric field lines are curved in contrast to straight light beams. We just begin to understand the physics of electric flows and their relevance for weakly electric fish behavior.

The EOD together with electroreceptive neurons form the active electrosensory system of weakly electric fish used for navigation, foraging and communication. The frequency of the EOD is specific for individual fish and conveys social information about species, sex, and potentially hierarchical position and motivational status. While objects evoke only tiny electrosensory signals, communication signals between individuals can be much larger in amplitude. Consequently, only close by objects are detected by the fish, whereas other fish are recognized over much larger distances. Thus, the latter gives rise to a specific electric flow which we call “social flow”. Both the relative movements of the fish as well as their relative EOD frequencies shape the electric flow signal that each fish is sensing.

We studied a population of weakly electric fish of the species *Apteronotus leptorhynchus* in a neotropical stream in Colombia. We used a grid of 64 electrodes to continuously record the electric signals of the population for several consecutive days. We use power spectrum analysis to extract EOD frequencies from the recorded data and developed a tracking algorithm based on EOD frequency and individual field structures, conducted from the signal strength on the different electrodes, to track individual fish.

In our data we detected at any time about 20 fish within the electrode array covering 3,50 by 3,50 m. So each fish's EOD is modulated by the presence of many fish. Unexpectedly, we found many fish with very similar EOD frequencies in close vicinity. This gives rise to complicated and slow EOD amplitude modulations, in the same frequency band as distortions caused by objects. We estimated the EOD amplitudes of each individual fish and used this data to compute the frequencies and amplitudes of the resulting sensory flows each fish receives while swimming within this population of fish.

Interaural time difference sensitivity at higher pulse rates in an early deafened auditory system

Nicole Rosskothen-Kuhl[12], Alexa N Buck[2], Jan W Schnupp[2]

[1]Neurobiological Research Laboratory, Section for Clinical and Experimental Otology, University Medical Center Freiburg, Freiburg, Germany

[2]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, SAR China

Sound localization is one of the major challenges for bilateral cochlear implant (CI) users. To date they have a limited perception of binaural cues, especially of interaural time difference (ITD) sensitivity. While studies on human implantees conclude that the issue lies in the lack of early sensory input, our recent study on neonatally deafened, CI-implanted rats has shown that ITD sensitivity can be developed independent from early sensory input if the bilateral CIs are synchronized. Here, we investigate to what extent the pulse rate influences the ITD performance of early deaf CI users.

We used the adult Wistar rat as a new model to investigate binaural hearing with electrical intracochlear stimulation. Deafness was induced neonatally by kanamycin and verified by measuring auditory brainstem responses. In young adulthood, CI-electrodes were inserted into the middle turn of both cochleae. Sensitivity for ITDs at different pulse rates (50, 300, 900 Hz) was studied by training CI-implanted rats on a two-alternative forced choice stimulus lateralization task. During training, CI-rats were connected to an external stimulator and trained on ITD discrimination by using binaural, biphasic stimuli.

In a matter of a few weeks of training, all neonatally deafened CI-rats showed microsecond ITD sensitivity when provided with precise ITD cues right from the start of first stimulation. Within the rats' physiological range of +/- 160 µs, surprisingly good ITD discrimination was found independent from the pulse rate although the performance was slightly better for lower rates. This ITD performance compares with that achieved by normal hearing rats.

Using synchronized CI stimulation immediately after bilateral CI implantation allows an inexperienced, adult auditory system to develop ITD discrimination within the normal range. Thereby, electric stimulation rates of up to 900 Hz do not prevent the development of ITD discrimination. These findings suggest that, given synchronized CI stimulation from the time of implantation, even early deafened CI patients with clinical stimulation rates should be able to use ITDs for sound localization.

Sensory Flow in Electrolocation: Characterizing the Responses of Electroreceptors to Moving Gratings

Carolin Sachgau[1], Jan Benda[1], Jan Grewe[1]

[1]Universität Tübingen, Neurobiologie, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Sensory flow, the spatiotemporal shift of a stimulus across a sensory surface, is a rich source of information which is crucial for guiding appropriate sensorimotor and behavioural responses. Sensory flow is well documented in the visual sense, but is less well understood in the electrosense. The gymnotiform wave-type weakly electric fish is known for its ability to actively sense objects in the water, a sense which is influenced by the object's size, shape, material, lateral distance, and speed, and the conductivity of the surrounding water. In contrast to visual images, electric images are blurry, because unlike the visual sense there is no mechanism to focus the image. When a weakly electric fish is presented with two objects separated by a gap, the electrical signal of this gap becomes weak in the electric image on the fish's skin surface at very short distances lateral to the fish. Yet, weakly electric fish are able to behaviourally distinguish gaps at much better spatial acuity than these electric images suggest, even in the presence of large background signals. It has been shown that these fish extract information from spatio-temporal patterns such as motion parallax. Here, we aim to characterize the neuronal mechanisms that underlie sensory flow in electroreception. Similarly to the well-studied examples of optic flow, we pass simple gratings along the longitudinal axis of the gymnotid weakly electric fish *Apteronotus leptorhynchus*, which stimulate electroreceptors distributed across its skin surface and elicit a sensory flow pattern. We record responses via intracellular recordings from tuberous electroreceptors (P-units) in the lateral line nerve of the fish. By varying the size of gaps, lateral distance, and the speed of the grating, and by varying the water conductivity of the surrounding water, we attempt to elucidate some of the spatiotemporal dynamics involved in the neural encoding of sensory flow in electroreception. Through this, we hope to establish the thresholds of these varying parameters via electroreceptor responses. This may lead to future work on the multimodal integration of sensory flow in higher brain regions, speciation of electrosensory flow in related fish species, and the finding of commonalities in the neural structures involved in sensory flow in other vertebrates and other sensory systems, ultimately including human sensory flow processing.

Retreat site quality outweighs compromised sensory perception in the weakly electric fish *Apteronotus leptorhynchus*.

Juan Felipe Sehuanes[1], Till Raab[1], Jorge A. Molina[2], Jan Benda[1]

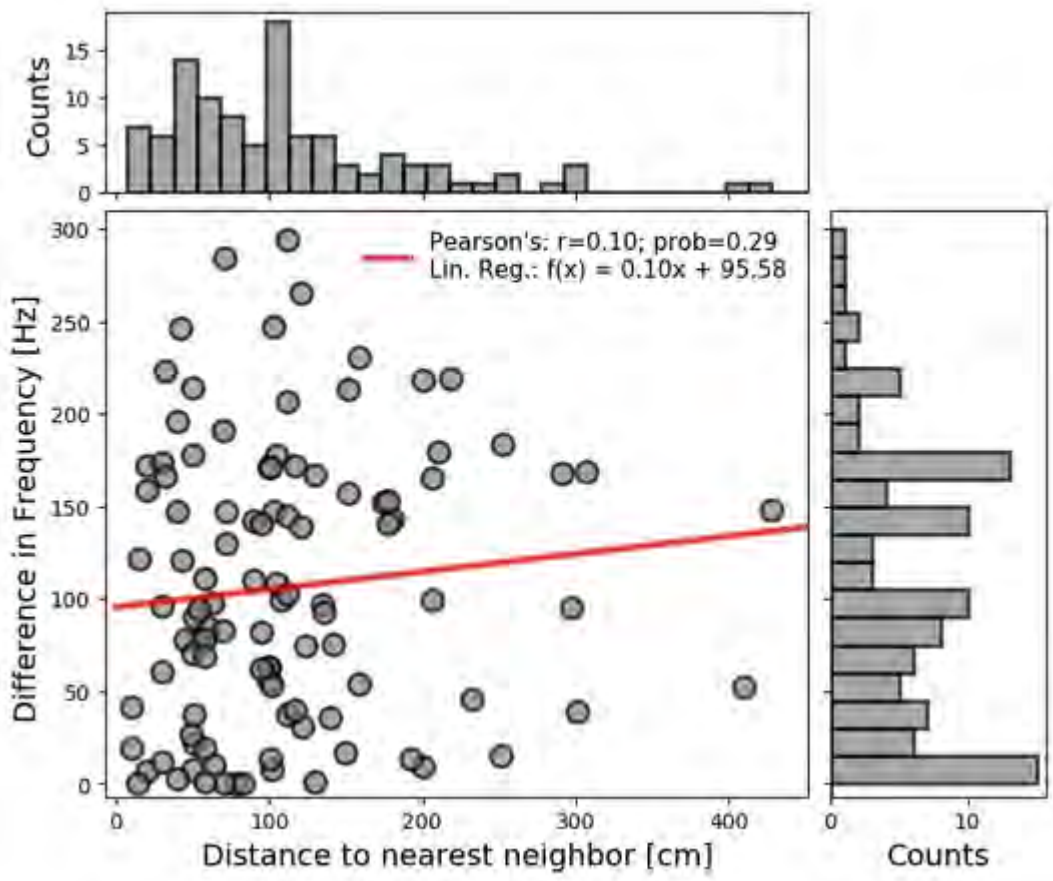
[1]Department of Neuroethology, Eberhard Karls Universität Tübingen, Germany

[2]Centro de Investigaciones en Microbiología y Parasitología Tropical CIMPAT, Universidad de los Andes, Colombia

The physiology and performance of every animal is influenced by the resting habitat it chooses to settle in during phases of inactivity. Establishing a proper retreat site can boost fitness, especially if it grants good protection from adverse weather or potential predators. Gymnotiforms are South American weakly electric fishes that use electric organ discharges (EOD) for electrolocation and communication. During the day these nocturnal animals are commonly found hidden in tree root systems at river banks, under fallen leaf litter, submerged logs, or stones. Wave-type weakly electric fish, like the brown ghost knife-fish *Apteronotus leptorhynchus*, continuously produce a periodical electric field that does not shut off during their resting phase. The fields of nearby fish with similar EOD frequencies cause a potential jamming signal that could impair the fish's electrolocation performance.

In this study we examined retreat site preferences of the brown ghost knife-fish in their natural habitat at a small stream of the Orinoco basin within the Colombian Llanos. We sampled 270m of this small river by carrying out diurnal transects in which we recorded 174 fishes during their inactive phase. Nearly all individuals preferred resting under stacked layers of stones that created underwater tunnel-systems, regardless if they had to share it with several conspecifics. Contrasting with previous studies that described A. leptorhynchus as a territorial species, we found brown ghost knife-fishes at densities higher than expected, with 50% of neighboring fishes hiding within 100 cm of each other. The distribution of the fishes within the habitat was rather independent, consistent with a spatial Poisson process. We registered EOD differences in frequency of adjacent individuals were as low as 3 Hz and were not correlated with distance between fish (see Figure).

Our results suggest that for A. leptorhynchus, the benefits of a tunnel-system micro-habitat as retreat sites that are resilient against floods and ballast outweigh the potential impairment of their electrolocation ability caused by jamming signals of multiple conspecifics.


Counts
15
10
5
0
Pearson's: r=0.10; prob=0.29
Lin. Reg.: f(x) = 0.10x + 95.58
Difference in Frequency [Hz]
300
250
200
150
100
50
0
0
100
200
300
400
Distance to nearest neighbor [cm]
0
10
Counts

Exploring the neural basis of pattern recognition in the cricket brain

Xinyang Zhang[1], Berthold Hedwig[1]

[1]University of Cambridge, Department of Zoology, CB2 3EJ, Cambridge, United Kingdom

A long-standing question in cricket neurobiology is what kind of neuronal mechanism the females use, to recognise the temporal pattern of the male calling song (Weber and Thorson 1989). Recent intracellular recordings of auditory brain neurons indicate a delay-line and coincidence detector circuit underlying auditory pattern recognition in the bi-spotted cricket Gryllus bimaculatus (Schöneich et al 2015). Behavioural tests with systematic variations of single pulse durations or inter-pulse intervals provide support for such a mechanism. These tests also led to the design of a chirp pattern with 5-20-50 ms pulses that is attractive when played forward, but non-attractive when the same pulses are played backwards as a 50-20-5 ms chirp pattern (Hedwig and Sarmiento-Ponce 20117). Here we use these two different chirps to further explore neuronal processing in the pattern recognition network in the brain. We expect that the response of pattern recognition neurons to these chirps will be substantially different and will reflect the different phonotactic responses.
Intracellular recordings and staining of auditory brain neurons are performed in crickets that are walking on a trackball. We aim to characterise the activity of the crucial neurons in the pattern recognition network, - i.e. the activity of the non-spiking delay-line neuron LN5, the coincidence detector neuron LN3 and the final feature detector LN4 - when these are exposed to the attractive and the non-attractive chirps.

First recordings of LN5 – the non-spiking neuron - show that tested by the attractive sound pattern (5-20-50) there is always an EPSP elicited at the second sound pulse (20ms) whereas the neuronal activity is fully suppressed during the full chirp tested with the non-attractive pattern (50-20-5). Meanwhile, the coincidence detector neuron LN3 and feature detector neuron LN4 constantly show a stronger activity at the 20ms pulse when tested with the attractive pattern but a decreasing spike rate with the non-attractive pattern.

Poster Topic

T18: Auditory System: Subcortical and Cortical Processing

T18-1A Complex Sound Processing by Multi-peaked Neurons in Mouse Auditory Brain Centers
Alexander Grigorievich Akimov, Marina Alexandrovna Egorova, Guenter Ehret

T18-2A Optogenetic stimulation of the ventral tegmental area affects early intracolumnar and late cortico-cortical tone-evoked processing in gerbil primary auditory cortex
Michael GK Brunk, Katrina E Deane, Martin Kisse, Mathias Deliano, Michael T Lippert, Frank W Ohl, Max FK Happel

T18-3A EEG neurofeedback: Emphasis on source activity from auditory cortex in patients with chronic tinnitus
Manuel Czornik, Azim Malekshahi, Herbert Bauer, Jürgen Dax, Christoph Braun, Niels Birbaumer

T18-4A Tone-evoked current source density patterns between the awake and anesthetized auditory cortex of Mongolian gerbils indicates differential recruitment of inhibitory microcircuitry
Katrina E Deane, Michael GK Brunk, Marina M Zempeltzi, Frank W Ohl, Max FK Happel

T18-5A Improved spectral resolution of optogenetic vs electric stimulation of the auditory nerve
Alexander Dieter, Marcus Jeschke, Tobias Moser

T18-6A Compensatory Activity in the Auditory Midbrain after Acoustic Trauma Indicates Hidden Hearing Loss
Eva B.S. Dunkel, Natascha Hofmann, Manuela Nowotny, Bernhard H. Gaese

T18-8A Assembly of auditory circuits in the absence of neurotransmission
Lena Ebbers, Christoph Körber, Hannes Maier, Marc August Willaredt, Heiner Hartwich, Hans Gerd Nothwang

T18-9A Recovery from Adaptation in Mouse Auditory Midbrain Neurons: Frequency Effects of Deviant Tones in Tone Sequences
Marina Alexandrovna Egorova, Alexander Grigorievich Akimov, Gleb Dmitrievich Khorunzii, Guenter Ehret

T18-10A Population Responses to Single and Competing Stimuli in the Barn Owl's Auditory Space Map
Roland Ferger, Michael V Beckert, Keanu Shadron, Brian J Fischer, José L Peña

T18-11A Processing of fast temporal modulations in bat auditory cortex matches communication call specific sound features.
Uwe Firzlaff, Stephen Hörpel

T18-12A The modulatory effect of pentobarbital in the auditory brainstem: evidence against GABAergic synapses in the lateral superior olive
Jonas Martin Fisch, Ayse Maraslioglu, Eckhard Friauf

T18-13A Neuronal coding of natural distress sequences in the inferior colliculus
Eugenia González Palomares, Francisco García-Rosales, Manfred Kössl, Julio C Hechavarría

T18-1B Effects of low-level activation of parvalbumin-positive interneurons on cortical processing in mouse A1
Tina Gothner, Pedro J. Gonçalves, Maneesh Sahani, Jennifer F. Linden, K. Jannis Hildebrandt

T18-2B Event-related EEG correlates of the processing of a metrical beat: in search for components of entrainment and prediction.
Manon Grube, Tamer Ajaj, Benjamin Blankertz, Kai Alter

T18-3B Localization of sound source approaching and receding in case of high-frequency hearing loss modeling in humans
Alisa Petrovna Gvozdeva, Elena Alexandrovna Ogorodnikova, Irina Germanovna Andreeva

T18-4B Reduced sound-evoked and resting-state BOLD fMRI connectivity in tinnitus
Benedikt Hofmeier, Stephan Wolpert, Ebrahim Saad Aldamer, Moritz Walter, John Thiericke, Christoph Braun, Dennis Zelle, Lukas Rüttiger, Uwe Klose, Marlies Knipper

T18-5B Auditory illusion in owls predicted by a probabilistic model of rival neuron populations
Lutz Kettler

T18-6B Role of of peripheral BDNF for auditory perceptional learning?
Marlies Knipper, Philipp Eckert, Marie Manthey, Lucas Matt, Philine Marchetta, Wibke Singer, Michael Walter, Peter Ruth, Thomas Schimmang, Peter Pilz, Lukas Rüttiger

T18-7B Strain as a Risk Factor for Tinnitus and Noise-Induced Hearing Loss in Rats
Lisa Koch, Bernhard H. Gaese, Manuela Nowotny

T18-8B Cellular neuroenergetics in the lateral superior olive
Lars Kunz, Sonja Brosel, Rebecca Hessmer, Benedikt Grothe

T18-9B Properties of Endbulb of Held Synaptic Transmission in the Mongolian Gerbil
Thomas Künzel, Kerstin Doerenkamp, Stefanie Kurth, Charlene Gillet

T18-10B Auditory Brainstem Responses Originating from Axonal Terminal Zones in the Auditory Brainstem of the Barn Owl
Paula T. Kuokkanen, Anna Kraemer, Nadine Thiele, Richard Kempter, Christine Köppl, Catherine E. Carr

T18-11B Neuronal Encoding of Behaviorally Relevant Sound Source Locations in Primary Auditory Cortex
Diana Inês Lopes Amaro, Michael Pecka

T18-12B Localization of AP-2δ Expression in the Chicken Embryo
Harald Luksch, Carina Schaub, Markus Moser, Benjamin Schusser

T18-1C State dependence of stimulus adaptation in the auditory cortex of Mongolian gerbils
Jing Ma, Michael GK Brunk, Marina Zempeltzi, Katrina E Deane, Max FK Happel, Reinhard König, Matthias Deliano

T18-2C A real-time EEG source activity from auditory cortex in patients with chronic tinnitus
Azim Malekshahi, Manuel Czornik, Herbert Bauer, Jürgen Dax, Christoph Braun, Niels Birbaumer

T18-3C Basic response properties in the auditory cortex and the frontal auditory field of the fruit-eating bat *Carollia perspicillata*
Adrian Mannel, Francisco Garcia-Rosales, Julio Hechavarria

T18-4C Transcriptional profiling of auditory brainstem nuclei in developing mice
Ayse Maraslioglu, Kathrin Kattler, Domenico Del Turco, Eckhard Friauf

T18-5C Comparison of Single Cell Spike Rate and Timing in the Brainstem in Response to Cochlear Implant and Acoustic Stimulation
Michaela Alisa Müller, Barbara Beiderbeck, Benedikt Grothe, Michael Pecka

T18-6C Development of specific functional axon and myelin morphology in auditory brainstem circuits
Alisha L. Nabel, Hilde Wohlfrom, Olga Alexandrova, Michael Pecka, Benedikt Grothe

T18-7C Accelerated recovery of ABR hearing thresholds after mild acoustic trauma in $Ca_V1.3$-$DCRD^{HA/HA}$ mice
Fahmi Nasri Abuqutheileh, Kerstin Blum, Jutta Engel, Simone Kurt

T18-8C Urocortin 3 at the Calyx of Held Increases Excitatory Postsynaptic Currents in the lateral MNTB
Sara Pagella, Conny Kopp-Scheinpflug

T18-9C Neural correlates of visuo-auditory sensory recalibration.
Hame Park, Christoph Kayser

T18-10C Cortical Activation Patterns in Electric Auditory Midbrain Stimulation
Gunnar Lennart Quass, Andrej Kral

T18-11C Laminar activity in the auditory cortex of vocalizing bats
Dennis Röhrig, Francisco García-Rosales, Manfred Kössl, Julio C. Hechavarría

T18-12C The influence of hearing impairment on the McGurk illusion
Stephanie Rosemann, Marie Dewenter, Dakota Smith, Christiane M Thiel

T18-1D Central gain is reduced with Tinnitus but remains unaltered with Hyperacusis in noise-exposed rats
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T18-3D More input = more information?
Acoustic signal processing in a small network
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T18-5D Auditory adaptation to high-frequency mating calls in eneopterine crickets
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T18-6D Rolr of Insular Cortex in Hyperacusis in Rat
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T18-7D Mice lacking the extracellular matrix protein brevican show impaired temporal processing in the inferior colliculus
Mira Türknetz, Jutta Engel, Simone Kurt

T18-8D Neurophysiological evidence for the stochastic resonance model of tinnitus development
Konstantin Tziridis, Evelyn Hammele, Patrick Krauss, Achim Schilling, Sönke Ahlf, Holger Schulze

T18-9D A model system to investigate sensory gating during sleep
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T18-10D Cathodic-leading and anodic-leading intracortical microstimulation differentially activates the auditory cortex
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T18-11D Detection Learning of Optogenetic and Electrical Stimulation in the Auditory Cortex of Mongolian Gerbils
Theresa Christiane Sofia Weidner, Nabila Alam, Martin Deckert, Gonzalo Arias Gil, Armin Dadgar, Frank W Ohl, Kentaroh Takagaki, Michael T Lippert

T18-12D Layer-specific entrainment to acoustic sequences in the Auditory Cortex
Kristin Lisa Weineck, Mira Röhm, Francisco Garcia-Rosales, Julio Hechavarria

Complex Sound Processing by Multi-peaked Neurons in Mouse Auditory Brain Centers

Alexander Grigorievich Akimov[1], Marina Alexandrovna Egorova[1], Guenter Ehret[2]

[1]I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Laboratory of Comparative phisiology of sensory systems, Torez ave., 44, 194223 St.Petersburg, Russia

[2]Institute of Neurobiology, University of Ulm, 89069 Ulm, Germany

Numerous studies of auditory brainstem centers, thalamus and cortex described neurons with multi-peaked frequency receptive fields, i.e. those having more than one excitatory characteristic frequency (see e.g. reviews in Ehret and Romand 1997; Winer and Schreiner, 2005, 2011). Multi-peaked neurons were found in the dorsal cochlear nucleus, auditory midbrain (central and dorsomedial nuclei of the inferior colliculus), auditory thalamus (medial geniculate body), and primary auditory cortex. In present study, we compare basic spectro-temporal characteristics of multi-peaked neurons from several auditory brain centers of the house mouse: auditory midbrain (central and dorsomedial nuclei) and primary auditory cortex (primary and anterior auditory fields) in order to avoid possible species differences in the evaluation of the development of a seemingly general spectral coding strategy for complex sounds in the ascending auditory pathways, from the periphery to higher centers.

The percentage of neurons with two characteristic frequencies increased from the auditory midbrain nuclei (6% of the central nucleus, 24% of the dorso-medial nucleus) to the primary auditory cortex (33% in the primary and anterior auditory fields). Areas of maximal sensitivity in frequency receptive fields of multi-peaked neurons often corresponded to second and third harmonics in mouse communication calls (mouse pups wriggling call, male's agonistic call and female's defense call; Ehret, 2013) in the low- and mid-frequency range, i.e. less than 30 kHz. Regression analysis revealed strong correlations between low and high characteristic frequencies of multi-peaked neurons with regression coefficients of 1.5 in both midbrain and cortical areas. Sounds of two tones whose frequencies corresponded to the characteristic frequencies in the auditory midbrain multi-peaked neurons often evoked facilitative responses, i.e. response rate was 20% more than sum of responses to each separate tone.

Thus, the functional specialization of multi-peaked neurons in the mouse auditory midbrain and even more in primary auditory cortical fields supports the detection and enhancement of soft second and third harmonics in mouse harmonic communication calls.

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Optogenetic stimulation of the ventral tegmental area affects early intracolumnar and late cortico-cortical tone-evoked processing in gerbil primary auditory cortex

Michael GK Brunk[1], Katrina E Deane[1], Martin Kisse[1], Mathias Deliano[1], Michael T Lippert[1,2], Frank W Ohl[1,2,3], Max FK Happel[1,3]

[1]Leibniz Institut for Neurobiology, Dept. Systems Physiology of Learning, Magdeburg, Germany

[2]Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany

[3]Otto-von-Guericke-University, Magdeburg,Germany

Dopamine belongs to the key neuromodulators regarding learning and task associated processing. Previous studies revealed that dopaminergic levels increase within primary auditory cortex (A1) during initial acquisition learning in a Go/No-Go auditory discrimination task. However, the impact of dopamine on cortical circuits of sensation are still elusive. In a first study, we have shown based on current-source density (CSD) recordings that topical application of the D1/D5 receptor agonist SKF-39383 in A1 promoted a gain enhancement of auditory inputs based on recurrent corticothalamic feedback. In a recently performed multi-taper spectral and time-frequency analysis, we additionally revealed that dopamine increased phase locking of auditory processing in granular layers III-IV within the gamma-band (88-97 Hz) at the columnar best frequency.

In the current study, the goal was to reveal the contribution of the circuitry between the main source of cortical dopamine, the ventral tegmental area (VTA) and A1 to the described effects. We transfected the ipsilateral VTA with an adeno-associated virus containing a C1V1 opsin with an YFP-tag, that expression is controlled by a CAMαII promotor sequence and implanted a fiber for optogenetical stimulation of the VTA. Successful recruitment of reward-related brain circuits was evaluated by self-stimulation behavior. Subsequently, we characterized auditory cortex circuitry activation after pure tone presentation by CSD analysis in anaesthetized animals before, with and after a coupled optical VTA stimulation. Based on linear mixed models, we demonstrate a significant increase of overall columnar current flow and corresponding effects on infra- and supragranular layers during laser stimulation that lasted over half an hour later on, verifying cortical effects of VTA-A1 projections. Our study thereby contributes to a better understanding of the circuit effects of the VTA-based dopaminergic system and its impact on learning-dependent plasticity in sensory cortex.

EEG neurofeedback: Emphasis on source activity from auditory cortex in patients with chronic tinnitus

Manuel Czornik[1], Azim Malekshahi[1], Herbert Bauer[5], Jürgen Dax[2], Christoph Braun[2,3,4], Niels Birbaumer[1,6]

[1]Institute of Medical Psychology and Behavioural Neurobiology, University of Tübingen, Germany
[2]MEG-Center, University of Tübingen, Germany
[3]CIN, Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Germany
[4]Department of Psychology and Cognitive Science, University of Trento, Rovereto 38068, Italy
[5]Faculty of Psychology, Social, Cognitive and Affective Neuroscience Unit (SCAN-Unit), University of Vienna, Vienna, Austria
[6]WyssCenter for Bio and Neuroengineering, Geneva, Switzerland

Subjective chronic tinnitus is a prevalent symptom caused by an auditory sensation in absence of a corresponding acoustic source which can lead to discomfort, pain, and various psychological problems like depression or anxiety. However, even though many attempts have been used, no method of treatment could prove its efficacy universally and in the long term (Dobie, 1999).
One promising approach which could possibly fill this lack of a general effective treatment in the long term is electroencephalography (EEG) neurofeedback. This technique allows patients to learn voluntary control over ordinarily autonomous physiological parameters by operant conditioning and could thereby produce encouraging results (e.g., Dohrmann, Weisz, Schlee, Hartmann, & Elbert, 2007; Gosepath, Nafe, Ziegler, & Mann, 2001; Schenk, Lamm, Gündel, & Ladwig, 2005). The mentioned studies could show a significant reduction in tinnitus-related distress by an upregulation of alpha activity (8–12 Hz) and downregulation in the beta band (14–30 Hz), respectively the delta band (1–4 Hz).
However, the approach used in the present study differs substantially not only due to its focus solely on the auditory cortex alpha band power but also to its innovative setup enabling the suppression of visual and somatosensory alpha activity through visual stimulation and air flow as tactile face-stimulation. Furthermore, the setup included pink noise, also called 1/f noise, as acoustic tinnitus masker. Using this approach, 8 patients underwent 15 neurofeedback sessions at the Institute of Medical Psychology and Behavioural Neurobiology, Tübingen. Each session comprised 4 runs, starting with 10 seconds of pink noise stimulation as tinnitus maskers and alpha rebounder in auditory cortex. Following, 5-second rest and 12 seconds of alpha-regulation are proceeded. This procedure is repeated.
For some of the patients the presented treatment delivered very promising results leading to a broader use of the setup. Also, the implementation in the therapy of additional symptoms or disorders seems conceivable. Summarized, the utility of the constituted neurofeedback in tinnitus treatment could be conclusively shown. Yet, to determine if it could supersede the present gold standard in tinnitus therapy, cognitive behavioural therapy (CBT), future research is required.

Acknowledgements

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Tone-evoked current source density patterns between the awake and anesthetized auditory cortex of Mongolian gerbils indicates differential recruitment of inhibitory microcircuitry

Katrina E Deane[1], Michael GK Brunk[1], Marina M Zempeltzi[1], Frank W Ohl[1,2,3], Max FK Happel[1,2]

[1]Leibniz Institute for Neurobiology, Systems Physiology of Learning, Brenneckestr. 6, 39118 Magdeburg, Germany

[2] Otto-von-Guericke University, Magdeburg, Germany

[3] Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany

Research on sensory coding has built its foundation on animal models under anesthesia and must now transfer its gained insight into awake experimentation. Many of the studies seeking to bridge the gap between these two contrasting states have done so at a single- or multi-unit level rather than at a population level. In this study, local field potential recording and current source density (CSD) analysis were used to provide a mesoscopic measure of cortical microcircuitry in consideration of spatial and temporal synaptic activity. CSD profiles of primary auditory cortex (ACx) of ketamine-anesthetized Mongolian gerbils (*Meriones unguiculatus*) were compared before and after cortical silencing with muscimol and further compared with CSD data from awake recordings in passively listening animals.
Cortical silencing with muscimol showed significant reduction of tone-evoked activity across all cortical layers. Sink activity was only found in lemniscal thalamocortical input layers IV and Vb/VIa. In comparison to awake recordings, our main finding in the ketamine anesthetized ACx was increased recurrent excitation of early granular sink activity in layers III/IV. We further used analysis of the temporal overall current flow (averaged rectified CSD) and relative residual imbalances of the CSD in order to quantify the contribution of lateral input at a given cortical patch. Under anesthesia we found a sharper columnar frequency tuning mainly due to granular gain enhancement after best-frequency stimulation. Also, we observed that in awake ACx the columnar activity is less stimulus-onset-locked, as compared to the anaesthetized state. We suppose that distinct differences between layer-dependent tuning properties are due to a differential recruitment of the interacting excitatory and inhibitory microcircuitry between the two global brain states. Specifically, strong amplification of preferred frequency in layers III/IV might be prevented in passively, awake listening gerbils by differential recruitment of lateral inhibition.

Improved spectral resolution of optogenetic vs electric stimulation of the auditory nerve

Alexander Dieter[1,2], Marcus Jeschke[1,3,4], Tobias Moser[1,2,3]

[1]University Medical Center Göttingen, Institute for Auditory Neuroscience, Robert-Koch-Straße 40, 37075 Göttingen, Germany

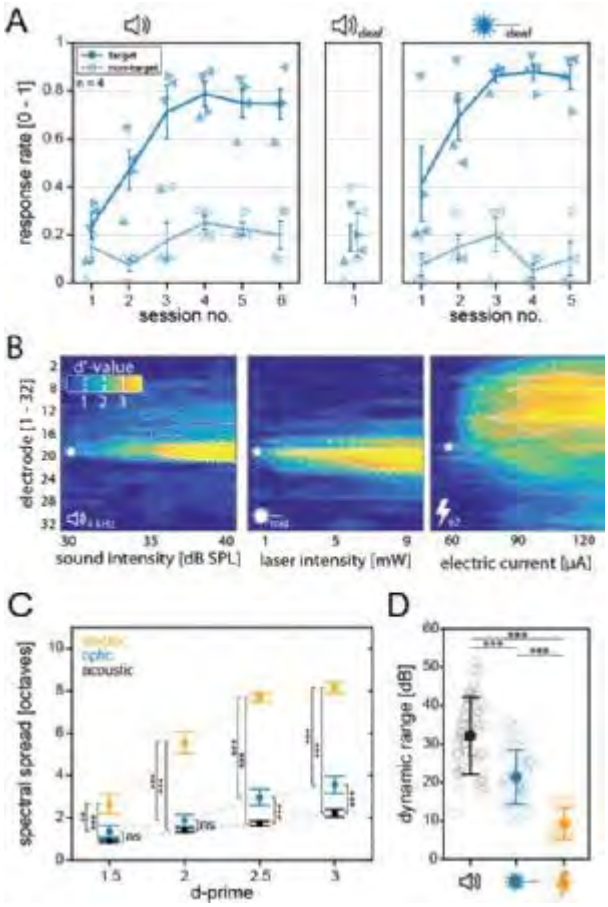
[2]Göttingen Graduate School for Neurosciences and Molecular Biosciences, University of Göttingen, 37075 Göttingen

[3]Auditory Neuroscience and Optogenetics Laboratory, German Primate Center, 37077 Göttingen

[4]Cognitive Hearing in Primates Group, German Primate Center, 37077 Göttingen

Cochlear implants (CIs) electrically stimulate spiral ganglion neurons (SGNs) and partially restore auditory function in half a million profoundly hearing impaired users. However, wide current spread from each stimulation electrode in the cochlea leads to channel crosstalk, limiting the number of independent stimulation channels and thus frequency resolution of artificial sound encoding. Optogenetic stimulation of SGNs – which enables activation of genetically modified photosensitive cells with light – might be a way to overcome this mayor limitation of electrical CIs. Since light can conveniently be confined in space, cochlear optogenetics holds the potential to increase the number of independent stimulation channels and thus frequency resolution of artificial sound encoding.

In this study, SGNs of adult Mongolian gerbils were transduced with AAVs carrying the channelrhodopsin-2 variant CatCh. We performed 32-channel electrophysiological recordings of multi-unit activity in the tonotopically organized inferior colliculus of anesthetized gerbils while stimulating SGNs acoustically, optogenetically or electrically. We then performed an activity-based analysis in order to compare the spread of cochlear excitation upon stimulation with the different stimulation modalities. Our results demonstrate that optogenetic stimulation of the cochlea indeed happens with a higher spectral resolution than monopolar electric stimulation and thus provides a potential approach to overcome the major limitation of electrical cochlear implants.


A
response rate [0 - 1]
session no.
B
d'-value
electrode [1 - 32]
sound intensity [dB SPL]
laser intensity [mW]
electric current [µA]
C
spectral spread [octaves]
d-prime
D
dynamic range [dB]

Compensatory Activity in the Auditory Midbrain after Acoustic Trauma Indicates Hidden Hearing Loss

Eva B.S. Dunkel[1], Natascha Hofmann[1], Manuela Nowotny[1], Bernhard H. Gaese[1]

[1]Cell Biology and Neuroscience, Neurobiologie und Biosensorik, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

Demographical changes in industrial countries with increasing numbers of the elderly and a rising influence of noise pollution (Hoffman et al., 2017) leads to a growing number of people with high risks of having hearing issues. While many issues are related to reduced hearing sensitivity, a clinically inconspicuous audiogram does not guarantee a daily life without hearing problems. The phenomenon of hidden hearing loss (HHL) is associated with normal hearing thresholds, but a reduced speech intelligibility, especially in noisy environments. This does neither affect hearing thresholds, nor is it easily noticeable by a patient, other than tinnitus. In addition, the temporal processing of information is affected (Plack et al, *Trends Hear* 10:1; 2014).

In order to investigate the mechanisms underlying HHL in the rat auditory pathway, we measured ABR and acoustic startle response before and after inducing a trauma with acoustic overstimulation at around 16 kHz. Differences in auditory processing between sensitive and high threshold pathways were investigated by testing acoustic stimuli with and without background noise before and after trauma.

Physiological measurements from the auditory brainstem were taken in eleven female Sprague Dawley rats to determine changes in hearing sensitivity and changes in the activation of auditory structures at different levels of the pathway. A strong elevation of auditory thresholds at and above trauma frequency was found, as expected. Thresholds mostly recovered over a period of three weeks. When measured with a background noise level of 70 dB SPL, we found almost no trauma-induced changes in auditory sensitivity.

In addition, we performed a more detailed analysis of individual peak components in the ABR waveform. The expected reduction of early ABR waves representing the auditory nerve (Schaette McAlpine, *J Neurosci* 31:13452; 2011) was not found. Despite of that, we discovered a compensation in higher auditory centers during recovery over a period of eight weeks. This compensation occurred in ABR wave III, strongly related to the activity of the inferior colliculus in the midbrain. Since this increased activity was only found when stimuli were presented with background noise, the results can indeed be related to characteristics of HHL. The measurements can be used to characterize and track HHL. This can be further applied in following studies, perhaps in combination with prepulse inhibition measurements using a gap in background noise to examine the behavioral effects of this phenomenon.

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Assembly of auditory circuits in the absence of neurotransmission

Lena Ebbers[1], Christoph Körber[2], Hannes Maier[3], Marc August Willaredt[1], Heiner Hartwich[1], Hans Gerd Nothwang[1]

[1]Department of Neuroscience, Carl von Ossietzky University Oldenburg
[2]Institute of Anatomy and Cell Biology, Heidelberg University
[3]VIANNA, Hannover Medical School

Ever since the pioneering work of Hubel and Wiesel in the visual system, the importance of neuronal activity has been recognized as a key determinant for proper development and maturation of neuronal circuits. Peripheral deafness results in reduced neuronal activity in auditory processing centers in the brain. Many studies on the role of activity in the central auditory system used cochlear ablations to analyze the impact of peripheral dysfunction. However, this approach represents a rather drastic procedure which may cause alterations in the central auditory system not present in human patients with congenital or age-related hearing loss.

In order to explore the role of neuronal activity in a more systematic way, a mouse model with inducible and spatially flexible genetic silencing of auditory circuits was generated. This transgenic mouse reversibly expresses the tetanus toxin light chain (TxLC) in a Cre recombinase dependent manner in defined circuits of the central auditory system. TxLC is a protease cleaving the synaptic vesicle associated membrane protein 2 (VAMP2) and thereby blocking neurotransmitter release.

Mice expressing the Cre recombinase under the control of the *Atoh7* promoter (*TxLC*^{Atoh7} mice) show robust expression of TxLC in small spherical bushy cells and globular bushy cells of the anteroventral cochlear nucleus complex (aVCN) in the auditory brainstem. Both bushy cell types are involved in brainstem circuits important for directional hearing. While small spherical bushy cells project to the lateral superior olive (LSO) ipsilaterally, globular bushy cells innervate the contralateral medial nucleus of the trapezoid body (MNTB), which in turn inhibits the ipsilateral LSO.

Electrophysiological analysis of this circuit in *TxLC*^{Atoh7} mice revealed a severe reduction in the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in mature MNTB neurons, confirming functional expression of TxLC in globular bushy cells of the aVCN.

On the anatomical level, there was no significant difference in volume of the MNTB and the LSO in *TxLC*^{Atoh7} mice at P25 compared to wildtype littermates. In addition, assembly of the calyx of Held synapse formed by globular bushy cells onto the MNTB was unaffected at P16, but presynaptic markers were significantly reduced in adult animals.

In a second approach we expressed the Cre recombinase under control of the *Egr2* promoter (*TxLC*^{Egr2} mice) which results in a widespread TxLC expression in the auditory brainstem.

We conducted the same electrophysiological analysis as in *TxLC*^{Atoh7} mice, revealing disturbed sEPSCs, but surprisingly, MNTB neurons were not silenced.

Anatomically, *TxLC*^{Egr2} mice showed a significant reduction in volume of the MNTB and the LSO compared to wildtype littermates. This volume reduction was caused by a reduced number of neurons and decreased soma cross section area of remaining cells.

In addition auditory brainstem recordings in *TxLC*^{Egr2} mice revealed disturbed auditory processing.

In conclusion, our mouse models allow for systematically investigating the impact of absence of neurotransmission on development, maturation and maintenance of discrete auditory circuits in the brainstem.

Recovery from Adaptation in Mouse Auditory Midbrain Neurons: Frequency Effects of Deviant Tones in Tone Sequences

Marina Alexandrovna Egorova[1], Alexander Grigorievich Akimov[1], Gleb Dmitrievich Khorunzii[1], Guenter Ehret[2]

[1]I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Laboratory of Comparative phisiology of sensory systems, Torez ave., 44, 194223 St.Petersburg, Russia

[2]Institute of Neurobiology, University of Ulm, 89069 Ulm, Germany

Recently, we showed that adaptation in mouse auditory midbrain neurons supports perception of tonal streams (Malinina et al., 2016). Equally strong adaptation from the first to the next (second, third, fourth) tones in the sequence of identical tones was noted for inter-tone intervals of 4 – 500 ms. For intervals longer than 500 ms, spike rates to tone bursts did not differ significantly, i.e. adaptation was largely absent. The release from stimulus-specific adaptation in the auditory system has been discussed as main source for detecting deviants in a stream of sounds (Ayala and Malmierca, 2013; Pérez-González and Malmierca, 2014). Here, we study the influence of deviant tone frequencies in tone sequences to which neurons in the mouse auditory midbrain (central nucleus of the colliculus inferior) showed a largely adapted response.

In anesthetized (ketamine/xylazine) mice, single-unit responses were recorded to series of five 100 ms tones with 4 ms inter-tone intervals and spike rates analyzed. The frequency of the first four tones in each series corresponded to a neuron's characteristic frequency (CF). The frequency of the fifth tone varied within the range of -/+ one octave with respect to CF. Thus, the frequencies of the first four and the fifth tones were either in one and the same or in two different critical bands of mouse hearing (Ehret, 1976; Egorova and Ehret, 2008). In 2/3 of the 75 neurons, responses to the second, third and fourth tones were considerably reduced or totally absent compared to the response to the first tone, indicating strong adaptation. Responses to the fifth tone of deviating frequency recovered from adaptation. On average, recovery was strongest when the tone frequency was at a distance of 0.2 octaves from the neuron's CF. Responses to the fifth tone decreased near the border of neuron's excitatory response area. When the excitatory response area extended beyond 1/3 octave from the CF, i.e. beyond the one critical band from the CF, responses to the fifth tone were larger when frequencies of the fifth tone and the CF fell in two non-overlapping critical bands.

In conclusion, frequency-specific adaptation in the mouse inferior colliculus seems to be the neurophysiological origin for novelty reactions to deviating tones. The auditory critical band mechanism emphasizes responses to novelty if the frequency of the deviating tone is at least one critical band away from the adapted ones.

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Population Responses to Single and Competing Stimuli in the Barn Owl's Auditory Space Map

Roland Ferger[1], Michael V Beckert[1], Keanu Shadron[1], Brian J Fischer[2], José L Peña[1]

[1]Albert Einstein College of Medicine, Dominick P. Purpura Department of Neuroscience, Bronx, New York
[2]Seattle University, Department of Mathematics, Seattle, Washington

Since the discovery of a midbrain map of auditory space, the barn owl, a nocturnal hunter with outstanding sound localization abilities, has become a model organism to study neural circuits computing and representing binaural cues relevant for sound localization. Neurons in the owl's midbrain respond maximally to acoustic stimuli with distinct combinations of interaural time and level differences (ITD, ILD). Together, these neurons form a topographic representation of auditory space which supports sound-orienting behavior. However, open questions regarding how the neural population in the map is read out on a trial-by-trial basis remain unanswered. This work, using a microelectrode array to record multiple units across the map, reaches beyond responses of single neurons to investigate the relationship between the activity pattern across the neural population and behavior.

Recent work has shown that the read out of population activity by a population vector (PV) predicts orienting head saccades, and approximates Bayesian statistical inference by integrating the overrepresentation of frontal directions and the differential shape of spatial tuning curves across the map. We show that the trial-by-trial variability of neural activity matched predictions made under a Bayesian model, which matches a PV to the behavioral output. When a stimulus becomes less reliable (e.g. by decorrelation of the binaural signal) an animal’s performance becomes less accurate. The Bayesian model predicts that this should manifest in a broadening of the population response in the map. An alternative hypothesis that could explain this behavioral effect is that the reduced reliability induces shifts in the population response, which are indistinguishable from a response to a different ITD, termed differential correlations. We tested which model was better matched by the trial-by-trial population activity across a number of stimulus conditions. This analysis showed that the population activity and correlation structure matches predictions of the Bayesian/PV model.

Additionally, the PV model could break down when competing stimuli from different directions are presented at the same time. We explored whether the global inhibition network described in the owl's optic tectum for selecting dominant stimuli, could restore population activity patterns consistent with a PV readout. We found that the population activity under two-sound stimulation remained consistent with predictions of a PV readout of the higher priority stimulus.

Processing of fast temporal modulations in bat auditory cortex matches communication call specific sound features.

Uwe Firzlaff[1], Stephen Hörpel[1]

[1]Technische Universität München, Department Tierwissenschaften, Lehrstuhl für Zoologie, Liesel-Beckmann-Str. 4, 85354 Freising, Germany

Bats use a large repertoire of calls for social communication. In the bat Phyllostomus discolor, social communication calls are often characterized by temporal amplitude and/or frequency modulations in the range of 100-150 Hz. However, in the mammalian auditory cortex modulation transfer functions are typically limited to modulation frequencies below 100 Hz.
We investigated the coding of sinusoidally amplitude modulated (SAM) sounds in auditory cortical neurons in P. discolor by constructing rate and temporal modulation transfer functions. Neural responses to playbacks of various communication calls were additionally recorded and compared to the neurons' responses to SAM sounds.
The results show that amplitude modulations around 130 Hz characteristic for e.g. aggression calls evoked best rate responses in a population of neurons located in the posterior dorsal field auf the auditory cortex of P. discolor. Significant phase-locking (quantified as vector strength, VS) of these neurons was observed, with a mean best phase-locking frequency of 93 Hz. Parts of this population responded stronger to aggression calls than to communication calls without strong temporal modulations. Roughly correlating with VS, temporal response patterns often reflected the fast temporal envelope fluctuations of aggressions calls. Thus, the P. discolor dorsal auditory cortex shows an extraordinary ability to encode temporal modulations, which might support species-specific communication.

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The modulatory effect of pentobarbital in the auditory brainstem: evidence against GABAergic synapses in the lateral superior olive

Jonas Martin Fisch[1], Ayse Maraslioglu[1], Eckhard Friauf[1]

[1]Animal Physiology Group, Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany

Glycine and GABA are major inhibitory neurotransmitters in the mammalian central nervous system. By binding at postsynaptic receptors, they elicit inhibitory chloride currents. To distinguish GABA-mediated- from glycine-mediated transmission, receptor antagonists are often employed. However, antagonist-binding sites of $GABA_A$ and glycine receptors are similar. Consequently, unspecific binding of the antagonist is unavoidable, e.g. for gabazine and strychnine (Jonas et al., 1998). The problem can be circumvented by using modulators which bind more specifically. One such modulator is the barbiturate pentobarbital (Pbt), which selectively modulates $GABA_A$ receptors and below concentrations of 100 µM, it has little or no agonist effect on its own (Muroi et al., 2009). Via whole-cell patch-clamp recordings in acute slices of postnatal day (P) 11 mice, we investigated the influence of Pbt on miniature inhibitory postsynaptic currents (mIPSCs) in the inferior colliculus (IC) and the lateral superior olive (LSO). The effect of Pbt on $GABA_A$ receptor mediated currents has recently been shown in the IC (Moore & Trussell, 2017). Therefore, we first analyzed IC neurons to validate Pbt effects on mIPSCs in a proof of concept approach (positive control). In accordance with the findings of Moore & Trussel, Pbt (30 µM) increased the weighted decay time constant τ_w of mIPSCs by > 200 % (Ctrl: 5.59 ± 0,13 ms; Pbt: 12.73 ± 0,30 ms, p = 2*10^-86). However, it neither affected the frequency of mIPSCs (2.0 ± 0.2 vs 1.7 ± 0.4 events/s, p = 0.487) nor the quantal size (55.4 ± 7.0 vs 58.0 ± 6.2 pA, p = 0.804). These results demonstrate prolonged open times of $GABA_A$ receptors by Pbt and thus confirm the existence of functional $GABA_A$ receptors in the IC.

As the positive control experiments had shown that Pbt at 30 µM is able to modulate receptor kinetics in our hands, we turned to the analysis in the LSO. In contrast to the results obtained in the IC, τ_w remained virtually unchanged in the presence of Pbt in the LSO (Ctrl: 2.59 ± 0,05 ms; Pbt: 2.74 ± 0,06 ms, p = 0.061). Like in the IC, there was no change in the frequency of mIPSCs (6.7 ± 0.7 vs 7.4 ± 2.0 events/s, p = 0.768) and the quantal size (49.1 ± 3.5 vs 46.8 ± 2.7 pA, p = 0.711). The results imply the absence of functional synaptic $GABA_A$ receptors in the LSO of P11 mice. However, they cannot rule out the existence of extra synaptic GABAA receptors. We conclude that inhibitory neurotransmission to P11 mouse LSO neurons appears to be mediated purely by glycine. Our results provide some new insights into inhibitory neurotransmission in the auditory brainstem. To investigate whether there is a developmental switch or shift from purely GABAergic to purely glycinergic transmission (cf. Kotak et al., 1998; Nabekura et al., 2004), we currently analyze the inhibitory synaptic inputs to LSO neurons in P4 mice. The results will be demonstrated on the poster.

Neuronal coding of natural distress sequences in the inferior colliculus

Eugenia González Palomares[1], Francisco García-Rosales[1], Manfred Kössl[1], Julio C Hechavarría[1]

[1]Goethe Universität Frankfurt am Main, Institut für Zellbiologie und Neurowissenschaft, , Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

Auditory stimuli are important environmental cues, crucial for animal behaviour. Animal communication calls are a type of such behaviourally relevant sounds. For example, in a menacing situation, animals can emit distress vocalizations to intimidate predators or alert conspecifics. The neuronal responses to these stimuli undergo several computations before they reach the auditory cortex (AC), the highest stage in the auditory hierarchy dedicated specifically to acoustic processing. However, the nature of these neuronal computations at a subcortical level is still not fully understood. Recent work in the auditory cortex of the bat *Carollia perspicillata* demonstrated the multiscale representation of temporal features present in conspecific distress calls, with a population of neurons tracking the slow temporal dynamics of the calls (bout structure) and another one marking their fast temporal structure (individual syllables). Consequently, AC neurons representing complementary temporal scales provided independent information about the natural sequences, based on an information-theoretic framework. Here, we address whether the same task parcelation for the processing of multiple timescales embedded in natural stimuli occurs in lower auditory stages in addition to the AC. For that, we conducted extracellular recordings in the inferior colliculus (IC) while awake bats listened to natural distress vocalizations from conspecifics. Specifically, we addressed the following questions: (1) is the multiscale temporal structure of natural communication sequences represented in the IC with distinct neuronal subpopulations? (2) how does the precision of collicular auditory neurons compare to that of the AC? and (3) does the neuronal spiking in the IC provide independent information, or is it a cortical property that does not appear, at least, up to this level of the auditory pathway? We show that IC neurons represent the stimulus in a more rigorous manner that the AC in terms of synchronization and precision, and that the representation of embedded timescales in natural vocalizations is not a common feature of this lower auditory structure.

Effects of low-level activation of parvalbumin-positive interneurons on cortical processing in mouse A1

Tina Gothner[1], Pedro J. Gonçalves[2], Maneesh Sahani[3], Jennifer F. Linden[4], K. Jannis Hildebrandt[1,5]

[1]Department of Neuroscience, University of Oldenburg, Oldenburg, Germany
[2]Research Center Caesar, Max Planck Society, Germany
[3]Gatsby Computational Neuroscience Unit, University College London, London, UK
[4]Ear Institute and Department of Neuroscience, Physiology, and Pharmacology, University College London, London, UK
[5]Cluster of Excellence Hearing4all, University of Oldenburg, Oldenburg, Germany

Parvalbumin-positive (PV+) interneurons typically synapse onto the axon, soma or proximal dendrites of pyramidal cells and therefore are thought to have a major influence on cortical processing. The modulation of PV+ cell activity provides a means for top-down control of sensory processing and cortical state. While previous work has suggested several possible effects of PV+ interneuron activation using direct and brief optogenetic excitation of PV+ cells, the impact of modulatory, more subtle changes in PV+ interneuron activity on cortical processing remains unclear. We postulate that the overarching effect of PV+ interneuron activation is to suppress recurrent intracortical activity, and therefore that low-level PV+ interneuron activation may dampen intracortical synaptic transmission while leaving thalamocortical synaptic transmission largely unchanged. We therefore predict that prolonged low-level PV+ activation may produce: (1) sharpening of spectral tuning due to reduced intracolumnar spread of activity, (2) more transient responses via a reduction of spontaneous and sustained responses driven in part by intracortical activity, and (3) decorrelation of activity of pairs of cortical neurons.

To test the hypotheses, we recorded auditory cortical response to tones in awake and chronically implanted mice. We increased the activity of PV+ interneurons by prolonged, low-level optogenetic depolarization, using a Stable Step-Function Opsin (SSFO). This enabled us to decouple the timing of optogenetic activation of PV+ interneurons from the timing of the auditory stimulation. We recorded neural data in periods of auditory stimulation either without or with SSFO activated, to compare cortical activity under control conditions and under conditions of sustained activation of PV+ interneurons.

We recorded from 1356 single units in eight animals. We analyzed the responses with respect to changes in (1) spectral tuning, (2) transient vs. sustained responses, and (3) pairwise correlations.

(1) The bandwidth of frequency tuning decreased after PV+ activation, mostly due to stronger reduction of responses at frequencies flanking the units' best frequencies.
(2) Sustained firing rates during acoustic stimulation were more strongly affected by PV+ activation than spontaneous firing rates, while onset responses to sound were least affected. The ratio between onset and spontaneous activity increased after activation for the majority of units.
(3) For the majority of simultaneously recorded cell pairs, both noise and signal correlations were reduced after PV+ activation. For a small fraction with unusually high pairwise correlations, these correlations increased after activation.

In summary, our data on the effects of low-level modulation of PV+ cell activity are consistent with overall suppression of recurrent and intracortical activity, shifting cortical processing towards a more sensory, feed-forward input and away from intracortical processes.

Event-related EEG correlates of the processing of a metrical beat: in search for components of entrainment and prediction.

Manon Grube[1,2,3], Tamer Ajaj[3], Benjamin Blankertz[3], Kai Alter[4]

[1]Aarhus University, MIB, Norrebrogade 44, 8000 Århus C, Denmark
[2]TU Berlin, Machine Learning Group, Marchstr. 23, 10587 Berlin, Germany
[3]TU Berlin, Neurotechnology Group, Marchstr. 23, 10587 Berlin, Germany
[4]Newcastle University, Auditory Group, Framlington Place, Newcastle-upon-Tyne, NE2 4HH, UK

Aims. This work seeks to seeks electrophysiological event-related potentials (EPRs) in the human EEG that are indicative of the processing of a metrical beat, based on purely temporal characteristics of basic rhythmical tone sequences. Methods. Motivated by pre-existing behavioural work (Povel and Essens, 1985; Grube and Griffiths, 2009) on temporal processing accuracy and subjective perception of the catchiness of rhythmic patterns with a strongly vs. a weakly metrical beat, a total of 784 sequences were composed (each containing 7 or 8 tones distributed over 16 beat units of 200 ms). Evaluated based on behavioural ratings, an optimally separable set of 28 strongly and 28 weakly metrical sequences was chosen and employed in a passive listening EEG paradigm looking at the processing of sequences in their original format and with a violated ending. In order to analyse ERP markers of metrical processing, continuous EEG data were epoched and averaged across subjects (n=20). Results. Grand average responses of initial analysis at FZ display differences in the EEG for strongly compared to weakly metrical sequences that indicate the beat-based analysis of the unfolding of sequences, their metrically plausible endings and violation of those. Conclusions. The findings provide evidence for the beat-based predictive encoding of such minimalistic rhythms and the temporal prediction of their metrically plausible endings. On-going analyses tease aim to tease out the individual components and test the classification of strongly vs. weakly metrical beat processing in a machine learning-based multiple electrode approach.

Localization of sound source approaching and receding in case of high-frequency hearing loss modeling in humans

Alisa Petrovna Gvozdeva[1], Elena Alexandrovna Ogorodnikova[2], Irina Germanovna Andreeva[1]

[1]Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, Saint-Petersburg, Russia
[2]Pavlov Institute of Physiology Russian Academy of Sciences, Saint-Petersburg, Russia

Localization of approaching and receding sound sources is based on the amplitude and spectral cues, which occur due to direction-dependent filtration of the sound by the pinnae and the body of the listener (Kolarik et al., 2015). The most prominent spectral changes in signals of moving sound sources are observed at high frequencies; the fact indicates significance of high-frequency hearing for moving sound sources localization (Andreeva, Nikolaeva, 2013). In case of sensorineural hearing loss (SNHL) damage to the cochlea occurs, which leads to high-frequency hearing loss and to the changes of suprathreshold signal coding (Moore, 1995). That is why separate estimation of the role of each of these processes in impairment of auditory motion localization draws attention. In this study we estimated temporal thresholds of approaching and receding sound images in 11 adult individuals with clinically normal hearing in case of high-frequency hearing loss modeling which corresponded to mild and moderate SNHL. It allowed us to evaluate separately the influence of peripheral component of SNHL on temporal thresholds of approaching and receding sound images. Modeling of high-frequency hearing loss was carried out by filtration of broadband signals, which formed illusions of approaching and receding sound sources during their listening by subjects. Amplitude frequency response of the filters corresponded to mild and moderate SNHL. Moving sound images were created by linear changes in amplitude of broadband noise bursts sequences, which were played back to listeners via two loudspeakers, placed in front of the listener at 1 and 4 m distance (Altman, Andreeva, 2004). Duration of sequences varied from 125 to 400 ms. Temporal threshold was determined as the shortest of employed durations which provided probability of mistakes in determining direction of motion to be equal or less than 25 %. Experiments were conducted in soundproof anechoic chamber which volume was 62.5 m3.

In experimental series with mild and moderate SNHL modeling the average temporal threshold was similar and equal to 150 ms, which coincided with the threshold for control broadband signals. However when estimated together, probabilities of mistakes in determining direction of approaching and receding sound images with subthreshold duration (125 ms) were significantly higher in series with mild (42 % of mistakes) and moderate (38 % of mistakes) SNHL modeling comparing to control series (30 % of mistakes, $p<0.01$, $p<0.05$, respectively). Overestimation by listeners of receding sound images with subthreshold duration was observed. Thus, in case of modeling of high-frequency hearing loss specific for mild and moderate SNHL in subjects with normal hearing we didn't reveal significant increase of temporal thresholds of approaching and receding sound images, but there was increased uncertainty for determining direction of motion of sound images with subthreshold duration, mostly approaching ones.

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Reduced sound-evoked and resting-state BOLD fMRI connectivity in tinnitus

Benedikt Hofmeier[1], Stephan Wolpert[1], Ebrahim Saad Aldamer[1], Moritz Walter[1], John Thiericke[1], Christoph Braun[2], Dennis Zelle[3], Lukas Rüttiger[1], Uwe Klose[4], Marlies Knipper[1]

1 Department of Otolaryngology, Head and Neck Surgery, Hearing Research Center Tübingen, Molecular Physiology of Hearing, University of Tübingen, Elfriede-Aulhorn-Str. 5, D-72076 Tübingen, Germany

2 MEG Center, University Hospital Tübingen, Otfried-Müller-Str. 47, D-72076 Tübingen, Germany

3 Section of Physiological Acoustics and Communication, Department of Otolaryngology, Head and Neck Surgery, University of Tübingen, Elfriede-Aulhorn-Str. 5, D-72076 Tübingen, Germany

4 Department of Diagnostic and Interventional Neuroradiology, University Hospital Tübingen, Hoppe-Seyler-Str. 3, D-73076 Tübingen, Germany

The exact neurophysiological basis of chronic tinnitus, which affects 10-15% of the population, remains unknown and is controversial at many levels. It is an open question whether phantom sound perception results from increased central neural gain or not, a crucial question for any future therapeutic intervention strategies for tinnitus.

We performed a comprehensive study of mild hearing-impaired participants with and without tinnitus, excluding participants with co-occurrences of hyperacusis. Therefore, we used audiological evaluation, fMRI measurements (during rest and with audiological stimuli) as well as body fluid analysis for cortisol.

For the audiological evaluation we did ear examination, tympanometry, acoustic reflex measurements, pure tone and speech audiometry as well as ABR measurements. For the tinnitus patients a tinnitus questionaire was used to assess different aspects of tinnitus strain (Hiller et al., 1994).

fMRI image acquisition was performed on a 3-Tesla scanner (Skyra, Siemens, Germany). Besides resting state measurements for functional connectivity, four different auditory stimuli (music and chirp sounds with different frequencies) were used for task evoked measurements.

A right-hemisphere correlation between tinnitus loudness and auditory perceptual difficulty was observed in the tinnitus group, independent of hearing threshold differences. This correlation was linked to reduced and delayed sound-induced suprathreshold auditory brain responses (ABR wave V) in the tinnitus group, suggesting subsided rather than exaggerated central neural responsiveness.

A profound reduction in positive interhemispheric connections of homologous auditory brain regions and a decline in the positive connectivities between lower auditory brainstem regions and regions involved in sound detection (hippocampus, posterior insula) were observed in the tinnitus group.

Auditory illusion in owls predicted by a probabilistic model of rival neuron populations

Lutz Kettler[1]

[1]TU München, Department of Zoology, Liesel-Beckmann-Straße 4, 85350 Freising, Germany

Recently, perception has increasingly been described by statistical inference from noisy sensory inputs, meaning that probabilistic predictions generate representation of uncertainty in the brain. In this new paradigm the brain is assumed to extract the statistical properties of sensory signals and generates percepts from a range of possible interpretations. In this study a neuronal population model on basis of representation of auditory space in the owl midbrain, which is consistent with Bayesian inference was tested to predict how the brain decodes ambiguous sensory information and how it uses combinations of cues to eliminate illusory perception. The barn owl was used as model because the auditory system and especially sound localization is well understood in this species, and because barn owls, like humans, perceive illusions. Previous studies in humans and owls showed that tonal and tone-like stimuli generate an illusion called a phantom source. It has been debated so far how the brain generates and resolves these illusions.

Behavioral responses to the illusion were well predicted by the population model, but also revealed that computation beyond Bayesian inference, specifically probabilistic sampling, is needed to explain the behavior. The question how combinations of different spatial cues help in resolving the illusion, were tested with virtual auditory worlds, using head related transfer functions (HRTFs). In the virtual auditory worlds, single cues can be manipulated, which allowed for showing that cue combination explains and resolves phantom sound sources. The results with manipulated auditory cues were also well predicted by the model. The data reveals, how sensory systems handle multiple, possibly unreliable, cues and that mechanisms underlying disambiguation resemble across species, including humans.

Role of of peripheral BDNF for auditory perceptional learning?

Marlies Knipper[1], Philipp Eckert[1], Marie Manthey[1], Lucas Matt[3], Philine Marchetta[1], Wibke Singer[1], Michael Walter[2], Peter Ruth[3], Thomas Schimmang[4], Peter Pilz[2], Lukas Rüttiger[1]

[1]University of Tübingen, THRC, Molecular Physiology of Hearing , Elfriede-Aulhorn-Str. 5, 72076 Tübingen, Germany

[2]Department of Animal Physiology, University of Tübingen, Tübingen, Baden-Württemberg, Germany

[3]Institute of Pharmacy, Department of Pharmacology, Toxicology and Clinical Pharmacy, University of Tübingen, Tübingen, Germany

[4]Instituto de Biologíay Genética Molecular, Universidad de Valladolid y Consejo Superior de Investigaciones Científicas, Valladolid, Spain

While novel studies indicate that perceptional learning is not linked with permanent changes in cortical circuitry but rather with change that are gated in a task- or context dependent fashion, the molecular substrate of perceptional learning is still elusive. It is currently speculated that perhaps changes in synaptic weights restricted to a limited set of task-specific synapses play a crucial role (Irvine DRF Hearing Res. 2018, Zhang Y-X et al. Amitay S 2016, Plos One). BDNF is upregulated in the cochlear and ascending auditory pathway gradually from prior hearing onset onwards (postnatal day P4) (Singer et al., Knipper, Neuropharmacol 2014). Previously, we showed that peripheral BDNF in the cochlea or lower brainstem regions, but not in the central higher cortical brain regions is fundamental for improved and optimized auditory fidelity with hearing onset [Zuccotti A et al. Knipper, 2012, J Neuroscience, Chumak T et al., 2016, Mol Neurobiol]. This improved auditory fidelity included greater sensitivity of auditory fibers, lower hearing thresholds, and enlarged dynamic range and inhibitory strength in the inferior colliculus related to lowering of spontaneous firing rate. We here asked to what extend these BDNF driven changes in auditory fidelity may influence memory-related shaping of auditory skills and auditory perceptional learning.

Methods:

Using conditional BDNF Pax2 KO mice, lacking BDNF in the cochlea, DCN and IC, we analyzed auditory fine structure analysis, auditory steady state response (ASSR), LTP and LTP-dependent task performance in BDNF Pax2 WT and KO mice, and investigated changes in markers for excitatory and inhibitory neuronal activity using high-resolution confocal microscopy and quantitative Westernblot approaches.

Results

Fundamental differences were found between BDNF Pax2 WT and KO on molecular, functional, behavioral level. The findings are discussed in the context of a role of BDNF in lower auditory brainstem regions for auditory perceptional learning

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Strain as a Risk Factor for Tinnitus and Noise-Induced Hearing Loss in Rats

Lisa Koch[1], Bernhard H. Gaese[1], Manuela Nowotny[1]

[1]Goethe University Frankfurt, Institute for Cell Biology and Neuroscience, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

Previous results in the literature suggest that pigmentation is associated with less sensitive hearing and a diminished vulnerability of the auditory system towards noise trauma in different rodent species. We investigated this hypothesis by studying strain- and pigmentation-dependent differences in the hearing ability and tinnitus development of different rat strains before and after an acoustic trauma.
Therefore, four rat strains of the species *Rattus norvegicus* were examined: i) Sprague Dawley (albino), ii) Wistar (albino), iii) Lister Hooded (pigmented) and iv) Long Evans (pigmented). For trauma induction, all animals were exposed to a narrowband noise centered at 16 kHz ± 0.25 oct at 115 dB SPL for one hour. Changes in hearing ability were investigated over about eight weeks after noise trauma by measuring auditory brainstem responses and the behavior-based acoustic startle reflex, which can be used to detect tinnitus.
Significant strain- and pigmentation-dependent differences were already found in the baseline hearing thresholds with pigmented strains showing higher hearing thresholds than albinotic strains. After noise exposure, hearing thresholds showed pigmentation-dependent differences regarding the frequency specificity and temporal progress of threshold recovery. Briefly, for three strains a permanent threshold shift was found that had a delayed onset in the pigmented strains. Only the Wistar strain showed fully recovered hearing thresholds eight weeks after trauma. Furthermore, the frequency range revealing significantly elevated thresholds started at 12 kHz for the pigmented and 16 kHz for the albinotic animals, respectively, spanning into the high-frequency region. The risk to develop a tinnitus sensation after the noise exposure showed a strain-dependency with tinnitus rates of 33% for the Wistar strain, 50% for Sprague Dawley and 75% in Long Evans rats. No convincing tinnitus risk was obtained for the Lister Hooded strain, which was due to inadequate parameters chosen for the measurement of the acoustic startle response.
In summary, confirming earlier suggestions, pigmented rats in our study had a less sensitive hearing but showed a delayed hearing threshold elevation after acoustic overstimulation. Furthermore, a strong strain- rather than pigmentation-dependency could be shown for several parameters of the hearing characteristics and the risk to develop a tinnitus sensation.

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Cellular neuroenergetics in the lateral superior olive

Lars Kunz[1], Sonja Brosel[1], Rebecca Hessmer[1], Benedikt Grothe[1]

[1]LMU Munich, Dept. Biologie II, Div. of Neurobiology, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

The brain is known for its high energy demand. This fact provides the bases for functional brain imaging, but also entails the brain's vulnerability to hypoxia and might explain the strong correlation of some neurodegenerative disorders with metabolic dysfunctions. The knowledge of neuronal metabolism on the cellular level is a prerequisite for understanding these relationships. With our studies, we aim at extending the knowledge of cellular neuroenergetics beyond the so far mainly studied regions (hippocampus, cerebral and cerebellar cortex). We monitored metabolic activity in neurons of the lateral superior olive (LSO), an auditory brainstem nucleus. The rational for choosing the LSO is on the one hand its simplicity since it contains only one neuron type, in contrast to the so far studied brain regions. On the other hand, LSO neurons exhibit extreme but well-studied biophysical specialisations (e.g. firing rates of several hundred hertz, low input resistances of a few megaohms). Using acute brainstem slices of Mongolian gerbils (Meriones unguiculatus), we monitored the changing of autofluorescence of two major metabolites (NADH, Nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide) upon electrical stimulation of LSO inputs. The LSO shows the typical biphasic NADH/FAD response known from hippocampal neurons; however, up to a frequency of 400 Hz, still physiologically relevant for LSO neurons. We compared for the first time the LSO with the hippocampal CA1 region and the cerebral cortex in the same animal. The LSO exhibited the slowest rate of NADH/$FADH_2$ consumption and regeneration. The frequency dependence within the three brain regions was only similar during the consumption phase, but different during regeneration. The observed changes in NADH and FAD levels, in combination with electrochemically monitored alterations in O_2 concentrations are a sign of pronounced contribution of mitochondrial oxidative phosphorylation in the LSO, which is known for the other brain regions as well. We applied the technique to test the hypothesis that slow feedback signalling described earlier may minimize energy expenditure in the LSO. Studying the spatial response to rather long stimulation pulse trains (20 s, 200 Hz), we observed single minima of NADH level changes in large parts of the LSO. However, we also detected regions with double minima. These double minima and the slow time course of NADH changes (time to 1st minimum: 4.6 s) point at GABA-B receptor mediated, activity-dependent gain control mechanism. Accordingly, blocking of GABA-B signalling with CGP 55845 resulted in the disappearance of double minima and larger energy consumption. Our data suggest that the spatially variable, GABA-B-mediated feedback signalling augments metabolic efficacy in the LSO.
In conclusion, we showed that the LSO represents an apt model for neuroenergetics and, by comparison with other brain regions, that biophysical properties define metabolic demands of neurons. This knowledge of specific metabolic demands should be of relevance for the interpretation of functional brain imaging data as well as for the understanding of neuroenergetical implications in health and disease.
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Properties of Endbulb of Held Synaptic Transmission in the Mongolian Gerbil

Thomas Künzel[1], Kerstin Doerenkamp[1,2], Stefanie Kurth[1,3], Charlene Gillet[1]

[1]RWTH Aachen University, Auditory Neurophysiology Group, Institute for Biology 2, Worringer Weg 3, 52074 Aachen, Germany

[2]RWTH Aachen University, DFG Research Training Group GRK2416 „MultiSenses-MultiScales"

[3]RWTH Aachen University, Department of Chemosensation, Institute for Biology 2, Worringer Weg 3, 52074 Aachen, Germany

The auditory nerve forms a giant axosomatic terminal, the Endbulb of Held, on spherical bushy neurons in the anteroventral cochlear nucleus. Only very few enlarged synaptic terminals contact a single bushy neuron. Auditory information processing that relies on temporal aspects of the sound stimulus is strongly dependent on high-fidelity synaptic transmission at the Endbulb of Held. Therefore the basic synaptic parameters like size of the readily releasable pool, quantal size and release probability, which determine the EPSC the synapse induces in the postsynaptic neuron, have been studied in some detail at this synapse. However, this was mostly done in animals that do not rely strongly on low-frequency temporal cues, for which the Endbulb of Held is thought to be specialized. We therefore set out to characterize stationary and dynamic properties of Endbulb of Held synaptic transmission in the Mongolian gerbil and compare our results to measurements in the mouse.

We used whole-cell voltage clamp recordings of evoked postsynaptic currents (eEPSC) and also spontaneous miniature postsynaptic currents (mEPSC) from spherical bushy cells of the rostral part of the anteroventral cochlear nucleus in acute gerbil brain slices. Gerbils were juvenile hearing animals (~P19).

We used the mean amplitude of mEPSC to directly measure the quantal size q per neuron. When no mEPSC could be obtained, we used the group average qav which was 43±13pA (n=27). Now we employed two methods to estimate the size of the readily releasable pool (RRP) of the gerbil Endbulb. The Schneggenburger method yielded a RRP estimate of 260±93 vesicles (n=18), the method devised by Elmqvist and Quastel yielded 303±166 vesicles (n=20). From these data we derived the initial release probability p of the gerbil Endbulb. We found p to be 0.42±0.15 (n=18) or 0.44±0.15 (n=20). Together these parameters determine the average initial eEPSC amplitude of the gerbil Endbulb to be 4.8±2.1 nA (n=21). Compared to data from our own recordings or the literature, the gerbil Endbulb has a larger RRP than mouse or rat endbulbs. The higher RRP could indicate a larger terminal area with more release sites. This is in accordance with histological findings in which gerbils appear to also show the large spherical bushy neuron type reported from the low frequency AVCN of the cat. The large bushy cells prominently project to the medial superior olive and have one or very few enlarged endbulb synaptic contacts. However, the quantal size we measured for the gerbil is lower than what is reported for the mouse. Given that the release probability is roughly identical, the lower q thus explains why initial eEPSC appear a lot larger in mice than in gerbil. Overall, the gerbil endbulb synaptic transmission seems to be biased to sustained activity while the mouse endbulb emphasizes powerful phasic release.

Auditory Brainstem Responses Originating from Axonal Terminal Zones in the Auditory Brainstem of the Barn Owl

Paula T. Kuokkanen[1,2], Anna Kraemer[2], Nadine Thiele[3], Richard Kempter[1], Christine Köppl[3], Catherine E. Carr[2]

[1]Humboldt-Universitaet zu Berlin, Institute for Theoretical Biology, Philippstr. 13, Haus 4, 10115 Berlin, Germany
[2]Department of Biology, University of Maryland, College Park MD 20742, U.S.
[3]Department of Neuroscience, Carl von Ossietzky Universitaet, 26109 Oldenburg, Germany

The auditory brainstem response (ABR) is an extracranially recorded potential, which is used for diagnosis of hearing loss, especially among newborns. The ABR is generated in the auditory brainstem by local current sources, which also give rise to extracellular field potentials (EFPs). The origins of both the ABR and the EFP are not well understood. Traditionally synaptic dipoles have been attributed as the main sources for both. We have recently found that EFPs, especially their dipole behavior, may be dominated by the branching patterns and the activity of axonal terminal zones in the nucleus laminaris of the barn owl (McColgan et al., 2017). Furthermore, our model suggests that the dipoles from axonal terminal zones can be strong enough to contribute to extracranial potentials. To test the hypothesis that axonal arbors also shape the ABR, we used the well-described barn owl early auditory system. We recorded the ABR and a series of EFPs between the brain surface and nucleus laminaris (NL) in response to binaural clicks. We furthermore recorded extracellular single-cell responses in the nucleus magnocellularis (NM). The ABR and the EFP within and around NL are correlated, as are the NM spikes and the ABR. Thus, our model of the dipole sources in the auditory brainstem is in accordance with the data. Together, our data and model suggest that axonal dipoles within the barn owl nucleus laminaris contribute to the ABR wave III.

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Neuronal Encoding of Behaviorally Relevant Sound Source Locations in Primary Auditory Cortex

Diana Inês Lopes Amaro[1], Michael Pecka[1]

[1] Division of Neurobiology, Department Biology II, Ludwig-Maximilians-Universitaet Muenchen, Munich, Germany

Sounds are a constant presence in our lives and help us navigate in complex sensory environments, e.g. crossing a busy road. The formation of meaningful auditory streams in a given behavioral scenario relies on our brain's capacity to extract the situationally relevant properties from sounds, such as the spatial location of their sources – a process known as auditory scene analysis (ASA). Despite its crucial importance for auditory perception, the neuronal mechanisms of ASA are still poorly understood since in most studies, animals are in a passive environment (in which sounds have no particular behavioral relevance) and are head-fixed (i.e. in an egocentric reference frame). In opposition, natural ASA is characterized by the selective listening to the sound source of relevance while moving, resulting in a continuous change of the position of this source relative to one's head. These ethological circumstances suggest that an allocentric-based neuronal code representing the relevant sound source would be advantageous. To investigate to what extent such a neuronal representation exists in the auditory cortex, we developed a goal-directed auditory localization task in which Mongolian gerbils freely move and are trained to forage in an arena for an auditory target. The target corresponds to sound coming from a particular sound source and is associated to an area in the arena, which is differently located across trials. The animals are continuously tracked during the trial and through a feedback-loop system, depending on their position, either the target or another sound source is active. With this task, we introduce behavioral relevance to different sound sources in an environment in which animals have to constantly change their position with respect to these sources. During task performance, we record neuronal activity in primary auditory cortex via multiple tetrodes connected to a wireless-transmitting headstage. During offline analysis, we calculated the head angle relative to the active source and correlated these angles with the recorded neuronal responses to construct spatial tuning functions. We find that tuning varies across neuronal population: as expected, some neurons are similarly tuned to both target and non-target sound sources, i.e. are not affected by the difference in their behavioral relevance. Interestingly, however, some neurons show differential tuning to the behaviorally relevant source. Since the only difference between the sound sources is their position in space, their distinct neuronal representations requires allocentric information already at the level of the primary auditory cortex. Thus, our novel behavioral paradigm allows the identification of a neuronal processing regime that facilitates the localization of relevant sound sources during ASA.

Localization of AP-2δ Expression in the Chicken Embryo

Harald Luksch[1], Carina Schaub[1,2], Markus Moser[3], Benjamin Schusser[2]

[11]Technical University of Munich, TUM School of Life Sciences, Liesel-Beckmann-Straße 4, 85354 Freising, Germany
[3]Max-Planck-Institute for Biochemistry, Martinsried, Germany
[2]Reproductive Biotechnology, TUM School of Life Sciences, Technical University of Munich, Germany

AP-2 is an important family of transcription factors implicated in many aspects of development, cell differentiation and the regulation of cell growth and death. AP-2δ; is a member of this family, specifying gene expression patterns required for the development of the posterior midbrain. In adult mouse brain, AP-2δ; is expressed in the posterior midbrain (inferior colliculus, IC), as well as in the cortex, dorsal thalamus and superior colliculus. The midbrain is one of the central areas in the brain where multimodal integration, i.e., the integration of information from different senses, occurs. AP-2δ; deficient mice are viable but lack part of the posterior midbrain due to increased apoptosis in this part of the brain starting at the end of embryogenesis. Despite the absence of the inferior colliculus in AP-2δ; deficient mice, these animals retain at least some higher auditory function, as neuronal responses to sounds were recorded in the neocortex, suggesting an alternate auditory route that allows responses to individual tones (Hesse et al., PLOSone 2011).
We here investigated the distribution of AP-2δ; in chicken embryos. We first identified and localized the AP-2δ expression in the chicken midbrain during embryogenesis with Western blot and immunohistochemical analysis. The results confirmed the presence of AP-2δ; in the Inferior colliculus (IC). In addition, AP-2δ; expression was found in the Optic tectum (TeO), specifically in young Shepherd's crook neurons that are an essential component of the isthmic network.
Additionally, we used the CRISPR/Cas9 system to generate AP-2δ; knockout (KO) in primordial germ cells (PGCs) which will allow the generation of AP-2δ; KO chickens in the future. Transfected PGCs were enriched by FACS sorting. The resulting pool demonstrated a total INDEL efficiency of 15.1 %. After performing limiting dilution and single clone expansion, clone number 4 (C4) exhibited a deletion of several nucleotides. The deletion let to an early stop codon causing a knockout of AP-2δ; in PGCs.
These modified PGCs will be transplanted into surrogate roosters in order to generate AP-2δ; KO chicken with selective lesion of the auditory midbrain. These animals will allow to study the auditory pathway of the chicken without an inferior colliculus and delineate its role in spatial and cognitive processing of sound.

Fig 1: Localization of AP-2δ in transversal sections of chicken midbrain at embryonic day 14. B: Auditory midbrain (inferior colliculus), inset with details of cells. C: Optic tectum with cells in layer 10.

State dependence of stimulus adaptation in the auditory cortex of Mongolian gerbils

Jing Ma[1,2], Michael GK Brunk[1], Marina Zempeltzi[1], Katrina E Deane[1], Max FK Happel[1], Reinhard König[2], Matthias Deliano[1]

[1]Leibniz Institute for Neurobiology, Department of Systems Physiology of Learning, Brenneckestraße 6, 39118 Magdeburg, Germany

[2]Leibniz Institute for Neurobiology, Special Lab Non-Invasive Brain Imaging, Brenneckestraße 6, 39118 Magdeburg, Germany

The amplitudes of evoked cortical responses decay with sensory stimulus repetition, which is often referred to as adaptation. Adaptation is one of the most robust features of sensory processing. Within auditory cortex, adaptation has been suggested as a reflection of short-term synaptic depression (STSD) and, thus, representing auditory sensory memory. This could be reflected by a dependence on stimulus presentation rate of auditory response. In general, time constant τ_{SOI} has been derived from the exponential increase of peak amplitudes of dominant auditory evoked waveform as a function of stimulus-onset interval (SOI) in humans.

Since there are inevitable methodological limitations of human studies using non-invasive techniques such as Magnetoencephalography (MEG) or Electroencephalography (EEG), we are further investigating auditory adaptation in animal using invasive electrophysiology. Specifically, we study the dependence of stimulus adaptation in the auditory cortex of Mongolian Gerbil (*Meriones unguiculatus*) in three different conditions: 1) when the animal is under anesthesia, 2) when the cortical activity is pharmacologically silenced by topical application of muscimol, a $GABA_A$ agonist, and 3) when the animal is passively listening to the stimuli while being awake. By means of layer specific in-vivo electrophysiology employing radial current source density (CSD) analysis, we first study whether the exponential increase of the peak amplitudes of auditory evoked responses as a function of SOI is also observable in gerbils. Note that CSD analysis reveals layer-dependent information of the synaptic activity in auditory cortex circuits, thus it allows to assess the cortical sources of adaptation. Secondly, by using an identical acoustic stimulation paradigm in the three conditions, we investigate the state dependence of stimulus adaptation. We expect that our results will provide further insight into the underlying mechanisms of stimulus adaptation.

So far, measurements in conditions 1) and 2) have been performed. There is clear indication showing that SOI-dependent auditory adaptation is also observable in anesthetized gerbils on the level of cortical generators, and that time constant τ_{SOI} lies in similar range around 2.5s, as in human M100/ N100; However, the latency of the CSD peak that reflects overall synaptically driven current flow within cortex, is at around 40 ms, which is much smaller than for the M100/ N100 in human MEG/ EEG studies, which appears at around 100 ms.

A real-time EEG source activity from auditory cortex in patients with chronic tinnitus

Azim Malekshahi[1], Manuel Czornik[1], Herbert Bauer[5], Jürgen Dax[2], Christoph Braun[2,3,4], Niels Birbaumer[1,6]

[1] Institute of Medical Psychology and Behavioural Neurobiology, University of Tübingen, Germany
[2] MEG-Center, University of Tübingen, Germany
[3] CIN, Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Germany
[4] Department of Psychology and Cognitive Science, University of Trento, Rovereto 38068, Italy
[5]Faculty of Psychology, Social, Cognitive and Affective Neuroscience Unit (SCAN-Unit), University of Vienna, Vienna, Austria
[6] WyssCenter for Bio and Neuroengineering, Geneva, Switzerland

Tinnitus as perception of sound without an actual external source is the consequence of multiple factors rather than a single disease entity[1,2]. Weisz et al. observed markedly reduced alpha activity (8-12Hz) and increased delta (1-4Hz) power in awake tinnitus patients relative to normal hearing controls[3]. The differences were most pronounced over perisylvian regions and were significantly correlated to tinnitus-related distress. Weisz et al. argue convincingly that an auditory cortex alpha signals has the same functional state as other sensory structures[4]. Since alpha activity in sensory cortices has been shown to be a direct indicator of ongoing inhibition and the balance of excitation and inhibition in a cortical structure[5,6], therefore, reduced auditory cortex alpha activity becomes a central key process in the chain of reactions that leads to the phantom perception of tinnitus. Here a new method for on-line detection of auditory alpha EEG-activity is proposed and tested with simulated data and illustrated with patient data. Aim of the proposed procedure is its use in an EEG-neurofeedback-treatment approach which allows on-line auditory alpha self-upregulation training in patients with chronic tinnitus. The first part of the method is localizer runs which are designed to find optimal channels which they reflect auditory cortex activity and also finding a pattern of primary auditory cortex activity. The online procedure as second part is calculation of correlation between the pattern of primary auditory cortex activity and pattern of alpha-upregulation in real-time as an index (Similarity Index(SI)) of how much the alpha oscillation is coming from auditory cortex. The multiplication of this index and alpha power from the optimal channels are final neurofeedback output. The third part of the method is to use a continuous tactile face-stimulation and visual stimulation which target suppression of somatosensory and visual alpha and facilitation of auditory alpha activity.

Acknowledgements

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Basic response properties in the auditory cortex and the frontal auditory field of the fruit-eating bat *Carollia perspicillata*

Adrian Mannel[1], Francisco Garcia-Rosales[1], Julio Hechavarria[1]

[1]Goethe Universität Frankfurt am Main, Institut für Zellbiologie & Neurowissenschaft, Neurobiology of auditory computations, Department of Neurobiology and biosensors, Max-von-Laue-Straße 13, 60438 Frankfurt am Main, Germany

Bats are used as a model organism for acoustic communication, echolocation and processing of auditory information. Their auditory system has been heavily studied to understand how biosonar information is processed in the brain. This processing of information so far was mostly studied in the inferior colliculus (IC) and the auditory cortex (AC). In this study, recordings were performed in the AC and in the frontal auditory field (FAF) of the fruit eating bat *Carollia perspicillata*. Studies on the bat FAF are scarce, and it remains unknown how tuning properties observed in the auditory midbrain and cortex relate to those observed in frontal auditory areas. Our goal was to compare response properties of AC and FAF neurons to unravel possible transformations to auditory processing along the cortical hierarchy. For that, neurons tuned to frequency and echo delay information (the latter is a type of tuning that allow bats to compute target distance) were tested. Results show clear differences between the AC and FAF. The AC shows fast, temporally clear responses to the auditory stimuli, while neurons in the FAF appear to be slower (longer latency), temporally unprecise, and unreliable across trials. Based on these results and previous studies, there seems to be a trend of deterioration of response precision as information travels up the auditory pathway. Our data indicates that this trend continues on the way to the FAF.

Transcriptional profiling of auditory brainstem nuclei in developing mice

Ayse Maraslioglu[1], Kathrin Kattler[2], Domenico Del Turco[3], Eckhard Friauf[1]

[1]Animal Physiology Group, Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany

[2]Genetics/Epigenetic Group, Department of Biological Sciences, Saarland University, Saarbrücken, Germany.

[3]Institute of Clinical Neuroanatomy, Neuroscience Center, Goethe-University Frankfurt, Frankfurt am Main, Germany.

In the mammalian auditory brainstem, the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO) are involved in sound localization and thereby fulfill distinct functions. MNTB neurons convert monaural excitatory input into inhibition, whereas LSO neurons integrate binaural excitatory and inhibitory inputs. In both nuclei, gene expression patterns depend on age and sensory experience. To capture these patterns and to assess molecular differences between these nuclei, we generated whole transcriptome profiles by sequencing the RNA of MNTB and LSO tissue isolated via laser capture microdissection at four ages (postnatal days (P)7, P12 = hearing onset, P21, P60). At each age, ≥3 biological replicates were analyzed. Principal component analysis revealed that global gene expression patterns were clustered by age and nucleus. Differential expression analysis showed distinct expression patterns in MNTB and LSO at all ages. Data of P60 samples were analyzed in detail, whereas P7-21 data are in progress and will be demonstrated at the poster. In the P60 dataset (4 biological replicates for each nucleus), 17,389 transcripts were identified. About 40% of >700 most abundant transcripts (RPKM >100) occurred in each of the two nuclei. Among the most abundant transcripts, gene set enrichment analysis (GSEA) showed enrichment of for example transcripts related to “oxidative phosphorylation”, reflecting the high energy demand of both nuclei. 1,043 transcripts were differentially expressed. 431 transcripts displayed >2-fold higher levels in the MNTB, and 612 did so in the LSO (>10-fold: 104 in MNTB, 152 in LSO). Gene ontology analysis revealed differential expression of transcripts involved in “ion transport”. A deeper look into these transcripts revealed different subsets of voltage-gated K+ channels causing fast spiking in the LSO and MNTB at P60. While some of these channels were described earlier in LSO and MNTB and thus validate our data, some novel candidates were also identified. GSEA also revealed abundance differences of various transcripts belonging to the “calcium signaling pathway”. Immunohistochemical validation of RNA-sequencing results suggests functional relevance of these findings. Together, we show high numbers of of differentially expressed genes between MNTB and LSO, including transcripts relevant for neuronal signaling. These emphasize the distinct functions of these nuclei. Analyzing various developmental stages will show how these differences emerge at the transcriptional level. The whole dataset will provide information about region-specific and age-specific markers as well as novel candidates for further investigations.

Comparison of Single Cell Spike Rate and Timing in the Brainstem in Response to Cochlear Implant and Acoustic Stimulation

Michaela Alisa Müller[1], Barbara Beiderbeck[1], Benedikt Grothe[1,2], Michael Pecka[2]

[1]Graduate School of Systemic Neurosciences, Ludwig-Maximilians-Universität München

[2]Biozentrum, Neurobiologie, Department Biologie II, Ludwig-Maximilians-Universität München

Hearing impairment and deafness is the most frequent sensory deficit today and deprives patients from interaction with their environment. Specifically, spatial hearing is essential for communication as well as navigation in everyday life. The cochlear implant (CI) allows for the functional restoration of hearing, in particular the basic understanding of speech. Unfortunately, sound localization in complex environments is still severely limited in patients with bilateral CIs and its restoration thus remains one of the central obstacles of CI research.

Interaural Time Difference (ITD) is the dominant binaural cue for sound localization and speech comprehension in noisy environments. Neuronally, ITD sensitivity is based on the integration of excitatory and inhibitory synaptic inputs from both ears by brainstem neurons in the medial superior olive (MSO) and the lateral superior olive (LSO). Current implantation and stimulation techniques as well as behavioral performance levels imply that CI users might predominately make use of the LSO pathway. Recent in vivo recordings in the LSO demonstrated that during normal hearing, inhibition and excitation interact with microsecond precision during ITD processing (Beiderbeck et al., 2018). However, the mechanisms underlying this precise temporal integration of individual inputs and potential differences in temporal precision during electrical stimulation are not understood.

Here, we obtained in vivo electrophysiological recordings from single neurons in the brainstem of Mongolian Gerbils (Meriones unguiculatus). We characterized the temporal precision of action potential firing in response to click-train stimuli of varying inter-click-intervals (ICI) in binaural neurons of the LSO and their upstream, monaural inputs, namely the cochlear nucleus (CN) and medial nucleus of the trapezoid body (MNTB). To assess potential differences in temporal precision between acoustic and electrical stimulation, complementary data are obtained in normal hearing animals and animals that underwent deafening and cochlear implantation.

Our results demonstrate that electrical stimulation differs from acoustical stimulation throughout the auditory brainstem. We observed dramatically higher spike probability and reduced jitter for the electrical stimulated cells both in CN and MNTB. Ultimately, we aim to identify basic principles of binaural integration in the auditory brainstem during acoustic and CI-based spatial hearing

Development of specific functional axon and myelin morphology in auditory brainstem circuits

Alisha L. Nabel[1,2], Hilde Wohlfrom[1], Olga Alexandrova[1], Michael Pecka[1], Benedikt Grothe[1]

[1]Division of Neurobiology, Department Biology II, Ludwig-Maximilians-Universität München, 82152 Planegg-Martinsried, Germany

[2]Graduate School of Systemic Neuroscience,Ludwig-Maximilians-Universität München, 82152 Planegg-Martinsried, Germany

Myelination patterns define action potential conductance and temporal precision. Precise timing of action potentials is a fundamental prerequisite for neuronal processing. However, it is an open question how myelination patterns are regulated during development.
In mammals, some of the most precisely timed inputs are realized in auditory brainstem circuits. Specifically, the processing of differences in the arrival of sounds at the two ears, the interaural time differences (ITDs), for sound location in the azimuth depends on sub-millisecond timed excitatory and inhibitory inputs. To accomplish this, the ITD circuit exhibits remarkable physiological and anatomical specializations to ensure fast, stable and precise synaptic transmission.

We recently reported that axons from globular bushy cells (GBCs) tuned to low frequencies, for which we depend on ITDs, show a unique myelination pattern that differs from the traditional assumption that internode length necessarily scales positively with axon diameter. The conduction velocity of these low-frequency GBC axons is enhanced due to an increase in axon thickness and a decrease in internode length compared to GBC fibers tuned to higher sound frequencies. This ensures rapid and highly precise action potential transmission, important for ITD processing (Ford et al., Nat Comm 2015; Stange-Marten et al., PNAS 2017).
The difference in myelination pattern in GBC fibers according to their frequency tuning raises the question of what mechanism underlies the development of the unique myelination pattern in low-frequency GBC axons. We therefore asked if this pattern is already present before hearing onset or if it develops due to hearing experience by analyzing axon diameter and internode length of GBC axons in the Mongolian gerbil (*Meriones unguiculatus*) before hearing onset at P10.

Before hearing onset, GBC axons tuned to high sound frequencies have already reached a mature-like diameter. In contrast, low-frequency GBC axons in P10 animals are much thinner than in adult gerbils – and even thinner than high-frequency GBC axons. Hence, in contrast to high-frequency fibers, low-frequency GBC axons dramatically increase their caliber after hearing onset. Both in high and low-frequency fibers of P10 animals we found myelination of axons to be already present, with internodes as expected given their P10 diameter. In general the internodal length did not change after hearing onset in both high- and low-frequency GBCs with one exception: We discovered a higher variability of internode length along the entire axon in P10 vs. adult gerbils. Hence, there is a refinement related to hearing onset. In conclusion, the myelination pattern of GBC axons is defined by the axon diameter before hearing onset. An increase of axon calibre of low-frequency fibers after hearing onset leads to unusual internode/axon diameter ratios and speeds up action potential conductance as required in the ITD circuit.

Accelerated recovery of ABR hearing thresholds after mild acoustic trauma in $Ca_V1.3$-$DCRD^{HA/HA}$ mice

Fahmi Nasri Abuqutheileh[1], Kerstin Blum[1], Jutta Engel[1], Simone Kurt[1]

[1]Saarland University, Department of Biophysics and CIPMM, Buildg. 48, Kirrberger Str. 100, 66424 Homburg, Germany

Mild noise trauma can cause irreversible damage to the cochlea despite complete recovery of the hearing threshold. The irreversible damage affects a subset of inner hair cell (IHC) - auditory nerve fiber synapses the loss of which does not impair hearing thresholds, which is described as noise-induced cochlear synaptopathy.

Here we analyzed noise vulnerability of $Ca_V1.3DCRD^{HA/HA}$ mice on C57Bl6/N background. $Ca_V1.3$ Ca^{2+} channels are responsible for ~90 % of the Ca^{2+} influx at IHC ribbon synapses. The genetic manipulation of the distal C-terminal modulatory domain in the C-terminus of $Ca_V1.3$ (DCRD) leads to altered gating and reduced current inactivation resulting in an increase of peak IHC Ca^{2+} currents without affecting frequency-dependent hearing thresholds (Scharinger et al., Front Cell Neurosci 2015).

$Ca_V1.3DCRD^{HA/HA}$ mice and wildtype littermates aged 8-11 weeks were subjected to a mild 8-16 kHz trauma (white noise) at 100 dB SPL for two hours. Auditory brainstem responses (ABR) were measured two days before trauma (-2), after trauma (0) and 1,2,3,5,7,14,21 and 28 days after trauma. Noise exposure caused a temporal threshold shift (TTS) in both mouse groups, which was slightly but significantly larger in $Ca_V1.3DCRD^{HA/HA}$ mice by 4 – 6 dB at 2, 4, and 11.3 kHz directly after trauma. Unexpectedly, hearing thresholds of $Ca_V1.3DCRD^{HA/HA}$ mice recovered significantly faster from acoustic trauma in a broad frequency range compared with wildtype mice. From these results, we conclude that larger $Ca_V1.3$ L-type Ca^{2+} currents in IHCs or spiral ganglion neurons lead to better recovery from cellular stress following acoustic trauma in $Ca_V1.3DCRD^{HA/HA}$ mice.

Preliminary immunohistochemical analysis of the number of ribbons at 28 days after trauma revealed that ribbons were largely preserved in both genotypes in contrast to the findings of Kujawa and Liberman in CBA/J mice (J Neurosci 2009) suggesting that noise vulnerability may strongly depend on the genetic background of the mouse lines used.

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Urocortin 3 at the Calyx of Held Increases Excitatory Postsynaptic Currents in the lateral MNTB

Sara Pagella[1], Conny Kopp-Scheinpflug[1]

[1]Graduate School of Systemic Neurosciences - LMU Munich, Neurobiology, Grosshadener strasse 2, 82152 Planegg-Martinsried , Germany

BACKGROUND

Urocortin 3 (Ucn3) is an evolutionarily-conserved neuropeptide of the corticotropin releasing factor (CRF) family. Its discrete distribution in the central nervous system (CNS) renders it an interesting neuromodulator of very diverse systems, from stress and anxiety, to sociability and feeding. Ucn3 expression in the auditory system has been recently detected, specifically in the Superior Olivary Complex. Interestingly, by using an Ucn3 reporter mouse model, we found a peculiar pattern of expression of this neuropeptide in the Calyx of Held on lateral neurons in the Medial Nucleus of the Trapezoid body (MNTB). However, to date, Ucn3 function in auditory brainstem is still obscure. Additionally, the specific synaptic actions and signal transduction pathways of Ucn3 and its receptor CRFR2 seem to depend on neuronal type, brain area, availability of specific G proteins and mouse strain. In this regard, one major hurdle relates to the fact that Ucn3 often acts at presynaptic terminals, which are notoriously difficult to access for direct electrophysiological recordings. However, due to its large size, successful recordings have been carried out at the Calyx of Held since the early 90s. Here we report our results in the elucidation of the presynaptic mechanisms of action of Ucn3 in the auditory brainstem at the Calyx of Held - MNTB synapse.

METHODS

Anatomical description of the localization of UCN3 expressing cells and axons was accomplished with the use of UCN3 TdTom mouse model. Whole-cell patch clamp experiments were carried out on wild-type C57BL6 mice aged P14 to P22 in the lateral MNTB. Excitatory postsynaptic currents (EPSCs) were evoked using a bipolar electrode placed at the midline over the trapezoid body fibers. 10uM SR95531 and 1uM Strychnine were added to the aCSF to inhibit IPSCs. Baseline data was acquired before the addition in the aCSF of UCN3 (600nM/300nM/200nM/100nM) or a CRFR2-specific inhibitor, K41498 (200nM), and after 10 minutes of drug wash out. Calyceal currents were identified as EPSC with an amplitude >1nA. mEPSCs were recorded with additional 1mM TTX. Spontaneous activity and intrinsic properties of recorded cells were studied in current-clamp mode.

RESULTS

600nM UCN3 significantly increased the amplitude of EPSCs (48.5% $p=< 0.005$) as well as the frequency of mEPSCs. Conversely, K41489 caused a decrease (25.5% $p=0.003$) in the amplitude of EPSCs and in the frequency of mEPSCs. None of the drugs caused changes in the amplitude of mEPSCs. Intrinsic properties of recorded cells remained unaffected by both drugs.

CONCLUSIONS

Our preliminary results clearly indicate a presynaptic action of Ucn3. Direct investigation of the Calyx of Held will allow us to untangle the molecular pathway involved. Possible mechanisms include increased release of intracellular Ca^{2+} through PLC or PKC activity, modulated conduction through P/Q Ca^{2+} channels, or inhibition of K^{+} channels.

In other synapses, Ucn3 was also reported to cause presynaptic depolarization. In this regard, depolarization of the Calyx of Held was shown to affect postsynaptic EPSCs and cause short-term

facilitation. Intriguingly, we found Ucn3 positive Calyxes only on the lateral MNTB cells, that are responsive to low frequency sounds and have different ion channels and membrane properties from the medial ones. Therefore, Ucn3 might represent a specialization of the lateral Calyxes that plays a crucial role in cases of auditory stress or energy imbalance.

Neural correlates of visuo-auditory sensory recalibration.

Hame Park[1,2,3], Christoph Kayser[1,2,3]

[1]Universität Bielefeld, Fakultät für Biologie, Universitätsstraße 25, 33615 Bielefeld, Germany
[2]Cognitive Interaction Technology – Center of Excellence, Inspiration 1, 33619 Bielefeld, Germany
[3]Institute of Neuroscience and Psychology, University of Glasgow, University Avenue, Glasgow G12 8QQ, United Kingdom

Simultaneously presented multisensory stimuli not only combine to guide immediate reactions, but also shape the perception of subsequent stimuli. During the ventriloquist effect (VE) a sound is mislocalized towards a visual cue. This VE also influences the perceived location of a subsequent sound - the ventriloquist after-effect (VAE), a form of sensory recalibration. We investigated the neural mechanisms of this VAE.

We measured behavioral performance and magnetoencephalography (MEG) while participants performed an auditory localization task designed to induce the VAE (Fig 1A). Psychometric data confirmed the VAE: the perceived sound location was biased by previous visual and auditory stimuli (Fig. 1B). Bayesian modelling suggested that the VAE arises from a systematic bias in auditory representations, not a change in a priori bias or the precision of sensory representations (Fig. 1C).

To understand the underlying neural mechanisms, we used cross-validated linear classification to localize MEG source activity encoding current and previous sensory information. We used regression modelling to determine, when and where in the brain, the encoding of a unisensory sound is influenced by the previous sensory information, and when and where the neural signature of the previous stimuli is directly predictive about the behavioral VAE (Fig. 1D-E).

This revealed that the encoding of sound locations in the left parietal cortex was significantly influenced by both the previous visual and auditory stimuli, starting at 80ms post stimulus onset (Fig. 1D). Furthermore, the perceptual VAE-bias was explained by the neurally encoded information about the previous stimuli in superior parietal cortex (Fig. 1E). This suggests that during the VAE previous auditory and visual information biases the encoding of a subsequent sound in parietal cortex and thereby induce a shift in the perceived sound location. Our results suggest an origin of rapid multisensory recalibration at the level of sensory encoding rather than decision making.

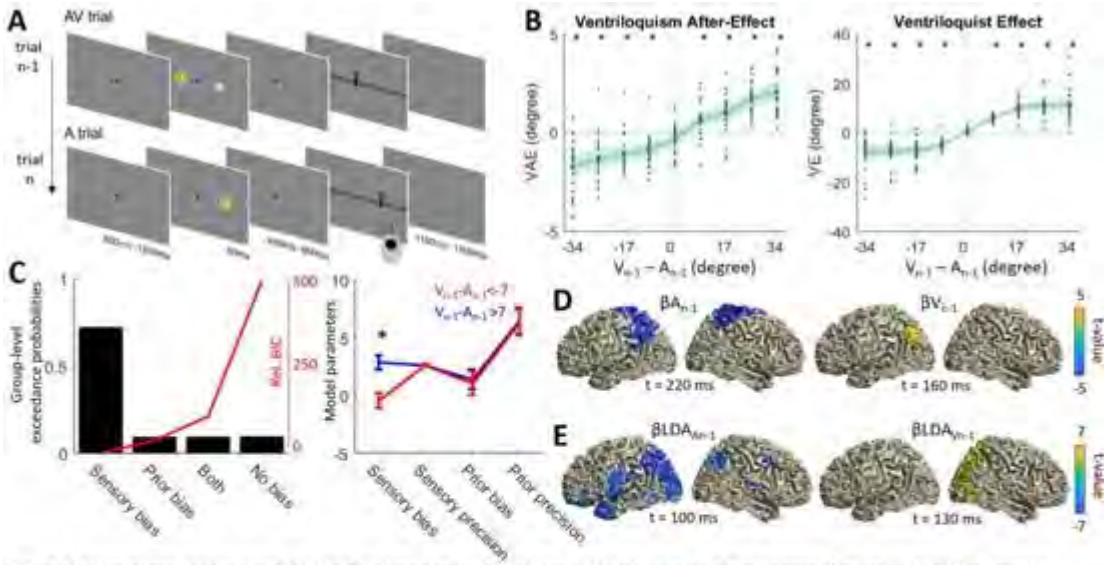

Figure 1. A Paradigm. Audio-visual (AV) trials alternated with auditory (A) trials. A trials always came after an AV trial. Task was to localize the perceived sound source. Stimulus positions were pseudo-randomized (within and across trials), each drawn from 5 locations. **B** VAE (in A trials) and VE (in AV trials) (total of 24 subjects). VAE was defined as the reported sound location minus the mean perceived location for the respective unisensory sound position (in A trials), shown as a function of the audio-visual discrepancy in the preceding trial (AV trial). VE is defined as the reported location minus the actual location of the sound (in AV trials), as a function of the audio-visual discrepancy of that trial. (*p<0.05). **C** Left: Model comparison results. Right: Influence of audio-visual discrepancy on model parameters. (*p<0.01). **D,E** Cross-validated linear discriminant analysis (LDA) was used to localize stimulus-selective MEG source activity (left vs. right lateralized stimuli), i.e. to characterize relevant neural representations. Group-level significance maps showing the significant contribution of previous auditory (A_{n-1}) and visual stimuli (V_{n-1}) to the encoding of the current sound in trial n **D** (LDA_{An}~1+βA_n+βA_{n-1}+βV_{n-1}; data for A_n not shown). **E** Map for VAE (VAE~1+βLDA_{An-1}+βLDA_{Vn-1}).

Cortical Activation Patterns in Electric Auditory Midbrain Stimulation

Gunnar Lennart Quass[1,2], Andrej Kral[1,2]

[1]Hannover Medical School, Institute for AudioNeuroTechnology , Stadtfelddamm 34, 30625 Hannover, Germany
[2]DFG CLuster of Excellence "Hearing4All"

Subjects using an auditory midbrain implant, placed in the inferior colliculus, fail to achieve open speech perception and their achieved independent channel separation is rather low. Current-focusing using bipolar and tripolar stimulation is an effective approach in cochlear implants. In this study, we therefore systematically compared the effect of the place of electric stimulation in monopolar, bipolar, and tripolar configurations in 10 adult NMRI mice using recordings in the primary auditory cortex. We further tested the cortical capacity to phase-lock to either of two presented stimulus trains.
We delivered electrical stimuli as single pulses or pulse trains using a 1x16 PtIrO Neuronexus electrode array, located along the tonotopy axis in the IC, while simultaneously recording cortical LFPs and spikes from primary auditory areas using a 4x8 PtIr Neuronexus array. When presenting pulse trains, we used two different electrode sites and two different pulse repetition rates.
We found no significant differences in any of the investigated parameters between monopolar, bipolar, and tripolar stimulation. When considering the spread of activation, we found a prominent stimulation place-tuning-asymmetry for all conditions. While the evoked rate increased with increasing frequency tuning alignment of midbrain and cortical sites, the highest spike rates and lowest thresholds were consistently recorded at a CF difference of up to +1 octave (AC-IC). Between 0 and -1 octaves, response strengths were significantly lower. The functional spread of activation was approximately 2 octaves.

Our results suggest a place- and intensity-dependent activation of the auditory cortex that is strongest in the frequency area of 0 to +1 octaves. The current-focusing technique using multipolar stimulation is not effective in direct-contact stimulation, presumably because of the direct contact of the stimulating electrode and the target neurons. Separation of different inputs remains a largely unsolved problem in electric midbrain stimulation and requires further investigation on order to enable speech intelligibility for Auditory Midbrain users.

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Laminar activity in the auditory cortex of vocalizing bats

Dennis Röhrig[1], Francisco García-Rosales[1], Manfred Kössl[1], Julio C. Hechavarría[1]

[1]Goethe University Frankfurt am Main, Institute for Cell Biology and Neuroscience, Max-von-Laue Str., 13, 60438 Frankfurt am Main, Germany

Bats are endowed with a sophisticated vocal control system that allows them to navigate and communicate with conspecifics. As one of very few animals, they possess the ability to echolocate by emitting ultrasound and receiving the returning echoes. The neural processing of echoes received by the bat largely depends on the analysis of their frequency content and is performed by the cochlea and the central auditory system. Auditory cortex neurons are typically tuned to spectral properties of sounds, and their spectral input can be best described with frequency tuning curves represented as spike count against stimulus frequency. However, current research suggests that tuning properties can be also quantified from neuronal population signals, such as local field potentials (LFPs). Since different cortical layers play very different roles for the processing of sensory information, it is necessary to record neural activity at many sites simultaneously. While most neurobiological studies have examined laminar differences in structure, less emphasis has been given to physiological differences between layers.

It was the aim of this study to explore the relative contribution of auditory cortical layers in representing pure tones as well as self-emitted vocalizations. For this purpose, we recorded spiking activity and LFPs in the auditory cortex of awake short-tailed fruit bats (*Carollia perspicillata*) across multiple translaminar electrodes. Columnar activity was observed during two distinct behavioral contexts: 1) while the bats were passively listening to sounds, and 2) while the bats were voluntarily self-emitting sounds.

First, we compared the frequency selectivity in neuronal population signals with the tuning of spiking responses across laminae. Second, we applied a common method to elucidate activation patterns in the cortex, by subjecting LFPs to current-source density (CSD) analysis. This method indexes the location, direction and strength of transmembrane current flow.

Our data show that the tuning relationship between neural spiking activity and the LFP, obtained from the same recording sites, follow a similar pattern. Whereas frequency tuning is weak and broad in superficial layers (i.e. layers I and II), it tends to be stronger and narrower in deeper layers. Further, our recordings revealed that auditory cortical columns represent both, the decision of vocalizing and self-emitted vocalizations in a layer-specific manner. While the main input layers of the auditory cortex (middle layers; i.e. layer III and IV) process incoming sounds, the superficial (I-II) and deep (V-VI) layers of the cortex encode the decision to produce voluntary calls. The representation of this preparatory audio-motor feature occurs at LFP frequencies below 20 Hz.

Altogether, our data shed light onto the potential role of the auditory cortex in the processing of sensory information and the control of vocalizations.

The influence of hearing impairment on the McGurk illusion

Stephanie Rosemann[1,2], Marie Dewenter[1], Dakota Smith[1], Christiane M Thiel[1,2]

[1]Biological Psychology, Department of Psychology, European Medical School, Carl-von-Ossietzky Universität Oldenburg, Oldenburg, Germany

[2]Cluster of Excellence "Hearing4all", Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany

Previous research investigating cortical plasticity in age-related hearing loss provides evidence for cross-modal reorganization in the auditory cortex, additional recruitment of the frontal lobe, and increased coupling of the visual and auditory cortices for matching audio-visual input. These changes already begin to occur when hearing impairment is only mild to moderate. In addition, we recently showed that hearing-impaired people are more prone to the McGurk illusion than those with normal hearing. Using functional magnetic resonance imaging (fMRI), we here investigated the influence of mild to moderate hearing impairment on neural correlates of the McGurk illusion.

Twenty hearing-impaired and twenty normal-hearing subjects aged between 50 and 75 years participated in the study. The group of hearing-impaired subjects showed a uniformly varying degree of mild to moderate and symmetrical age-related hearing loss and did not have any experience with hearing aids. The measurements taken included the McGurk illusion in the MRI, an assessment of hearing impairment by a pure tone audiometry, a test of speech-in-noise perception, and a questionnaire about everyday listening effort. Loudness was individually adjusted to 80% speech intelligibility to enable equal performance in both groups.

Our main aim was to relate hearing impairment (high-frequency hearing loss, speech-in-noise perception, and everyday listening effort) to BOLD response amplitudes elicited by audiovisual speech processing. Four different conditions were implemented in the experiment: auditory only, visual only, audiovisual congruent, and audiovisual McGurk (incongruent) stimulus presentation. Preliminary analysis of the fMRI data showed that normal-hearing participants engage visual, auditory, frontal, insular, and cingulate cortices, as well as the angular gyrus and precuneus, during presentation of the McGurk illusion. Hearing-impaired participants, however, only showed significant activation within the visual, auditory, frontal, and insular cortices. Listening effort was related to increased activity in the cingulate cortex during the presentation of incongruent audiovisual material (McGurk illusion) but not during congruent audiovisual presentation. Behaviorally, we found a significant correlation between everyday listening effort and the McGurk illusion, with higher listening effort leading to more McGurk illusion percepts.

We here provide evidence that listening effort plays a major role in audiovisual speech perception, especially under non-matching conditions, as higher listening effort is related to a stronger McGurk illusion. Increased activation in the cingulate cortex during the presentation of the McGurk illusion seems to be associated with increased listening effort in elderly participants. These results suggest that perception of the McGurk illusion and its neural correlates both change as a function of everyday listening effort.

Central gain is reduced with Tinnitus but remains unaltered with Hyperacusis in noise-exposed rats

Lukas Rüttiger[1], Dorit Möhrle[1], Benedikt Hofmeier[1], Marlies Knipper[1]

[1]University of Tübingen, Tübingen Hearing Research Centre, Elfriede Aulhorn Str. 5, 72076 Tübingen, Germany

A successful future therapy for Tinnitus and Hyperacusis will require a diagnostic subcategorization on the basis of objective neural correlates. In the present study, we used a refined operant conditioning animal model to divide equally noise-exposed rats into groups of animals that develop either Tinnitus, Hyperacusis, both, or no disorders.
Auditory processing (ABR wave I and IV latencies and central neural gain, ABR wave IV/I amplitude ratio), and cochlear functions (OHC dependent amplification) were determined by acoustic stimulus-evoked Auditory Brainstem Responses (ABR) and Distortion Product Otoacoustic Emissions (DPOAE), respectively.
Animals devoid of any disorders (no Tinnitus no Hyperacusis) showed signs of elevated central neural gain (increased ABR wave IV/I amplitude ratio), accompanied by shorter ABR wave I and IV latencies. To date, this is rather attributed to be a feature of Tinnitus. However – from our observations – animals with Tinnitus had rather a reduced neural response gain and delayed ABR wave I and IV latencies. Animals with Hyperacusis did not show these changes. Therefore, we think that identification of Tinnitus and Hyperacusis will substantiate reduced central gain with prolonged ABR latencies for Tinnitus.
Our findings outline a first step towards the development of a non-invasive diagnostic tool that can be used in humans and animals to objectively differentiate Tinnitus and Hyperacusis.

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Two-Pore Potassium channels in auditory processing

Mahshid Helia Saber[1], Christoph Körber[1]

[1]Institute of Anatomy and Cell Biology, Functional Neuroanatomy, Im Neuenheimer Feld 307 , 69120 Heidelberg, Germany

Auditory brainstem neurons are capable of ultrafast and precise information processing. This characteristic is a result of the ability of auditory brainstem neurons to fire action potentials at high frequency. It has been shown that this ability develops over the first two postnatal weeks before the onset of hearing. One important feature of high frequency firing is a precisely set neuronal excitability, which is determined by the resting membrane potential. The two-pore domain K+ (K2P) channels are voltage-insensitive potassium channels that form leak channels, which are crucial for the establishment of the resting membrane potential. TASK-5 is a K2P subunit, which is selectively expressed in auditory brainstem neurons and it is upregulated around the onset of hearing. However, at present the functional role of TASK-5 is unknown. We examined the role of TASK-5 with knock-down and knock-out approaches in rats and mice. Knockdown of TASK-5 by shRNA expression in the ventral cochlear nucleus (VCN) and medial nucleus of the trapezoid body (MNTB) suggests that TASK-5 has an impact on the action potential waveform as it reduces the duration of an action potential. Moreover, the AP threshold is reduced in globular bushy cells of the VCN with reduced TASK-5 expression. These results were confirmed in a knock-out mouse model. Thus, TASK-5 appears to correspond to the present knowledge of K2Ps to increase excitability upon inactivation.

More input = more information? Acoustic signal processing in a small network

Jan Scherberich[1], Manuela Nowotny[1]

Goethe University Frankfurt, Department of Neurobiology and Biosensors, Max-von-Laue Str. 13, 60438 Frankfurt am Main, Germany

In acoustic communicating bushcrickets, different mate finding strategies of several species can lead to specialized morphologies in their respective hearing organs. In *Ancylecha fenestrata* (Tettigoniidae: Phaneropterinae), males fulfill the tasks of song production (stridulation) and mate finding (phonotaxis), while females answer occasional to the male's song. We discovered a sex specific auditory fovea, an overrepresentation of the carrier frequency of the female call response at about 10 kHz, in the male's ear. Males use an unusually high amount of sensory units, about the half of the total number of auditory neurons in this hearing organ, to encode the female call frequency. To answer the question how this increased number in sensory cells, which respond to the same stimulus frequency and the resulting increased neuronal input to the auditory path, influence the signal processing along the auditory pathway, we compared the neuronal signals at different stages along the pathway in both sexes of *A. fenestrata* and an additional bushcricket species without a fovea, called *Mecopoda elongata* (Tettigoniidae: Mecopodinae). We used intracellular recordings in the primary sensory neurons in the ear (*crista acustica*) and extracellular recordings from the tympanal nerve to characterize the neuronal input to the first integration center (prothoracic ganglion), where ipsilateral and contralateral signals interact. In the ganglion, we used a multiunit array (32 channel) to identify spiking activity and local field potentials over the area of the entire ganglion. Preliminary data from our measurements in *M. elongata*, with pure-tone stimuli, exhibit a shift of the first spike latency, from about 3 ms in the sensory cell to 9 ms in the tympanal nerve and 11 ms in the first integration center. A stimulus with 20 ms duration lead to about six distinct spikes with an interspike interval of about 2 to 5 ms in the ear. In the ascending connective to the brain, the number of spikes is clearly decreased, in most cases to only one single spike after about 17 ms using the same stimulus signal. Our comparative study will further incorporate neuroanatomy by labeling (Neurobiotin, Dextran) sensory and processing neurons, to correlate these anatomical findings with the measured neuronal activity pattern in *M. elongata* and *A. fenestrata*. With these datasets we hope to better understand the underlying neuronal network and gain general knowledge about the question whether more input to a network leads to more or precise knowledge about sensory environment.

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Electrophysiological correlates of selective auditory spatial attention: effects of sex and menstrual cycle

Michael-Christian Schlüter[1], Stephan Getzmann[2], Jörg Lewald[1]

[1]Auditory Cognitive Neuroscience Laboratory, Department of Cognitive Psychology, Ruhr University Bochum, 44780 Bochum, Germany

[2]Leibniz Research Centre for Working Environment and Human Factors, Ardeystraße 67, 44139 Dortmund, Germany

Men show significantly better auditory spatial performance than women in so-called “cocktail-party” situations, that is, when a target sound source of interest has to be localized in the presence of distractor sources [1,2]. At present, the neural substrates of this sex difference are still unknown. Studies using non-spatial tasks have revealed changes in auditory event-related potentials (ERPs) across the menstrual cycle, with the most prominent changes occurring during the luteal phase [3]. Here, we addressed the questions of (1) sex differences in ERP correlates of auditory selective spatial attention and (2) effects of the menstrual cycle on these ERP correlates in women. Women were tested at three points in time of their menstrual cycle (menstrual, follicular, and luteal phases) and men were tested once. In the auditory “cocktail-party” task, a target sound source was presented among three distractors (animal vocalizations) at different locations while auditory ERPs were recorded. The subject had to localize the target by pressing one out of four buttons. Only correct trials were analysed. The results showed significant modulation of the N2 component in women depending on the phase of the menstrual cycle, with smallest amplitudes during menses. Group comparisons revealed most significant differences in P2 and N2 amplitudes between both sexes during menses of female participants, with more positive values (that is, greater P2 and smaller N2 amplitudes) in women than in men. As the N2 has been shown to be a correlate of selective auditory spatial attention [4], it seems as if changes in levels of sex hormones during the female menstrual cycle are able to modulate the brain processes underlying target localization in “cocktail-party” situations.
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Auditory adaptation to high-frequency mating calls in eneopterine crickets

Stefan Schöneich[1], Tony Robillard[2], Hannah M. ter Hofstede[3]

[1]University of Leipzig, Institute for Biology, Leipzig, Germany
[2]Muséum national d'Histoire naturelle, Paris, France
[3]Dartmouth College, Hanover, NH, USA

In field crickets (*Gryllus bimaculatus*), the brain receives auditory input from two ascending neurons; AN1 is sharply tuned to the low-frequency (5 kHz) calling song of the males and controls positive phonotaxis, whereas AN2 triggers negative phonotaxis and is tuned to high-frequency sounds like predatory bat calls. In one group of crickets (Eneopterinae: Lebinthini), however, males produce exceptionally high-frequency calling songs. We recently found that female lebinthines, instead of approaching singing males, produce vibrational responses after male calls, and males then track the source of vibrations to find females [ter Hofstede et al. 2015 Curr Biol: https://doi.org/10.1016/j.cub.2015.10.064]. In the eneopterine cricket *Cardiodactylus muria*, the dominant frequency of male calls is 14 kHz. Extracellular recordings from the neck connectives in *G. bimaculatus* showed similar activity levels in response to 5 and 14 kHz sound pulses due to AN1 and AN2 activity, respectively. Connective recordings in *C. muria*, however, showed no neural activity in response to 5 kHz and strong activity in response to 14 kHz pulses. As there is no low-frequency sound response forwarded to the brain in *C. muria* that corresponds with the narrow AN1 tuning known from field crickets, we assume that either AN1 has been lost in *C. muria* or its frequency tuning has also shifted to that of the AN2. Intracellular recording and staining experiments were conducted to compare morphologies and the directional- and frequency-tuning of auditory ascending interneurons in the brains of the two cricket species. For this, 20 ms pure-tone pulses of systematic frequency (2-20 kHz) and level (35-80 dB SPL) combinations were played by speakers positioned at 45° left and right in front of the cricket, while the animal was standing freely, only tethered at its head, on a walking sphere. In *G. bimaculatus* (N=20), we could unambiguously discriminate between AN1 and AN2 based on their frequency tuning and morphology. AN1 responses showed highest directionality (spike difference between ipsi- and contralateral sound stimulation) towards soft sound pulses of 45-50 dB at 5 kHz with directionality slightly decreasing at higher intensities, whereas AN2 directionality increased steadily with increasing intensity for 14 and 5 kHz. All ascending auditory interneurons recorded in *C. muria* (N=12) were only sensitive to high-frequency sounds with tuning curves resembling AN2 of *G. bimaculatus*. AN-responses in *C. muria*, however, showed only one small directionality peak just above its response threshold of 55 dB at 14 kHz, and almost no evidence of directionality at other frequencies and intensities. Whereas frequency and amplitude-dependent directionality is seen in *G. bimaculatus*, it appears that AN-directionality has been lost in *C. muria*, perhaps due to the evolution of a new communication system in Lebinthini crickets in which females do not require directional acoustic cues because males find the females based on their vibrational reply.

Rolr of Insular Cortex in Hyperacusis in Rat

Ali Shahbazi[1], Minoo Karimi[2], Farinaz Nasirinejad[3], Shohreh Jalaei[4], Helnaz Mokrian[2], Saeid Farahani[2]

[1]Iran University of Medical Sciences, Department of Neuroscience, Hemmat Exp Way, Tehran, Iran
[2]Tehran University of Medical Sciences, Department of Audiology, Engheleb St., Tehran, Iran
[3]Iran University of Medical Sciences, Department of Physiology, Hemmat Exp Way, Tehran, Iran
[4]Tehran University of Medical Sciences, Department of Physiotherapy, Enghelab St., Tehran, Iran

Introduction: Hyperacusis is defined as high sensitivity and extreme response to the intensity of sound that do not seem loud to others. The mechanisms underlying hyperacusis spicialy the role of insulae, a multimodal cortex with different auditory functions , is not well understood. The aim of this study is to investigate the role of insular cortex on hyperacusis.

Methods: Number of 33 male wistar rats weighting 170-200 gr. were assigned randomly in three experimental groups entitled; control group with no intervention, sham group with stereotaxic surgery without insular lesion, lesion group with bilateral stereotaxic lesion of insular cortex with NMDA (10 mg/ml). Startle responses to different intensities of auditory stimuli; 70, 80, 90, 100, 110 dBSPL, with and without background noise (70, 80 dBSPL) were measured before and one, two, four weeks after the insular lesion.

Results: our results showed an increase startle response at 100dBSPL stimulus without background noise one week after insular lesion, and increased responses to all intensities at weeks 2 and 4. Furthermore, there was a decrease in startle response to 110 dBSPL stimulus with 80 dBSPL background noise, one week after insular lesion, while no significant difference was detected in 70dBSPL background noise.

Conclusion: The findings indicated that following cortical excit0toxic lesion limited to insula there are an increase in startle responses in the absence of background noise, while a decrease in responses in the presence of background noise, representing increased sensitivity to the loudness perception and a hyperacusis-like phenomenon. In conclusion this study showed a relationship between insular cortex lesion and hyperacusis in rats.

Mice lacking the extracellular matrix protein brevican show impaired temporal processing in the inferior colliculus

Mira Türknetz[1], Jutta Engel[1], Simone Kurt[1]

[1]Saarland University, Dept. Biophysics and CIPMM, Klinikum, Geb. 48 CIPMM , 66421 Homburg, Germany

The proteoglycan brevican is a major component of the extracellular matrix of perineuronal nets and is highly enriched in the perisynaptic space suggesting a modulatory role in synaptic transmission. Brevican is part of perineuronal nets at various stages of the auditory pathway, e.g. at inner hair cells synapses and at the somata of bushy cells, octopus cells in the cochlear nuclear complex, medial nucleus of the trapezoid body (MNTB) neurons, medial superior olive (MSO) and lateral superior olive (LSO) neurons, neurons of the dorsal nucleus of the lateral lemniscus (DNLL) and some neurons of the inferior colliculus (IC) and the auditory cortex (AC). Here we study the impact of brevican on dynamics and reliability of temporal processing of neuronal response properties of IC neurons. To this aim, we performed in vivo electrophysiological recordings from neurons of the IC from systemic brevican knockout (brevican-/-) mice and their wildtype littermates. The responses of those neurons to pure tones and amplitude-modulated (AM) tones were characterized to examine the role of brevican for spectral and temporal coding.

Neurons of the IC in brevican-/- mice showed no differences in response properties to pure tones and frequency tuning compared to wildtypes. In contrast, evoked rates in response to AM tone stimulation were increased in brevican-/- mice. Further, we found an increased upper cut-off frequency of phase locking at the expense of a reduced temporal precision in brevican-/- mice in response to stimulation with AM tones.

Taken together, our results demonstrate that lack of the extracellular matrix protein brevican impairs auditory processing of AM tones. Because brevican is present at various stages of the ascending auditory pathway, the observed phenotype could result from an additive effect but could also be the result from a deficit at a specific location. This question can only be answered by a conditional, region-specific knockout mouse.

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Neurophysiological evidence for the stochastic resonance model of tinnitus development

Konstantin Tziridis[1], Evelyn Hammele[1], Patrick Krauss[1], Achim Schilling[1], Sönke Ahlf[1], Holger Schulze[1]

[1]Universitätsklinikum Erlangen, HNO-Klinik, Experimental Otolaryngology, Waldstrasse 1, 91054 Erlangen, Germany

Subjective tinnitus, the perception of a phantom sound in the absence of any physical sound source, affects about 10 to 15% of the adult population. Despite these high prevalence and intensive research there is still a controversial debate about the neurophysiological mechanisms that lead to the development of this phantom percept. Recently we have published, first, a new model for tinnitus development based on stochastic resonance (SR). We propose that SR is used to constantly optimize information transmission from the cochlea into the auditory pathway thereby improving hearing thresholds, e.g., after an acoustic trauma on the cost of evoking – as a byproduct – a tinnitus percept. Second, we published a new statistical method for analyzing tinnitus related behavior in our animal model. This new approach of analyzing the changes of the gap prepulse inhibition of the acoustic startle response (GPIAS) allows us not only to estimate the frequency range in which an animal might perceive a tinnitus but also the putative relative strength of the corresponding percepts, represented by the normalized effect size, i.e. Hedge's g.
In light of both, our new SR hypothesis and analytical approach, we correlated neuronal activity of the auditory cortex of Mongolian gerbils with the behaviorally determined GPIAS effect size. In line with the predictions of our model we found a significant correlation of neuronal thresholds with the behaviorally determined tinnitus strength. In other words, the stronger the tinnitus percept is, the stronger is the benefit for hearing thresholds.

A model system to investigate sensory gating during sleep

Philipp van Kronenberg[1,2], Linus Milinski[2], Livia de Hoz[1,2]

[1]Charité - University Medicine, Neuroscience Research Center, Charitéplatz 1, 10117 Berlin, Germany
[2]Max Planck Institute for experimental Medicine, Neurogenetics, Herman-Rein-Straße 3, 37075 Göttingen, Germany

Sleep is necessary to maintain homeostasis of essential physiological processes in the brain. At the same time, it poses a risk to the sleeping animal due to the associated immobility and decreased behavioural responsiveness. Continuous processing of auditory stimuli during sleep is, for this reason, essential in order to detect approaching dangers. To date little is known about the mechanisms and structures underlying gating of meaningful sounds during sleep. In order to investigate the circuit involved in this gating we first developed an experimental model to study sound perception in mice during sleep. In this model mice were conditioned to associate a sound with an approaching danger. During sleep different sounds, conditioning and control, were presented, while electroencephalogram (EEG) and electromyogram (EMG) signals were recorded to assess the mental state of the animal. We evaluated the effect of conditioned and control sounds on sleep patterns. We found that sound presentation during sleep could change the EEG pattern during both REM and non-REM sleep phases without necessarily awakening the animal. While during non-REM both the conditioned and the control sounds elicited a change in the EEG pattern, during REM only the conditioned sound did. This suggests that sensory gating is more specific during REM than during non-REM sleep. Overall the results indicate that mice can discriminate between behaviourally relevant sounds during sleep and offer a model to investigate the circuit underlying sleep-associated auditory information processing.

Cathodic-leading and anodic-leading intracortical microstimulation differentially activates the auditory cortex

Mathias B. Voigt[1], Andrej Kral[1]

[1]Hannover Medical School, Institute of AudioNeuroTechnology (VIANNA), Stadtfelddamm 34, 30625 Hannover, Germany

Current developments in cortical neuroprosthetics largely rely on intracortical microstimulation (ICMS), i.e., electrical stimulation inside the cortical tissue, to restitute a (lost) sense or more generally provide feedback directly to the brain. For stimulation safety reasons the electrical pulses delivered usually consist of two phases of opposite polarity. Previous *in silico* simulations predicted a differential effect of the polarity of the leading phase (cathodic-leading vs. anodic-leading) of the stimuli on the specific neuronal activation patterns. Here, we investigated the effect of the leading-phase polarity of single ICMS pulses in the auditory cortex *in vivo*.

In vivo experiments were performed in adult guinea pigs (n=15) under ketamine/xylazine anesthesia. We recorded local field potentials and multi-unit activity from the primary auditory cortex using linear multi-electrode arrays spanning all six cortical layers. We compared current source density profiles and multi-unit spiking patterns to acoustical (50 µs clicks, 15-95 dB) and both cathodic- and anodic-leading electrical (0.1-45.0 µA, 200 µs/phase) stimuli.

As in the case of electrical stimulation of subcortical auditory nuclei, the response of the primary auditory cortex to ICMS pulses of both leading-phase polarities demonstrated a significantly reduced dynamic range in comparison to the response to acoustic click stimuli. There was a strong contrast in effectivity between the two different leading-phase polarities. Cathodic-leading stimuli were being more effective than anodic-leading stimuli in evoking a cortical response. The effectivity of ICMS pulses depended on the stimulation depth (comp. Voigt et al., 2017, Brain Stim). This led to a differential effect of the leading-phase polarity when stimulating deep cortical layers: In contrast to cathodic-leading stimuli, anodic-leading stimuli failed to evoke local neuronal activity when stimulating the deep layers. Furthermore, the latency of the multi-unit response provided supportive evidence for a preferential activation of fibers of passage with cathodic-leading stimuli, which was absent in anodic-leading stimuli.

These results provided robust *in vivo* evidence for a difference in the neuronal activation mechanism and consequently the effectivity of anodic- and cathodic-leading stimuli in the auditory cortex. Cathodic-leading stimuli should be preferred for ICMS applications like cortical neuroprosthetics.

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Detection Learning of Optogenetic and Electrical Stimulation in the Auditory Cortex of Mongolian Gerbils

Theresa Christiane Sofia Weidner[1,2], Nabila Alam[1], Martin Deckert[2,4], Gonzalo Arias Gil[1], Armin Dadgar[2,5], Frank W Ohl[1,2,3], Kentaroh Takagaki[1,2], Michael T Lippert[1,2]

[1]Leibniz Institute for Neurobiology, Systems Physiology of Learning, Magdeburg, Germany
[2]Center for Behavioral Brain Sciences (CBBS), Otto-von-Guericke-University Magdeburg, Germany
[3]Institute of Biology, Otto-von-Guericke-University Magdeburg, Germany
[4]Microsystems Technology, Institute for Micro- and Sensor Systems, Otto-von-Guericke-University Magdeburg, Germany
[5]Department of Semiconductor Epitaxy, Otto-von-Guericke University Magdeburg

Loss of function in sensory organs and primary sensory pathways is dificult to treat and tissue damage is often irreversible. Using cortical stimulation, damaged lower sensory structures can be circumvented by writing information directly into the sensory cortex. Here, we test the parameter space of detectable optogenetic and electrical brain stimulation in the auditory cortex of Mongolian gerbils.
Animals underwent surgery to transduce the primary auditory cortex (A1) with ChR2(H134R) and to implant an optrode. Additionally, we implanted two recording-electrodes at the surface of A1. After one week of recovery, we trained the animals in an auditory detection task using frequency modulated tones in a trace conditioning paradigm. When an animal had reached criterion (performance>40% in 3 consecutive sessions), the task was switched to the detection of optogenetic and electrical stimulation of A1.
Animals learned to detect stimuli in all three modalities, despite an initial drop in performance during artificial stimulation Surface ERP recordings revealed that artificial cortical stimulation was only behaviorally detectable when the elicited responses were significantly larger than auditory stimulation.
Our results demonstrate that gerbils can switch from an acoustic detection task to cortical electrical and optogenetic microstimulation and provide candidate stimulation parameters for use in cortical neuroprostheses.

Layer-specific entrainment to acoustic sequences in the Auditory Cortex

Kristin Lisa Weineck[1], Mira Röhm[1], Francisco Garcia-Rosales[1], Julio Hechavarria[1]

[1]Institute for Cellbiology and Neuroscience, Goethe University Frankfurt am Main, Neurobiology of Auditory Computations Group, Department of Neurobiology and Biosensors, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany

Though distress calls, produced in dangerous situations, can be crucial for the animals' survival, the physiological processes taking place in the listeners' brain and especially within the columns of the Auditory Cortex are not well understood. This study investigates the electrophysiological differences in entrainment between cortical layers during the exposure to natural acoustic sequences (distress calls) in the bat species *Carollia perspicillata* using laminar silicon probes. During stimulus presentation, an auditory steady-state response established, which had the ability to precisely track time as well as energy information and was manually divided into the slow (4-12 Hz) and fast (76 – 125 Hz) components of the local field potential (LFP). The fast LFP revealed generally no temporal adaptation and showed differences in amplitude across layers. In contrast, the slow LFP showed additional interlaminar variations in the time domain, indicating the involvement of several local and global auditory processing mechanisms that aid the encoding of acoustic sequences at the cortical level. To quantify the strength of coherence between the neurophysiological response and the auditory stimulus, the stimulus field coherence (stimFC) was calculated and compared across layers. In total, major significant differences in the stimFC between upper and lower cortical layers in the alpha (8 - 11 Hz) and low beta (12 – 20 Hz) range were found. However, an especially high level of coherence was detected in the gamma band (around 60 – 100 Hz) which corresponds to amplitude modulation frequencies that were best represented in the distress sequence used as stimulus, and was found in the granular and upper infragranular layers. Together, our results demonstrate that the entrainment to the auditory stimulus is strongest in cortical layers receiving prominent input from other brain areas such as the thalamus, suggesting an important role of thalamocortical interactions in auditory processing.

Poster Topic

T19: Chemical Senses: Olfaction, Taste, Others

T19-1A Perception and representation of temporally structured odor stimuli in the mouse olfactory bulb
Tobias Ackels, Andrew Erskine, Debanjan Dasgupta, Izumi Fukunaga, Alina Christina Marin, Andreas T Schaefer

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Perception and representation of temporally structured odor stimuli in the mouse olfactory bulb

Tobias Ackels[1,2], Andrew Erskine[1,2], Debanjan Dasgupta[1,2], Izumi Fukunaga[1,2,3], Alina Christina Marin[1,2], Andreas T Schaefer[1,2]

[1]Neurophysiology of Behaviour Laboratory, The Francis Crick Institute, London, UK
[2]Department of Neuroscience, Physiology and Pharmacology, University College London, London, UK
[3]Okinawa Institute of Science and Technology, Okinawa, Japan

Intensity fluctuations inherent to natural odour plumes are strongly modulated by the turbulent airflows in which they are carried. Odour plumes therefore hold rich temporal dynamics of odour concentration variation. As turbulence continually builds the structure of an odour plume, these dynamics contain information about the distance the plume has travelled as well as its source properties. It has, therefore, been hypothesised that animals could process the temporal dynamics of natural odour plumes in order to navigate and perform odour scene segmentation.
We used dual-energy photoionisation recording to determine the temporal features of odour plumes that are of potential behavioural salience during odour scene segmentation, finding that temporal correlation of odour concentrations reliably predicts whether odorants emerge from the same or different sources. To test whether mice are capable of using these temporal correlations to determine odour source separation, we developed a high-bandwidth odour delivery device capable of replicating many of the temporal features found in odour plumes. In conjunction with a high-throughput behavioural conditioning system (AutonoMouse) we trained mice (n=36) to discriminate between pairs of odours with a range of temporal correlations. Trained mice were capable of discriminating correlation structure at frequencies well over the sniff rate (>40 Hz).
Examining reaction times during behaviour and disrupting correlation structure of the stimulus onset revealed that the animal's performance did not rely on the early part of the stimulus but that longer sampling time correlated with better performance.
The high-throughput nature of these behavioural experiments allowed us to determine the psychophysical limit of perception for temporal correlation between odours, and therefore to define an appropriate stimulus range to investigate olfactory bulb representation of these stimuli. In vivo Ca2+ imaging and extracellular recordings from mitral/tufted cells showed segregated responses depending on the correlation of odour stimuli with populations of 10s of neurons sufficient to accurately reach behavioural performance.
We conclude that information of temporal correlations between odours is present in the output neurons of the olfactory bulb at sub-sniff resolution. Mice are capable of utilising this information in behaviour, suggesting that perception of temporal features of odour stimuli may be a mechanism by which animals perform odour scene segmentation. Thus, olfaction is a high bandwidth sense with temporal structure containing information about olfactory space that is indeed accessible to mice.

Broadly overlapping, but distinctly different expression domains for V1R-related zebrafish ora genes

Shahrzad Bozorg Nia[1,2], Daniel Kowatschew[1,2], Sigrun I. Korsching[1]

[1]University at Cologne, Institute of Genetics, Zülpicher Str. 47a, 50674 Cologne, Germany

[2]shared first author

The vertebrate sense of smell is carried by four main olfactory receptor families, among them the V1R/ORA family, which is small and highly conserved in teleost fish [1] – an unusual feature, since olfactory receptor gene families in general and mammalian V1Rs in particular are characterized by dynamic evolution including frequent gene birth and death events. We wished to investigate whether the principles guiding spatial expression patterns for the ORA family would be similar to those observed for larger olfactory receptor families [2, 3]. Zebrafish possess a typical teleost olfactory organ, cup shaped, with lamella covered with sensory epithelium protruding into the cup from a median raphe. We have performed quantitative in situ hybridization on complete series of horizontal cryostat sections of adult zebrafish olfactory epithelium, and have analysed the location of ora-expressing cells in three dimensions, radial diameter, height within the lamella, and height within the organ. We report broad, but distinctly different distributions for all ora genes, with preferred positions in different dimensions independent of each other. This is even valid for the closely related gene pairs ORA1/ORA2 and ORA3/ORA4. Together, the different distributions span nearly the full range of the sensory surface within the olfactory organ, suggesting that expression of ora genes is intermingled with those of other olfactory receptor families such as odorant receptors and V2R-related olfC genes.

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The expression pattern of two “Sensory neuron membrane proteins” emphasizes different roles of the CD36-related proteins in the olfactory system of moths

Sina Cassau[1], Stefanie Blankenburg[1], Jürgen Krieger[1]

[1]Martin Luther University Halle-Wittenberg, Institute of Biology/Zoology - Dept. of Animal Physiology, Hoher Weg 8, 06120 Halle (Saale), Germany

The ability to recognize distinct olfactory cues in the environment is of vital importance for most animals and largely determines their behavior. In moths, reproductive behavior heavily depends on female- or male-released pheromones and their sensitive detection by specialized olfactory sensory neurons (OSNs) residing in the antenna of conspecifics. Previous studies have shown that pheromone-responsive OSNs express a distinct pheromone receptor (PR) and moreover are characterized by the so-called “sensory neuron membrane protein 1” (SNMP1). In accordance with a proposed function of SNMP1 as a co-receptor involved in the transfer of pheromones to adjacent PRs, our studies revealed the co-expression of SNMP1 and receptors for female sex pheromone components in the noctuid moth *Heliothis virescens*. In addition, we found that support cells (SCs) of pheromone-responsive sensilla exhibit transcripts encoding a related protein, named SNMP2. Like SNMP1, SNMP2 belongs to the CD36-family of two-transmembrane domain receptors and transporters for lipophilic compounds. Towards a better understanding of the role of the two SNMP types in the olfactory system of moths, we generated polyclonal antibodies against SNMP1 and SNMP2 of *H. virescens* and conducted in-depth immunhistochemical analyses of their distribution and subcellular localization in the antenna. In line with the proposed function, SNMP1 was immunolocalized in the somata and the dendrites of OSNs in subsets of trichoid sensilla. In males, these sensilla trichodea generally contain one SNMP1-positive OSN whereas clusters of 2-3 labelled cells were found in females. In contrast, experiments with anti-SNMP2 antibodies revealed a broad expression of this protein in SCs of likely all trichoid and basiconic sensilla independent of sex. More detailed confocal and electron microscopic investigation of olfactory sensilla demonstrates SNMP2-like immunoreactivity close to the apical membrane of SCs and interestingly inside the lymph space of the sensillum. This expression pattern suggests a more general function of SNMP2, possibly in processes related to cleaning of olfactory sensilla. To assess the general validity of our findings we used the anti-HvirSNMP antibodies to scrutinize the antenna of two related moths species, *Helicoverpa armigera* and *Spodoptera frugiperda*. The present results indicate similar expression patterns for SNMP1 and SNMP2 as in *H. virescens* and further emphasize distinct functions of the two SNMP types in the olfactory system of moths.

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Long-term dietary experience of *Drosophila* results in structural modification in mushroom body-related dopaminergic neurons

Büsra Coban[1], Haiko Poppinga[1], Thomas D. Riemensperger[1], Andre Fiala[1]

[1]University of Göttingen, Molecular Neuroscience of behaviors, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

The experience of food with high or low nutritional value can influence behavior, e.g. food-related memory formation and feeding prevalence. The neuronal basis of these alterations that are dependent on the flies' dietary remain unclear. In this study, we use *drosophila melanogaster* to address the question how the caloric value of the animals' dietary affects behaviors such as olfactory associative learning and food uptake. In particular, we investigated functional and structural changes in neuronal connectivity between dopaminergic neurons (DANs) and intrinsic mushroom body neurons (Kenyon cells).

In order to analyze the effects of long-term feeding experience, three days old adult flies were kept on either hypocaloric, isocaloric or hypercaloric food for seven days. Subsequently, we have quantified the flies' food uptake using a capillary feeder assay (CAFE assay), and we have analyzed the flies' appetitive olfactory learning and short-term memory. Our results show that the calorie restriction causes an increased food uptake and enhancement in appetitive learning, while aversive olfactory conditioning remained unaffected when the flies were starved for 6 hours before the experiment.

The mushroom body (MB) is a key brain structure involved in associative learning and memory formation. The reinforcing effects of rewarding sugar stimuli or punitive signals are mediated by modulatory DANs innervating distinct compartments of MB. We investigated if modulatory DANs undergo structural rearrangement dependent on the dietary of the flies by measuring the intensity of reconstituted split GFP ("GRASP") across putative synaptic contacts between DANs and Kenyon cells. We found that DANs innervating specifically the γ3 compartment of the MB undergo structural remodeling in dependence of the nutritional value of food, whereas DANs innervating the other compartments remain unmodified. Upon calorie restriction γ3 DAN innervations onto Kenyon cells decreased significantly. Furthermore, we have investigated if there is any functional change in γ3 DANs which can perhaps be causative for their restructuring. Thus, we have measured intrinsic calcium activity of the cells over a long-term period in a cumulative way using the GFP- based Ca2+ indicator CaLexA. We found an increase in the activity of the γ3 DANs upon restriction of the caloric food value. In summary, the caloric value of food re-structures brain connectivity in that it shapes the contacts between specific DANs and Kenyon cells.

Functional and morphological diversity of projection neurons in the olfactory bulb of larval *Xenopus laevis*

Daniela Daume[1], Thomas Offner[2], Thomas Hassenklöver[1], Sara J. Hawkins[1], Lukas Weiss[1], Ivan Manzini[1,2]

[1]Animal Physiology, Animal Physiology and Biomedicine, Gießen, Germany

[2]Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany

The olfactory system is among the most ancient sensory systems and the blueprint of its neuronal circuitry is highly conserved across phyla. Olfactory sensory neurons detect odorant molecules and subsequently generate electrical activity patterns. The encoded information is then conveyed via the sensory neuron axons to the olfactory bulb. A population of projection neurons receives excitatory synaptic input of the sensory neuron axons and acts as first relay station of olfactory information processing. In mammalian olfactory systems, projection neurons were historically subdivided into mitral and tufted cells according to their morphology or localization in the olfactory bulb's histological layers. More recent research revealed that those populations are not only morphologically distinct, but also vary in terms of odor coding, axonal projection fields and their role in the circuitry. In earlier diverging vertebrates like amphibians, the clear distinction between projection neurons based on morphology and localization is more challenging. This is mostly due to high variability in shape and number of primary dendrites and less obvious cellular layering in the olfactory bulb. In this work, we provide a functional and morphological characterization of the projection neuron population in larval *Xenopus laevis*. We used a broad spectrum of methods ranging from neuronal tracings over immunohistochemistry to functional calcium imaging. We found differences in projection neuron dendritic configurations, axonal projection patterns, neuronal marker expression and odor tuning. The larval amphibian olfactory system seems particularly suited to investigate projection neuron specialization from an evolutionary and developmental perspective.

Investigation of calcium-mediated signaling in different compartments of mouse vomeronasal sensory neurons

Rudolf Degen[1], Marc Spehr[1]

[1]RWTH Aachen University, Biology II, Department of Chemosensation , Aachen, Germany

In mammals, the vomeronasal system detects a large number of semiochemicals. These cues convey information about sexual, social and reproductive status and thus regulate behavior. Within the vomeronasal organ, vomeronasal sensory neurons (VSNs) translate chemosensory information into electrical activity which is processed in the brain. This signal transduction starts with activation of G-protein coupled receptors in VSN microvilli. A complex biochemical cascade is triggered leading to generation of different messenger molecules through phospholipid turnover. Ultimately, diacylglycerol-dependent gating of a Ca^{2+}–permeable ion channel is thought to complete signal transformation. However, our understanding of many basic VSN signaling mechanisms is still rather fragmentary. Using photoactivatable chemical constructs, we investigate the effects of Ca^{2+} influx into different regions of the cell. Focal laser-assisted uncaging experiments with o-nitrophenyl-EGTA allows subcellular control of Ca^{2+} concentrations at high spatiotemporal resolution. Experimental focus is placed on Ca^{2+} signal profiling within various neuronal compartments. Combining Ca^{2+} uncaging, Ca^{2+} imaging, pharmacological blocking and whole-cell patch clamp recordings, we generate a detailed activity map of Ca^{2+}-mediated signaling in mouse VSNs.

Experience-dependent plasticity of an aversive olfactory circuit in *Drosophila melanogaster*

Benjamin Fabian[1,2], Veit Grabe[1], Rolf Beutel[2], Bill Hansson[1], Silke Sachse[1]

[1]Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Hans-Knoell-Strasse 8, 07745 Jena, Germany

[2]Friedrich-Schiller-Universität Jena, Institut für Zoologie und Evolutionsforschung mit Phyletischem Museum, Ernst-Hackel-Haus und Biologiedidaktik, Erbertstraße 1, 07743 Jena, Germany

The sense of olfaction is very crucial for insects in order to navigate in a complex environment of volatile odorants. Olfactory cues play a major role in locating suitable substrates for feeding and oviposition and are necessary for finding potential mating partners or for the avoidance of predators and parasitoids. While the structure and function of the olfactory system of *Drosophila melanogaster* is well understood and documented, little is known about whether and to which extent individual experience is able to modify certain parts of this system. In our study we focus on the aversive olfactory circuit that is activated by geosmin, an ecologically highly relevant odorant of toxic mold. Flies are cyclically exposed to geosmin over the duration of four days and afterwards their brains are analyzed via two-photon imaging techniques. In the presented poster we show which parts of the olfactory circuit are able to undergo plastic changes and which parts seem to be hardwired.

In search for pheromone receptors in the desert locust *Schistocerca gregaria*

Joerg Fleischer[1], Pablo Pregitzer[2], René-Sebastian Lemke[1], Xingcong Jiang[2], Ewald Grosse-Wilde[3], Heinz Breer[2], Jürgen Krieger[1]

[1]Martin Luther University Halle, Institute of Biology/Zoology, Halle (Saale), Germany
[2]University of Hohenheim, Institute of Physiology, Stuttgart, Germany
[3]Czech University of Life Sciences, Faculty of Forestry and Wood Sciences, EXTEMIT-K, Prague, Czech Republic

The desert locust (*Schistocera gregaria*) is feared for its ability to form swarms of billions of animals causing massive destruction of crop yields. For reproductive and aggregation behaviors that underlie swarm formation in locusts, pheromones have been reported to be of critical relevance. The mechanisms and signaling proteins mediating pheromone detection in locusts and other hemimetabolan insects are largely unknown. In holometabolan insects (such as flies and moths), pheromones are detected by olfactory sensory neurons (OSNs) on the antennae expressing pheromone receptors (PRs) that belong to the superfamily of odorant receptors (ORs). Moreover, pheromone-reactive OSNs are characterized by the expression of the "sensory neuron membrane protein 1" (SNMP1) that is considered to serve as co-receptor transferring pheromones to the relevant PR proteins.
In search for olfactory receptors involved in locust pheromone detection, the OR repertoire of *Schistocerca gregaria* was analyzed by sequencing the antennal transcriptome, leading to the identification of 119 OR types. To unravel candidate PRs, potential co-expression with SNMP1 was investigated for a larger number of the ORs. Thus, several ORs were found to be co-expressed with SNMP1 in antennal OSNs; these receptors are therefore considered as candidate PRs. To assess a possible involvement of the SNMP1-co-expressed OR types in locust pheromone detection, activation by the pheromonal compounds phenylacetonitrile and acetophenone from *Schistocerca gregaria* was scrutinized for several of them using "DREAM" (deorphanization of receptors based on alteration of mRNA concentration) experiments. These approaches indicated that some of the ORs co-expressed with SNMP1 are activated by either phenylacetonitrile or acetophenone. Currently, heterologous expression systems are utilized to further investigate the responsiveness of these OR types to locust pheromones.

Is the olfactory code combinatorial or multidimensional?

C Giovanni Galizia[1]

[1]University of Konstanz, Biology, Universitätsstr. 10, 78457 Konstanz, Germany

Color vision can create an almost infinite number of colors with just three photoreceptor types (in humans – some species have less or more photoreceptor types). Olfaction also creates an infinite number of odor percepts – but the number of olfactory receptors is much larger, with approximate values of 50 in Drosophila, 350 in humans, or 1000 in mice. However, while it is easy to distinguish two very similar colors when they are next to each other, it is difficult to identify them when presented alone the next day. Olfaction, on the other hand, allows recognizing a particular stimulus also years later. Does this qualitative jump from "diversity" to "identity" come with a large number of receptors alone, or does it relate to the way that odor information is encoded and memorized in the brain?

Most animals have large numbers of olfactory receptor types. Their axons project to olfactory glomeruli in the antennal lobe or olfactory bulb. An olfactory stimulus elicits a complex pattern of activated glomeruli – with different activity strength. These activity patterns have been characterized in many species for a variety of odorants, including mice, Drosophila and the honeybee. Despite the large amount of data available, the logic of how these complex patterns are read out by the brain remains elusive. In Drosophila, an increasing number of receptors appears to have a single (or very few) exceptionally good ligands, prompting some colleagues to propose a "labelled line" scheme for some odorants. Also in humans some anosmias are known that are caused by a single missing receptor gene. A system based on "labelled line" receptors is combinatorial, i.e. the combination of activated glomeruli/receptors can be decoded into the combination of odorants that form a stimulus blend. On the other hand, a system based on broad response spectra is multidimensional (such as color vision), where only the comparison across receptor types can be used to identify an odorant.

However, the presence of both highly specific and broadly tuned receptors might suggest that two systems coexist. The brain, then, could use a separate, parallel decoding system for combinatorial and multidimensional olfactory coding.

Dose-dependent modulation of olfactory transduction in mice

Kira Gerhold[1], Marc Spehr[1]

[1]RWTH Aachen University, Dept. of Chemosensation, Worringerweg 3, 52074 Aachen, Germany

Repeated or prolonged odor exposure typically leads to adaptation, stimulus-specific decreases in olfactory sensitivity by negative feedback modulation. Previous studies that investigated adaptation phenomena in olfactory sensory neurons (OSNs) have mostly been executed using saturating concentrations of stimuli. Here, we investigate the dose-dependency of OSN response modulation. We use cell-attached patch-clamp recordings from visually identified OSNs in an intact slice preparation of the mouse main olfactory epithelium. Acute sections preserve the epithelial structure and functional OSN properties, providing physiological conditions. Using IBMX/forskolin as a 'broadband' stimulus that bypasses receptor activation, we analyze OSN dose-response relationships. Our data reveal that IBMX/forskolin concentrations in the nano- to low micromolar range are sufficient to activate a substantial OSN population. Upon repetitive stimulation with such low stimulus concentrations, the majority of neurons exhibits no sign of adaptation. Some OSNs even display substantial response potentiation. However, the fraction of neurons showing such response potentiation declines with increasing stimulus concentrations, demonstrating that OSN response modulation is dose-dependent and subject to bidirectional regulation, i.e., to potentiation and adaptation. Therefore, OSNs share the mechanistic tools to adjust their sensitivity and, thus, to accommodate a broad range of odor concentrations.

Alarm pheromone modulates odor responses in the antennal lobe of the European honeybee (*Apis mellifera*)

R. Keating Godfrey[1,3], Jean-Marc Devaud[2], Martin Strube-Bloss[3]

[1]Department of Neuroscience, University of Arizona, Tucson, USA
[2]Centre National de la Recherche Scientifique (CNRS), Centre de Recherches sur la Cognition Animale (UMR 5169), Paul Sabatier University, Toulouse, France
[3]Department of Behavioral Physiology & Sociobiology, Biocenter University of Würzburg, Würzburg, Germany

In social organisms cues from conspecifics have the power to override other sources of information, even when these other stimuli are salient or rewarding. This dominance of social cues is observed in a number of vertebrates and invertebrates, but the neuronal mechanisms are not well-understood in any species. Honeybees, renowned for their ability to rapidly associate odors with reward, are also highly responsive to social cues. Alarm pheromone reduces appetitive learning performance in honeybees, an effect not explained by impaired sensitivity to odorants nor to the food reward. Interestingly, pre-exposure to the main component of alarm pheromone, isopentyl acetate (IPA), may enhance odor discrimination; bees pre-exposed to IPA responded more frequently to the particular training odor than closely related compounds which are more easily generalized by non-treated bees. In this project we investigate the underlying neuronal mechanisms of these effects by characterizing neural representation of odorants in the antennal lobe (AL) prior to and following IPA exposure. We use multi-unit extracellular, long-term recordings in combination with a subsequent spike sorting to obtain single unit activity. Preliminary results indicate that single AL-units became significantly modulated after IPA exposure, resulting in an altered population code in the recorded AL ensemble. Despite this significant modulation, the AL-network still separates the tested odors after IPA exposure.

Active olfactory sensing in the American cockroach, Periplaneta americana

Antoine Hoffmann[1,2], Jahn Nitschke[1], Giovanni Galizia[1], Einat Couzin-Fuchs[1]

[1]Neurobiologie - Universität Konstanz
[2]Max Planck Inst. für Ornithologie & IMPRS for Organismal Biology

Olfaction has traditionally been studied as a slow and static sense, even though it is clear that animals actively enhance their odor perception with specific behavior (sniffing, flight manoeuvres, antennal movements, etc.). We use the American cockroach (Periplaneta americana), who actively oscillates its long and highly mobile antennae during search, to study active olfactory sensing strategies. Naturalistic olfactory environments are turbulent, containing mixed odorants and multiple sources. We hypothesize that animals therefore need to actively adapt their movement to these changing parameters in order to extract relevant information and make decisions. In that regard, active sensing can be viewed as the adjustment of sampling behaviors to a changing olfactory landscape. Despite some research on antennal movements patterns in cockroaches (Okada and Toh 2004, Willis and Avondet 2004, Nishiyama 2007), it has still not been shown whether and how they use them in such a manner. Using behavioral wind-tunnel experiments that include antennae and body tracking and odor plume manipulations, we study the relation between odor encounter, plume structure and movement decisions. Our first sets of experiments demonstrated that both onset and offset of different odor stimuli modify body and antennal movements. We observe odor-specific antennal oscillation patterns with slow (0.5-2 Hz) and fast sweep cycles (4-5 Hz) as well as altered sweep ranges. There is a correlation between walking behavior and antennal frequency in an odor-dependent manner. Furthermore, by mapping odor concentrations in the wind-tunnel using a Photoionization Detector (PID) and systematically shifting the odor plume, we observe odor-specific plume-following behavior. These results indicate that there is an active adaptation of movement patterns according to the nature of the odor stimulus and its structure.

Steroid binding proteins in the human vomeronasal organ

Gustav Jirikowski[4], Martin Voß[3], Veronika M. Gebhart[2]

[1]Universitätsklinikum Jena, Inst. Anatomie II, Teichgraben 7, 07743 Jena, Germany

The persistence of a functional vomeronasal organ (VNO) in adult humans has been recently shown. Here we studied postmortem tissue samples of nasal mucosa and tissue samples obtained after surgical correction of nasal septum. We examined histological sections with antibodies to sex hormone binding globulin (SHBG) and to estrogen receptor alpha (ER), to corticosteroid binding globulin (CBG) and to glucocorticoid receptor (GCR) and to vitamin D binding protein (DBP) and to vitamin D Receptor (VDR). mRNA was extracted from tissue homogenates and subjected to RT- PCR in order to verify the respective transcripts.

In all samples we found epithelial cells within the mucosa of the lower part of the nasal septum which exhibited the morphological features of sensory neurons and which showed immunostaining for olfactory marker protein (OMP). These cells were interposed by ciliated cells, goblet cells and small capillaries. Only occasionally we found such cells within a defined epithelial duct. Many of the OMP- positive cells were immunoreactive for one or more of the steroid binding proteins. SHBG, CBG or DBP and their respective steroid receptors revealed differential distribution in sensory neurons: While steroid receptor staining was found in sensory zilia and in nuclei, the binding globulins were found in sensory dendrites and in dendritic protrusions. There was some overlap of the different steroid binding proteins, suggesting that sensory VNO neurons can be targeted by various steroids.

Similar to the VNO of microsmatic animals the human VNO may be involved in detection of steroidal pheromones.

Large scale evolutionary analysis of TAAR olfactory receptors in the aquatic lineage

Sigrun I. Korsching[1], Milan Dieris[1]

[1]Universität zu Köln, Institut für Genetik, Zülpicher Str. 47a, 50674 Köln, Germany

Trace amine-associated receptors (TAARs) have recently been shown to function as one of four major olfactory receptor families in vertebrates [1]. Members of the TAAR family regulate several important behaviors both in tetrapods and teleosts [2]. Chemosensory receptor families have arisen independently several times during evolution, but the time of birth is often not clear due to limited availability of key genomes. The origin of taar genes has been variously described to occur in jawed vertebrates, vertebrates, and even chordates [2, 3, 4]. Here we performed a thorough phylogenetic analysis making use of a plethora of recently available genomes. We report that olfactory functionality has arisen twice independently within the TAAR family, once in jawed and once in jaw-less fish. In lamprey, an ancestral gene expanded to generate a large family of olfactory receptors, while the sister gene did not expand in jawed vertebrates and appears not to serve an olfactory function. Both the lamprey receptors and their sister genes in jawed vertebrates do not exhibit the defining TAAR motif, thus we suggest to name them TAAR-like receptors (TARL). We have identified the evolutionary origin of both taar and tarl genes in a duplication of an much older serotonergic receptor in the most recent common ancestor of vertebrates. We infer two ancestral genes in bony fish for a subgroup of taar genes, class II. These two clades are present in both teleost fish (TAAR12, TAAR13) and the tetrapod lineage - in fact all mammalian olfactory TAAR receptors belong to class II. TAAR13 was found in both early-diverging and late-diverging bony fish at varying levels of gene expansion, suggesting many evolutionary late gene birth events and over half a dozen independent gene death events. TAAR12 showed the largest gene expansions within all class II taar genes, resulting in 15 different taar12 genes for the common carp, a close relative of zebrafish, and 13 genes in an eel species. However TAAR12 has been lost completely in later-derived fish such as neoteleosts. The loss of TAAR12 in all and TAAR13 in most of the neoteleost fish species may suggest compensation of functionality by class I and/or class III TAARs. Alternatively, a corresponding change in ecological requirements of more modern fish may have occurred.

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Dynamic Representations of Categories in the Mouse Olfactory Bulb

Elena Kudryavitskaya[1], Eran Marom[1], David Pash[1], Adi Mizrahi[1]

[1]The Hebrew University, Neurobiology department,The Edmond J. Safra Campus Givat Ram. Jerusalem 9190401. Israel.

Categorical perception is a canonical feature of sensory systems. In earlier studies of category perception learning , distinguishable objects on each side of the category boundary are often treated as the same. In real life, however, even when we spontaneously group two items into the same category, we do not necessarily treat them as identical for all purposes. For example, objects with different contexts can be assigned into different categories. In our study, we tested the impact of learning on category formation in the mouse OB. We trained mice using a Go No-Go paradigm using two different odour classification tasks: 1. An easy 2-category task where animals learned to classify binary odor mixtures in two groups according to the dominant component in the mixture. 2. A hard task with multiple discrimination boundaries intermingled along the space of binary odor mixtures. To study the neural correlates that accompany these tasks we used in vivo two-photon calcium imaging of Mitral Cells (MCs) in head-fixed awake mice engaged in the task. Morphing of one odour into another resulted in abrupt transitions between odour representations in ensembles of MCs, in accordance to what is known in the literature. We found that MCs responses were not only informative about molecular identity of odours but also represented the judgment of an animal for the task. When mice were engaged in the task, neuronal responses of MCs were more closely tied to perception than physical properties of the stimulus. Moreover, time lapse imaging of MCs showed task-dependent bidirectional changes at both the single cell level and the population level. Specifically, when mice were engaged in the 2-category task, MC odor responses reflected the perceptual boundary. This sharp 2-category transition was completely abolished in the 8-category learning. Our data thus underscores that categorical representation of odors in the mouse OB is dynamic and can be reorganized following learning.

Dual-color imaging for isolating olfactory bulb output streams in mice

Kim Chi Le[1], Daniela Brunert[1], Markus Rothermel[1]

[1]RWTH Aachen University, Dept. Chemosensation, AG Neuromodulation, Worringer Weg 3, 52074 Aachen, Germany

Mitral and tufted cells (MTCs) are the main output neurons of the olfactory bulb (OB) and project to different areas within the olfactory cortex (OC). Tufted cells predominantly send afferents to the anterior OC whereas mitral cells innervate the whole OC. It is known, that different brain areas can process segregated aspects of the sensory space as for example the “what & where” pathway in vision. Whether different regions in the OC receive different functional input from MTCs however, remains unknown.
Here, we established a dual-color imaging approach to visualize activity from anatomically defined MTC subpopulations. We performed AAV-mediated retrograde tracing from two regions of the OC (anterior olfactory nucleus (AON) & anterior piriform cortex (aPC)) to isolate MTC output streams based on their axonal targets. In order to separate these output streams we used green or red genetically encoded fluorescent calcium indicators; GCaMP6 and jRCaMP1a. Retrograde tracing from the AON or aPC yielded a strong expression in OB output neurons: labeled somata were mainly localized in the mitral cell and external plexiform layer.
Using widefield imaging we visualized AON or aPC traced MTC activity at the population level and compared their responses with PCD-GCaMP animals expressing GCaMP6 in olfactory sensory and OB output neurons. Odor presentation in PCD-GCaMP mice exhibited defined OB activity maps, consisting primarily of discrete glomerular foci, likely reflecting pre- and postsynaptic activity. In contrast, OB maps from AON or aPC traced MTCs showed a pronounced diffuse component. This was likely mediated by long range secondary MTC dendrites thereby confirming the specificity of our tracing approach.
Next, we compared target-defined OB output streams at a single-cell level using two-photon microscopy. So far, studies classifying mitral and tufted cells mainly based on somata position in the OB showed that TCs convey fast signals with short onset latency whereas MCs transmit late-onset signals. To our knowledge, here we present the first physiological data from MTC output streams isolated based on their axonal targets in the OC. Individual MTCs projecting to the AON showed a higher odor selectivity compared to MTCs innervating the aPC. Moreover, MTCs targeting the aPC displayed a broader range of onset latencies also containing slower responses. These data indicate that distinct regions in the OC may receive different odor information allowing for a parallel processing of olfactory information as already documented for other modalities.
Future dual-color imaging experiments from simultaneous traced MTC populations will directly compare the physiological properties of defined MTC output streams.

Expression of SNMP1 and candidate pheromone receptors in palps of the mouthparts from the desert locust *Schistocerca gregaria*

René-Sebastian Lemke[1], Pablo Pregitzer[2], Xingcong Jiang[2], Heinz Breer[2], Jürgen Krieger[1], Jörg Fleischer[1]

[1]Martin Luther University Halle-Wittenberg, Institute of Biology/Zoology, Department of Animal Physiology, Halle (Saale), Germany

[2]University of Hohenheim, Institute of Physiology, Stuttgart, Germany

In the gregarious phase, upon massive reproduction and aggregation, the desert locust (*Schistocerca gregaria*) can form giant swarms that tremendously endanger crop yields in the invaded areas. In locusts, reproduction and aggregation have been described to be affected by pheromones. In insects, pheromone detection is usually mediated by specialized olfactory sensory neurons (OSNs) on the antennae that are equipped with pheromone receptors (PRs). Pheromone-responsive OSNs are characterized by the expression of the “sensory neuron membrane protein 1” (SNMP1). Hence, SNMP1 is considered as a molecular marker for pheromone-reactive OSNs. Intriguingly, in *Schistocerca gregaria*, our PCR approaches showed that SNMP1 expression was not restricted to the antennae; in fact, expression of SNMP1 was also found for the labial and maxillary palps of the mouthparts. By in situ-hybridization experiments, we detected SNMP1 expression in a number of cells located in the tip regions of palps. These cells were adjacent to so-called “terminal sensilla” that are known to be partially olfactory. Subsequent double fluorescence in situ-hybridizations demonstrated that SNMP1 expression in both types of palps was confined to a subset of cells positive for the odorant receptor co-receptor (Orco). Since Orco is generally regarded as a marker for OSNs, these findings indicate that SNMP1 is expressed by olfactory neurons. To further investigate a possible relevance of SNMP1-positive olfactory neurons of palps in the detection of pheromones, we scrutinized the co-expression of recently identified candidate PRs from *Schistocerca gregaria* in these cells. PCR experiments indicated that most of the investigated candidate PRs were expressed in labial and maxillary palps. Performing two-color in situ-hybridizations, it was observed that similar to the expression of these candidate PRs in SNMP1-positive antennal OSNs, some of them were also expressed in subsets of SNMP1-positive olfactory neurons from both types of palps.

In summary, these observations suggest that certain olfactory neurons of palps could contribute to pheromone detection in *Schistocerca gregaria*. As gregarious locusts huddle and their palps are supposed to mediate contact chemosensation, such pheromones might be transferred during direct physical contact.

Probing honey bees' olfactory repertoire: a new approach toward OR deorphanization

Julia Mariette[1], Thierry Louis[1], Amélie Noël[1], Virginie Larcher[1], Thomas Chertemps[2], Nicolas Montagné[2], Emmanuelle Jacquin-Joly[3], Frédéric Marion-Poll[1], Jean-Christophe Sandoz[1]

[1]CNRS, Laboratory Evolution, Genomes, Behavior, Ecology, 1 rue de la Terrasse, 91198 Gif-sur-Yvette, France

[2]Sorbonne Universités—UPMC University Paris 06, Institute of Ecology & Environmental Sciences of Paris, Department of Sensory Ecology, Paris, France

[3]INRA, Institute of Ecology & Environmental Sciences of Paris, Department of Sensory Ecology, Versailles, France

Many aspects of insect behavior rely on olfaction allowing them to survive and reproduce. In the honey bee, decades of studies have shown the crucial role of olfaction for social interactions, foraging and reproduction. Although the neuroanatomy and the physiology of bees' olfactory system have been extensively studied, the molecular basis of honey bee olfaction is still poorly described. Indeed, the olfactory capacities (i.e. all the odorants that can be detected by a particular species) depend on the olfactory receptor (OR) repertoire expressed at the periphery within olfactory sensory neurons. The honeybee genome contains about 170 OR genes and to date, only 3 of them have been deorphanized (i.e. their ligands have been identified): AmOR11, AmOR151 and AmOR152. This knowledge gap is due in part to the difficult implementation of the techniques commonly used to deorphanize ORs, combining heterologous expression systems and electrophysiology or imaging recordings. In this project, we aim to develop an experimental platform for honey bee OR deorphanization. As a first step, we focus on ORs putatively involved in honeybees' mating behavior. Honey bee mating takes place at so-called congregation areas where thousands of males gather and mate with virgin queens. This behavior is driven by olfaction but apart from one odorant, 9-ODA, the principal component of the queen mandibular pheromone, little is known about the olfactory signals involved in this behavior. Interestingly, four ORs are strongly overexpressed in males' antennae compared to workers'. One of them, AmOR11, was deorphanized more than a decade ago and shown to detect 9-ODA, but nothing is known about the other three ORs: AmOR10, AmOR18 and AmOR170. The first aim of our project is thus to discover the ligands for the three orphan male-biased ORs. To do so, we couple the use of the Drosophila "empty neuron" system with calcium imaging to improve the speed of data acquisition. Our long-term aim is to build a high-throughput platform for the deorphanization of the entire honey bee OR repertoire and possibly those of other insect species.

Calcium in Kenyon Cell Somata as a Plausible Substrate for an Olfactory Sensory Memory in *Drosophila*

Alja Lüdke[1], Georg Raiser[1], Johannes Nehrkorn[2], Andreas V.M. Herz[2], C. Giovanni Galizia[1], Paul Szyszka[1]

[1]Department of Biology, Neurobiology, University of Konstanz, Konstanz, Germany
[2]Bernstein Center for Computational Neuroscience and Fakultät für Biologie, Ludwig-Maximilians-Universität München, Martinsried, Germany

Animals are able to link positive or negative experiences with stimuli that they encountered in the past. This time-bridging associative ability requires a sensory stimulus memory, i.e., information about the stimulus that persists after stimulus offset. The presence of sensory stimulus memories has been demonstrated in trace conditioning experiments, in which animals learn to associate a cue with a temporally separated reinforcing stimulus: *Drosophila* and other insects are able to solve an olfactory trace conditioning task, revealing a sensory odor memory in insect olfactory systems which can last for several seconds. However, the neural substrate of this sensory odor memory is still unknown. Searching for this substrate, we investigated odor-evoked activity after odor offset at the different stages of the olfactory pathway in *Drosophila* (Lüdke *et al*., 2018). Using *in vivo* Ca^{2+} imaging we measured response patterns in consecutive processing stages: in olfactory receptor neurons (ORNs, using the Orco driver line) and projection neurons (PNs, GH146 line) in the glomeruli of the antennal lobe, in PN somata, and in Kenyon cell (KC, OK107 line) dendrites and somata (in mushroom body calyces). We show that the post-odor responses in ORN axons, PN dendrites, PN somata, and KC dendrites are odor-specific, but different to the response patterns evoked during the stimulus, and not predictive of the chemical identity of the previous olfactory stimulus. In contrast to that, the odor-specific response patterns in KC somata persist after stimulus offset and the post-odor responses carry information about the identity of the previous olfactory stimulus. These findings show that the Ca^{2+} dynamics in KC somata could encode a sensory memory of odorant identity and thus might serve as a basis for associations between temporally separated stimuli.

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Beetles possess three primary olfactory processing centers

Laura Mähn[1], Florian Matyschik[1], Björn Trebels[1], Martin Kollmann[1], Stefan Dippel[1], Joachim Schachtner[1]

[1]Philipps-University Marburg - Biology, Department of Neurobiology, Karl-von-Frisch-Straße 8, 35043 Marburg, Germany

Beetles represent the largest insect order, which includes several serious agricultural and forestry pests. Despite their ecological and economic importance, little is known about their olfactory system.
A recent study in the red flour beetle *Tribolium castaneum* (Dippel et al., 2016) suggested three primary olfactory processing neuropils. Typical for insects, antennal olfactory information is processed within the paired antennal lobes, but in *T. castaneum*, olfactory input from the palps is processed independently in an unpaired glomerular neuropil in the gnathal ganglion – the gnathal olfactory center (GOC) - and the paired glomerular lobes (LGs) in the tritocerebrum.
We started to test whether these neuropils are also present in other beetles, by using immunohistochemistry in combination with palpal and antennal backfills, confocal laser scanning microscopy, and 3D reconstruction in different beetle species including the two main suborders Adephaga and Polyphaga.
We identified three central olfactory neuropils not only in closely related species to *T. castaneum* (*Zophobas morio* and *Tenebrio molitor*), but also in members of other polyphageous families, such as the dung beetle *Geotrupes stercorarius* and the asian ladybird *Harmonia axyridis*, as well as in the ground beetle *Abax parallelepipedus* and the diving beetle *Ascilius sulcatus* that both belong to the suborder Adephaga.
Our results indicate a tripartite olfactory processing system as a common feature of the beetles.

Physiological analysis of oscillatory microcircuits in the mouse accessory olfactory bulb

Sebastian Tobias Malinowski[1], Julia Mohrhardt[1], Chryssanthi Tsitoura[1,2], Yoram Ben-Shaul[3], Marc Spehr[1]

[1]Department of Chemosensation, Institute of Biology II, RWTH Aachen University, Germany
[2]Sainsbury Wellcome Centre for Neural Circuits and Behaviour, UCL, Great Britain
[3]School of Medicine, Department of Medical Neurobiology, The Hebrew University of Jerusalem, Israel

For mammals, and rodents in particular, chemosensation is essential for obtaining information about the environment. The accessory olfactory bulb (AOB) represents the first stage of information processing in the accessory olfactory system. Mitral cells are the AOB´s sole projection neurons. They receive input from peripheral vomeronasal sensory neurons and provide the only output pathway to higher brain areas. AOB mitral cells (AMCs) directly innervate regions in the medial amygdala and hypothalamus that control neuroendocrine and behavioral processes. Together with local inhibitory interneurons (granule cells), AMCs are organized in a complex network. Despite the physiological importance of AOB signaling for sensory information processing, little is known about coding strategies and signal integration within the network.
Surprisingly, we recently discovered that AMCs exhibit spontaneous infra-slow oscillatory activity at rest. It remains elusive, however, whether and how this additional temporal dimension of coding capacity contributes to chemosensory processing.
For systematic investigation of AMC signaling, we performed network-scale calcium imaging using confocal laser scanning microscopy. Here, we used Cre-loxP mouse genetics to selectively express the genetically encoded Ca2+ indicator GCaMP6f in AMCs. We provide a detailed characterization of AMC oscillation activity, both under control conditions and during selective pharmacological isolation from synaptic input. Furthermore, we describe AOB network properties based on extraction of temporal features and AMC ensemble correlation analysis. We also analyze the spatial organization of neuronal ensembles that display correlated activity. Together, our data indicate that AMCs assemble into distinct and partly overlapping microcircuits that share slow oscillatory activity patterns at rest.

AON top-down projections modulate olfactory bulb output activity in the mouse

Renata Medinaceli Quintela[1], Lutz Wallhorn[1], Jennifer Bauer[1], Markus Rothermel[1]

Department of Chemosensation, AG Neuromodulation, Institute for Biology II, RWTH Aachen University, 52056 Aachen, Germany[1]

The olfactory bulb (OB) is the target of massive cortical top-down projections from the anterior olfactory nucleus (AON) whose role in modulating early olfactory processing remains elusive. Here, we examined the effect of AON modulation on both a cellular and behavioral level in anesthetized and awake mice.
First, we investigated how top-down projections from the AON modulate OB output neuron activity using electrophysiological recordings in combination with optogenetic manipulations in anesthetized mice. Optogenetic activation of AON axon terminals in the OB led to a significant decrease in mitral/tufted (MT) cell spiking in the absence of inhalation-driven sensory input. The population time course showed a fast reduction of MT cell activity during light stimulation that was followed by a long lasting increase, reminiscent of OB offset responses. Since sensory input was shown to elicit AON feedback activity to the OB, we also tested for AON modulation effects during odor presentation. AON stimulation effects were independent of the strength and polarity of the odorant response and were observed across a variety of odors. The time course of the optical stimulation mediated reduction in MT cell spiking was similar to that observed in the absence of sensory input, arguing that our light stimulation protocol alone already strongly activated AON OB axons. Averaged normalized sniff-triggered spike histograms showed a decrease in both baseline and peak spike rate, consistent with an AON mediated effect on odor sensitivity rather than an influence on signal-to-noise ratio.
Next, we tested for behavioral consequences of extrinsically modulating AON activity. Mice implanted with an optical fiber targeting ChR2-expressing AON neurons were trained to report the presence of odorants. Unlike previous studies using optogenetic stimulation of piriform cortex neurons, AON stimulation did not seem to elicit an odor percept, since mice failed to lick to AON stimulation applied without sensory input. However, AON activation during odorant presentation reliably suppressed odor detection. This AON mediated effect was constant across odors and concentrations. Furthermore, testing different optical stimulation durations revealed that the AON modulates odor detection on a fast timescale.
Taken together, the strong inhibition of MTC activity observed during electrophysiological recordings might explain the suppression of odor detection observed in the behavioral experiments. These results support the hypothesis that the AON acts as a strong source of top-down input to the OB. Future experiments will focus on studying the effects of light-driven AON inhibition on OB output.

Functional Characterization of odor-driven modulation of olfactory perception by basal forebrain nuclei

Monika Müller[1], Inna Schwarz[2], Irina Pavlova[2], Manuel Mittag[1], Martin Schwarz[2], Martin Fuhrmann[1]

[1] Neuroimmunology & Imaging, DZNE e.V., Sigmund-Freud-Str. 27, 53127 Bonn, Germany

[2] Institute of Experimental Epileptology and Cognition Research, University of Bonn Medical Center, Sigmund-Freud Str. 25 53127 Bonn, Germany

The olfactory bulb (OB), the doorway to olfactory information into the mammalian brain, is innervated by centrifugal fibers originating in the horizontal diagonal band of Broca/magnocellular preoptic nucleus (HDB/MCPO), a basal forebrain area. Afferent projections from the HDB/MCPO connect to the granule cell layer as well as the glomerular layer, where they potentially influence olfactory processing within the olfactory bulb. However, little is known about the top-down modulation provided by the HDB/MCPO. Therefore, this project aims to further investigate the HDB/MCPO feedback circuit's modulatory influence on odor processing in the OB and olfactory-driven behavior.

Employing two-photon in vivo Ca^{2+}-imaging we monitored the activity of GCaMP6 expressing HDB axons in the OB of head-fixed mice during odor stimulation. We identified odor-responding axons originating in the HDB in the OB. Furthermore, we found different classes of odor-tuned axons responding to either one, both or none of the presented odors. Interestingly, axons with different response properties were spatially intermingled in the OB. Moreover, silencing the HDB by TeTx injection resulted in reduced glomerular odor-responses compared to sham-treated mice.

We conclude that the centrifugal projections from the HDB/MCPO innervating the OB are activated specifically by odors and modulate the activity of targeted cells within the olfactory bulb. Future investigation will focus on the role of different neuron populations in odor processing and the identity of the top-down modulatory network.

Volumetric calcium imaging of taste processing in the *Drosophila* brain

Daniel Münch[1], Carlos Ribeiro[1]

[1]Champalimaud Centre for the Unknown, Behavior and Metabolism Lab, Av. Brasília s/n (Doca de Pedrouços), 1400-038 Lisbon, Portugal

Maintaining nutrient homeostasis and adapting nutrient intake according to changing metabolic necessities is a challenge that all organisms are constantly confronted with. To face this challenge animals including *Drosophila melanogaster* are able to monitor their internal needs and integrate them with sensory input gathered from food sources in order to adapt feeding decisions and thereby control nutrient intake. While the taste sensory system of *Drosophila* is largely mapped and some parts of the internal state monitoring system are known, there is no good understanding of how taste information is processed in the brain to shape proper feeding decisions.

We have developed a method to perform high resolution volumetric imaging in the fly brain to observe taste processing across many neurons and across large brain areas. We use 2-photon *in-vivo* imaging in flies that express calcium indicators pan-neuronally. We perform manual stimulations with various tastes and from this data create maps of taste induced activity. We recorded first data sets and are currently investigating approaches for data processing and signal extraction. Evaluation of the activity maps will give us access to areas involved in higher order taste processing and allow us to directly target the involved circuitry.

Pheromone transduction and the role of the olfactory receptor co-receptor Orco in the hawkmoth *Manduca sexta*

Monika Stengl[1], Petra Gawalek[1], Katrin Schröder[1], Pavel Tyutyaev[1]

[1]University of Kassel, Biology, Animal Physiology, Heinrich Plett Str. 40, 34132 Kassel, Germany

Insect odor transduction employs 7-transmembrane olfactory receptors (ORs) that are inversely inserted into the ciliary membranes of the antennal olfactory receptor neurons. They hetero-multimerize with the evolutionary conserved 7-transmembrane molecule, called Orco (olfactory receptor-coreceptor). Orco appears to play several roles in odor transduction of the fruitfly *Drosophila melanogaster*. As chaperon it locates and maintains Orco in the membranes of the dendritic cilia. Furthermore, in heterologous expression systems it forms odor-gated OR-Orco receptor-ion channel complexes underlying ionotropic odor transduction. Finally, also as homo-multimer it forms a spontaneously opening ion channel that was suggested to control the spontaneous activity of the olfactory receptor neurons (ORNs). While it is clear that Orco does not couple to G-proteins, in *Drosophila* it is still under debate whether ORs, next to supporting fast, insensitive ionotropic odor transduction, also couple to G-proteins, allowing for sensitive metabotropic odor transduction. In the extremely sensitive pheromone-detecting ORNs of the hawkmoth *Manduca sexta* the detection of single pheromone molecules was demonstrated. Thus, it is likely that hawkmoths employ more sensitive pheromone transduction cascades that may differ from the ionotropic transduction of more abundant general odorants. Indeed, in hawkmoths we found no evidence for a role of Orco in ionotropic pheromone transduction. In contrast, we found evidence for the involvement of a G-protein-dependent phospholipase C, protein kinase C, and guanylyl cyclases in pheromone transduction. Next to the activation of at least three different pheromone-gated TRP-like ion channels we found a multitude of second messenger-gated ion channels in hawkmoth ORNs indicating that there are several parallel pheromone-transduction cascades that allow for the astounding sensitivity and the wide response range of pheromone-dependent hawkmoth ORNs. In addition, Orco appears to play an OR-independent role during development of the antennal ORNs that we started to examine with pharmacology. In tip-recordings of pheromone-sensitive sensilla Orco controls the spontaneous activity of ORNs by setting their membrane potential, apparently via voltage-dependent gating. So far, we found no effect of cyclic nucleotides on the open probability of Orco in the non-pheromone-stimulated ORNs. Currently, we compare the second-messenger and phosphorylation-dependence of Orco in the non-pheromone stimulated- and the pheromone-stimulated sensilla. [DFG grants STE 531/20-1,2 to MS]

Fuzzy topology for zebrafish V2R-like olfactory receptors: distinctly different, if broadly overlapping spatial expression zones

Adnan Shahzad Syed[1], Gaurav Ahuja[1,2], Vera Reichel[1], Daniel Kowatschew[1], Sigrun Korsching[1]

[1]University of Cologne, Institute for Genetics, Zuelpicher Strasse, 47a, 50674 Köln , Germany

[2]Current address: Center for Molecular Medicine Cologne (ZMMK) Robert-Koch-Str. 21, 50931 Cologne, Germany, and Max Planck Institute for Biology of Ageing Joseph-Stelzmann-Str. 9b, 50931 Cologne, Germany

The sense of smell is unrivaled in terms of molecular complexity of its input channels. Even zebrafish, a model vertebrate system in many research fields including olfaction, possesses several hundred different olfactory receptor genes, organized in four different gene families. For one of these families, the initially discovered odorant receptors proper, segregation of expression into distinct spatial subdomains within a common sensory surface has been observed both in teleost fish and in mammals. However, for the remaining three families, little to nothing was known about their spatial coding logic. Here we wished to investigate, whether the principle of spatial segregation observed for odorant receptors extends to another olfactory receptor family, the V2R-related OlfC genes. Furthermore we thought to examine, how expression of OlfC genes is integrated into expression zones of odorant receptor genes, which in fish share a single sensory surface with OlfC genes. We analyzed the spatial pattern of OlfC-expressing cells for seven representative receptors in three dimensions. We report non-random, if broad, distributions of labeled neurons for all OlfC genes analysed. Distributions for sparsely expressed OlfC genes are significantly different from each other in nearly all cases. For two of the three coordinates analyzed, OlfC expression zones are intercalated with those of odorant receptor zones, whereas in the third dimension some segregation is observed. Our results show that V2R-related OlfC genes follow the same spatial logic of expression as odorant receptors and their expression zones intermingle with those of odorant receptor genes. Thus, distinctly different expression zones for individual receptor genes constitute a general feature shared by teleost and tetrapod V2R/OlfC and odorant receptor families alike.

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Electrophysiological examination of neurons during taste reactivity test in the nucleus accumbens and medial orbitofrontal cortex of the rat

Istvan Szabo[1,2], Edina Hormay[1,2], Bettina Laszlo[1,2], Zoltan Karadi[1,2,3]

[1]Institute of Physiology, Medical School, University of Pecs, Pecs, Hungary
[2]Centre for Neuroscience, University of Pecs, Pecs, Hungary
[3]Molecular Neuroendocrinology and Neurophysiology Research Group, Szentagothai Research Center, University of Pecs, Pecs, Hungary

The nucleus accumbens (NAcc) and the medial orbitofrontal cortex (mOFC), parts of the forebrain limbic circuitry, play important role in the central regulation of feeding and metabolic control associated motivated behaviors. The orbitofrontal cortex has importance not only in the distinction of the different taste qualities but the neurons here also respond to the oral texture of fat. The present experiments were designed to provide complex characterization of the taste and texture detection of cells in the NAcc and mOFC in behaving animals.

In this study, extracellular single neuron activity is recorded in the NAcc and mOFC of male rats by means of tungsten wire microelectrodes while taste reactivity test is performed with the five primary taste qualities (NaCl, HCl, monosodium-L-glutamate [MSG], sucrose, QHCl) and with fat (milk, cream, vegetable oil) and nonfat but fat-like stimuli (paraffin oil, silicone oil). During the intraoral infusions, all ingestive and aversive mimics and postural-locomotor response patterns of the rats are recorded by video camera and later analyzed frame by frame.

In our previous examinations we proved the existence of taste-responsive neurons in both examined regions in anaesthetized rats. These data demonstrated that the NAcc and the mOFC are key structures in the integration of chemical and other signals arising from the endogenous and exogenous environments. In this poster we discuss the taste- and texture-responsiveness of neurons in these two regions and the associated taste- and texture-related behavioral patterns in freely moving animals. Our present data indicate that the neurons here utilize multiple functional attributes to play important roles in the adaptive organization of food and fluid intake behaviors.

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Linking crypt neurons to innate attractive behavior

Manish Tomar[1], Gaurav Ahuja[1,2], Maik Behrens[3], Riqui Liu[1], Wolfgang Meyerhof[4], Sigrun Korsching[1]

[1]Institute for Genetics, University of Cologne, Zuelpicher strase, 47a, 50647 Cologne, Germany

[2]Center for Molecular Medicine and Max Plank Institute for Aging Research, Robert Koch Strase, 50931 Cologne, Germany

[3]Leibniz-Institute for Food Systems Biology at the Technical University of Munich Lise-Meitner-Strasse 34, D-85354 Freising, Germany

[4] Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE) Arthur-Scheunert-Allee 114-116 14558 Nuthetal Germany

Crypt neurons constitute a small population of olfactory sensory neurons (OSNs) whose function is not well understood so far. We have shown previously that all crypt neurons express ORA4, one of seven genes in the V1R-related family of ora genes in zebrafish [1]. This constitutes an unusual mode of expression, more restricted than the one neuron/one receptor mode of expression typical for ciliated OSNs. Furthermore we showed that crypt neurons converge their axons in a single invariant glomerulus, mdg2 of the mediodorsal cluster of six glomeruli [2]. We have identified a ligand for ORA4 that elicits attraction in adult zebrafish. In an attempt to further characterize the function of ORA4 we have generated a knockout using CRISPR/Cas9 methodology. We report that crypt neurons as characterized by a specific marker survive the elimination of ORA4 and are still able to converge onto a glomerulus of the mediodorsal cluster. We are currently setting up an imaging system to study ligand-induced activity in projection neurons adjacent to mediodorsal glomeruli.

Reference
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Activity dependent adult neurogenesis in the mushroom bodies of the red flour beetle, *Tribolium castaneum*

Björn Trebels[1], Stefan Dippel[1], Magdalina Schaaf[1], Karthi Balakrishnan[2], Stefan Schütz[2], Ernst A Wimmer[3], Joachim Schachtner[1]

[1]Philipps-University Marburg, Department of Biology, Animal Physiology, Karl-von-Frisch-Str. 8, 35043 Marburg, Germany
[2]Department of Forest Zoology and Forest Conservation, Georg-August-University Goettingen, Buesgen-Institute, Buesgenweg 3, Goettingen 37077, Germany
[3]Department of Developmental Biology, Georg-August-University Göttingen, Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, GZMB, Ernst-Caspari-Haus, Justus-von-Liebig-Weg 11, Göttingen 37077, Germany

With its relative longevity and its susceptibility for genetic tools such as RNAi the red flour beetle *Tribolium castaneum* is ideally suited to study the development and plasticity of the nervous system. In the current study, we focus on cell proliferation in the adult mushroom bodies (MB). To visualize cell proliferation in adult *T. castaneum* MB we use the 5-ethynyl-2'-deoxyuridine (EdU) technique in combination with immunohistochemistry and the use of transgenic lines expressing neuron specific markers. We reliably labeled the progenies of the adult persisting MB neuroblasts in group-reared beetles, determined their identity and counted the newborn Kenyon cells within the first week after adult eclosion to determine the proliferation rate.
To address the question whether adult proliferation of Kenyon cells depends on olfactory input, we tested several environmental conditions. We isolated beetles as pupae and performed the same experiments as in group-reared beetles. Further, we enriched the environment of group-reared beetles during their adult life with the food related odor cis-3-hexenol or the beetles aggregation pheromone 4,8-dimethyldecanal (DMD). Stimulation experiments were repeated using beetles with a pupal RNAi mediated knock-down of the common odorant co-receptor (Orco).
Our data suggest at least two proliferation phases in the early adult. A first phase, direct after adult eclosion that lasts for about 4 days, is independent from olfactory stimulation. In contrast, a second phase that directly follows the first phase depends on olfactory stimulation.

The “hangry” fly larva: Internal state modulates network processing and switches olfactory preference in *Drosophila* larvae

Katrin Vogt[1], Matthew Berck[1,2], Luis Hernandez-Nunez[1], Guangwei Si[1], Aravinthan D.T. Samuel[1]

[1]Harvard University, Physics, Center for Brain Science, 52 Oxford Street, 02138 Cambridge, USA
[2]Janelia Research Campus, Ashburn, USA

Detecting and processing sensory cues allows us to select essential information from the environment and subsequently to enact purposeful behaviors. Appropriate decisions can however only be made when this sensory information is processed together with our internal state, such as hunger. Many studies have focused on sensory perception on the receptor level and how it influences motor output, while the impact of internal physiological state on sensory processing is less studied. Here we use the food-seeking behavior of fed and starved *Drosophila* larvae as a model to study internal state modulation of neuronal circuits and behavior. Olfactory information is essential to evaluate food quality before ingestion. This is especially important for the *Drosophila* larva, which mainly eats, grows, pupates, and then eventually becomes a fly. Using a normally aversive odor, we find that the odor response switches to attraction when larvae are starving, indicating that sensory processing is indeed modulated through internal state. We have recently created a detailed electron microscopic map of the entire neural circuit in the antennal lobe, the first odor processing center in the larval brain. However, the function of many of these anatomically characterized neurons remains unknown. Genetic silencing of some of these neurons reveals that they are only required to mediate the aversive odor response, but that they are dispensable under starvation. We also find other neuron types that have the opposite effect, they are not required for the odor aversion in fed animals, but their silencing abolishes odor attraction when larvae are starved. Two parallel projection neuron pathways mediate the odor information to different higher brain centers, the mushroom body and the lateral horn, which also seem to be differentially required for either the fed or starved innate response. Furthermore, we find that the recruitment of a prominent serotonergic neuron mediates the valence change in olfactory preference by modulating antennal lobe neurons via long distance diffusive transmitter release, a mechanism which could not be predicted by the connectome data. Thus, in summary, different subpopulations within neural circuits can be recruited under different conditions, allowing networks to shape behavioral responses in a dynamic manner. These findings underline that internal state, as a modulatory drive, needs to be taken into account as an important factor when exploring the full capacity of neural circuit computation.

The sense of smell on the transition from water to land: odor processing in the developing amphibian

Lukas Weiss[1], Paola Segoviano Arias[1], Sara Joy Hawkins[1], Katarina Dittrich[1], Thomas Offner[1,2], Thomas Hassenklöver[1], Ivan Manzini[1,2]

[1]Institute of Animal Physiology, Department of Animal Physiology and Biomedicine, Justus-Liebig-University Giessen, 35392 Giessen, Germany

[2]Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), University of Göttingen, 37073 Göttingen, Germany

The capacity to detect odorant molecules in- and outside of an aquatic environment makes amphibians a valuable model to study olfactory adaptations to different habitats. During the metamorphosis from an aquatic larva to a mostly terrestrial adult, the entire olfactory system is dramatically modified. In the secondary aquatic frog species *Xenopus laevis*, the larval olfactory system consists of a single nasal cavity detecting waterborne odorants, while the adult system is separated into a water- and an air-smelling system. It remains still elusive which adaptive strategies are applied by each of the two subsystems to detect odors in their respective medium. Additionally, many questions still remain as to how the drastic changes during metamorphosis are taking place without functionally disrupting the animal's sense of smell.

Here we describe the projections of the water- and air-smelling nasal epithelia to their respective target areas in the olfactory bulb using different neuronal tracing methods paired with functional Calcium imaging. The projection areas of the water-nose do not show any morphological or functional changes during metamorphosis, suggesting an undisrupted aquatic olfactory function from larval to adult state. In accordance with these results, behavioral responses to waterborne odorants also remain unchanged in the various developmental stages. In contrast, the air-bulb forms *de novo* during metamorphosis and shows projections that extensively cross between the two bulb-hemispheres, a feature that is absent in the water-system. This alternative wiring principle suggests an additional integrative function of the air-system in the adult animal with a putative behavioral relevance. In addition to neuronal adaptations, we found evidence that non-neuronal supporting cells in the two distinct sensory epithelia also display adaptive features that might be linked to the medium that they are exposed to.

The development of the olfactory system in amphibians grants intriguing insights into the evolutionary processes necessary for the adaptation to different environments and a successful transition from water to land.

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Poster Topic

T20: Somatosensation: Touch, Temperature, Proprioception, Nociception

T20-1A Abdominal sensing of substrate vibrations in locusts (*Schistocerca gregaria*)
Joscha Arne Alt, Reinhard Lakes-Harlan

T20-2A Neuronal mechanisms underlying sparse coding of passive touch – A combined *in vivo* and *in vitro* study
Claudia Barz, Pierre-Marie Garderes, Dan Ganea, Sven Reischauer, Dirk Feldmeyer, Florent Haiss

T20-3A Sensory profiles, pain characteristics and micro-RNAs as distinguishing factors in complex regional pain syndrome (CPRS) and fracture control patients
Christopher Dietz, Maike Müller, Lisa Karch, Ann-Kristin Reinhold, Bernhard Schwab, Claudia Sommer, Violeta Dimova, Frank Birklein, Heike L Rittner

T20-4A Subgroups of femoral chordotonal organ neurons differentially affect leg movements and coordination in *Drosophila melanogaster*
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T20-1B Characterization of campaniform sensilla on the legs of *Drosophila melanogaster*
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T20-2B Touch-induced affordances, spatial coordination of limbs and the definition of peripersonal space in insects
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T20-3B Correlative light and electron microscopy (CLEM) for investigating keratinocyte-nerve fiber contact zones in human skin
Christoph Erbacher, Philine Dinkel, Alexandra Gentschev, Sebastian Britz, Christian Stigloher, Nurcan Üçeyler

T20-1C Wisdom of the crowd vs. power of the few
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Sonja Meiser, Go Ashida, Birte Groos, Jutta Kretzberg

T20-1D Influence of cooling on C-fibres activation to rectangular and sinusoidal electrical stimulation
Julius Pakalniskis, Martin Schmelz, Richard W. Carr

T20-2D Central amygdala modulates nociception: optogenetic manipulation of network dynamics
Pinelopi Pliota, Isabel Wank, Sylvia Badurek, Klaus Kraitsy, Joanna Kaczanowska, Silke Kreitz, Andreas Hess, Wulf Haubensak

T20-3D The cortical signature of acute pain in rodent thalamus and somatosensory system
Jiaojiao Zhang, Simon Ponsel, Evgueni Ianovskii, Jurij Brankack, Andreas Draguhn

Abdominal sensing of substrate vibrations in locusts (*Schistocerca gregaria*)

Joscha Arne Alt[1], Reinhard Lakes-Harlan[1]

[1]Justus Liebig Universität Gießen, Institut für Tierphysiologie , Heinrich-Buff-Ring 38 , 35392 Gießen, Germany

Detection of substrate vibrations is an evolutionarily old sensory modality, with multiple functions, including predator detection. Insects use mainly scolopidial (chordotonal) sense organs for the perception of substrate vibrations. These organs can be found in different numbers and sizes in the body and appendages of probably all insects. Chordotonal organs occur in serial homology in each abdominal segment.

Here we investigate the electrophysiological response properties of these abdominal chordotonal organs of the locust *Schistocerca gregaria* during vibrational stimulation. Therefore a minishaker is attached to the sternit of the last abdominal segment and the responses to sinusoidal vibration (100 ms duration, 5 Hz repetition rate, carrier frequencies from 10 to 1000 Hz, in some cases up to 10kHz) of the nerves from the first to fifth abdominal segment are recorded.

The recordings show a sensitive reaction of all tergal and sternal nerves to vibrations. The lowest thresholds are from 10 to 800 Hz in the tergal nerves and 10 to 200 Hz in the sternal nerves. Also the tympanal organ (as one specialized organ in the series of homologous chordotonal organs) reacts strongly to body vibrations.

Behavioural observations show that the animals often touch the substrate surface with their abdomen, either during rest, but also during movement. Therefore the reported vibration sensitivity might add to the response of the presumably most sensitive vibration receptor, the tibial subgenual organs in the three leg pairs.

Neuronal mechanisms underlying sparse coding of passive touch – A combined *in vivo* and *in vitro* study

Claudia Barz[1,2,3,4,5,6], Pierre-Marie Garderes[3,4,5,7], Dan Ganea[3,4,5], Sven Reischauer[8], Dirk Feldmeyer[1,2,6], Florent Haiss[3,4,5,7]

[1] Institute of Neuroscience and Medicine, INM-10, Research Centre Jülich, Jülich, Germany
[2] Department of Psychiatry, Psychotherapy and Psychosomatics, Medical School, RWTH Aachen University, Aachen, Germany

[3] Department of Neuropathology, Medical School, RWTH Aachen University, Aachen, Germany
[4] Department of Ophthalmology, Medical School, RWTH Aachen University, Aachen, Germany
[5] IZKF, Medical School, RWTH Aachen University, Aachen, Germany
[6] Jülich-Aachen Research Alliance - Translational Brain Medicine, Jülich, Germany
[7] Department of Neuroscience, Pasteur Institute, Paris, France
[8] Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

Previous research has indicated that sensory information can be encoded by only a small number of active neurons (Olshausen & Field, 2004). This sparse coding strategy is a hallmark of layer 2/3 neurons in rodent somatosensory cortex, where a small fraction of excitatory neurons has been found to fire the majority of the spikes (O'Connor et al., 2010). The precise cellular and network mechanisms underlying sparseness are currently not well understood, largely because a direct link between the neurons activated *in vivo* and their cellular properties investigated *in vitro* has been missing. We used 2-photon calcium imaging to identify neurons in L2/3 somatosensory cortex that are highly activated by principal whisker vibrotactile stimulation. The individual high responders were then tagged using photo-convertible GFP for subsequent targeting in the brain slice using intracellular patch-clamp recordings and biocytin staining. This approach allowed us to investigate the physiological and morphological properties of high responders that distinguish them from less active control cells. *In vivo* imaging showed that high responders had strong, fast and reliable activation by whisker stimulation as well as increased levels of spontaneous activity. With and without stimulation, pairs of high responders were consistently more strongly coupled than other groups of active neurons. Furthermore, *in vitro* intracellular measurements of tagged neurons revealed reduced intrinsic excitability as well as larger and more frequent excitatory network inputs in high responders compared to control neurons. Both groups displayed similar passive cell properties and similar morphological characteristics pertaining to different excitatory subclasses. Our findings support the view that a subset of excitatory L2/3 neurons are preferentially targeted by excitatory network inputs, leading to increased activation both in the presence and absence of sensory stimulation and to a compensatory down-regulation in intrinsic excitability in this population. Thus, the choice of which neurons participate in stimulus encoding may largely depend on network connectivity rather than on stimulation or morphological and physiological properties of the neurons.

Sensory profiles, pain characteristics and micro-RNAs as distinguishing factors in complex regional pain syndrome (CPRS) and fracture control patients

Christopher Dietz[1], Maike Müller[1], Lisa Karch[1], Ann-Kristin Reinhold[1], Bernhard Schwab[1], Claudia Sommer[3], Violeta Dimova[2], Frank Birklein[2], Heike L Rittner[1]

[1]Department of Anesthesiology, University Hospitals Wuerzburg, 97080 Wuerzburg, Germany
[2]Department of Neurology, University Hospitals Mainz, Mainz, Germany
[3]Department of Neurology, University Hospitals Wuerzburg, 97080 Wuerzburg, Germany

Delayed diagnosis of CRPS is a clinical problem that puts patients at risk of secondary damage such as chronic disease or disability (Breivik et al., 2016; Lunden et al., 2016). It is therefore of great interest to evaluate existing diagnostic tools towards their ability to differentiate CRPS patients and fracture patients (FC). Clinically CRPS presents as chronic pain disease that is preceded by a trauma. Pain is often accompanied by inflammatory symptoms like edema, reddening or skin color changes. Based on this, we investigated two microRNAs (miRs) known to be involved in immune response and barrier function. The aim of the study was to find biomarkers in CRPS patients and fracture control patients (FC) to distinguish the two groups. All patients were recruited following the ncRNApain study protocol after ethical approval. Patients undertook a structured clinical examination where the CRPS severity score (CSS) was calculated, Quantitative Sensory Testing (QST) (Rolke et al, 2006) for the assessment of the sensory function and a blood withdrawal for investigating micro-RNAs. In order to analyze exosomal hsa-miR-144-5p and hsa-miR-223-5p expression, plasma exosomes were purified followed by RNA isolation and quantification via qPCR. Patients were also given self-administered questionnaires (patient reported outcomes). This included the Graded Chronic Pain Scale, the Neuropathic Pain Symptom Inventory (NPSI), the State Anxiety Inventory and the Beck Depression Inventory 2. In our final analysis, we included 148 patients with upper extremity CRPS and 35 FCs . Only complete data sets with no missing values were included. Table 1 shows demographics and patient characteristics. We did not find significant effects of age and sex on our data. All patient reported outcomes including the CSS were higher in CRPS patients indicating more pain and higher psychological burden. Especially the NPSI was significantly higher in CRPS patients indicating pain characteristics similar to neuropathic pain. In the QST analysis CRPS patients were significantly more sensitive to heat pain and mechanical pain than the FCs. Allodynia was also more likely within the CRPS cohort. CRPS patients were characterized by an increased sensitivity towards painful stimuli and a decreased sensitivity towards non-painful stimuli on the affected extremity. FC had mostly identical QST profiles on the affected and unaffected extremity. Only exosomal hsa-miR-223-5p was decreased in CRPS patients. Increased miR-223 is anti-inflammatory, barrierprotective and associated with lower risk of persistent pain after lumbar disc herniation (Moen et al, 2017).

In this large cohort, we confirmed disturbances in sensory function measured by QST of CRPS patients. The analysis of miRs illustrates that the two groups differ not only in clinical parameters but also on a molecular level and point towards an involvement of barriers and immune function in CRPS pathophysiology. In summary, assessing allodynia, the patient´s sensitivity towards heat pain/mechanical pain and the patient reported outcomes (e.g. NPSI) can be helpful in differentiating CRPS patients and fracture patients to avoid delayed diagnosis in CRPS patients.

Variables	CRPS (n=148)	Fracture Controls (n=35)	p value[1]
Age ± SD (range)	52.7 ± 12.3 (20;91)	47.7 ± 13.9 (20;78)	0.037
Sex (n (%) male/n (%) female)	31 (20.9)/117 (79.1)	17 (48.6)/18 (51.4)	0.001
CSS[2]	10.6 ± 2.8 (3;16)	1.86 ± 2.30 (0;12)	< 0.001
mean pain last week (NRS 0-10)	4.9 ± 2.2 (0;10)	1.6 ± 2.0 (0;6)	< 0.001
NPSI sum[3]	0.4 ± 0.2 (0;1)	0.1 ± 0.1 (0;0.43)	< 0.001

Table 1 Baseline demographic, CSS, pain and NPSI.

[1] independent t-test (Pearson Chi Square for categorical data), all p values two-tailed, $p < 0.05$ significant; [2] CSS, CRPS symptom severity score; range 0-17; [3] NPSI, Neuropathic pain symptom inventory; range 0-1

Subgroups of femoral chordotonal organ neurons differentially affect leg movements and coordination in *Drosophila melanogaster*

Alexander S. Chockley[1], Sara Ratican[2], Ansgar Büschges[1], Till Bockemühl[1]

[1]University of Cologne, Department of Animal Physiology, Zülpicher Str. 47b, 50674 Köln, Germany
[2]Geisel School of Medicine, Dartmouth University, 1 Rope Ferry Rd., Hanover, NH, USA

Animal behavior, specifically locomotor behavior, is continuously modulated by sensory systems. This modulation introduces a degree of adaptability to general motor output variability, unforeseen obstacles, and variations in the walking surface; this adaptability is needed to produce successful locomotor behavior. Among the sensory structures in the insect leg that contribute to this, the femoral chordotonal organ (fCO), an internal proprioceptor in the proximal femur, contains dozens to hundreds of neurons, depending on the species. Studies have shown that, in addition to detecting substrate vibrations, fCO primary sensory neurons provide information about the leg's state during walking by measuring the position, velocity, and acceleration of the tibia relative to the femur[1]. We characterized the morphology and function of groups of fCO neurons in Drosophila melanogaster to investigate how leg position information is encoded and how this information is used to influence locomotor behavior. As functional groups of neurons have been found in the fCO of other insects[2] that respond to similar kinematic parameters of tibial movement, we first investigated whether fCO neurons can be genetically grouped and if these groups have distinct effects on leg movement. Using the resources available at the Bloomington Drosophila Stock Center and the FlyLight database (Janelia Research Campus), we have tested Gal4 lines that label subgroups of fCO neurons and have characterized their morphology within the legs as well as their projection patterns in the ventral nerve cord (VNC). The distributions of these neurons within the fCO as well as their central projection patterns vary between Gal4 lines, but seem to reflect the categorization into club, claw, and hook groups[3]. Activation of these subsets using Chrimson[4] produced leg movements in some lines (tibial extension or flexion), with some lines showing no obvious effects. Inhibiting these fCO neurons optogenetically (GtACR1[5]) in a free-walking paradigm showed mostly mild effects on walking, with clear qualitative differences between the various Gal4 lines, suggesting that these subsets of neurons encode different movement parameters of the femorotibial joint. For example, one line that showed tibial extension during activation demonstrated deficits in tibial flexion during inhibition. This was seen as increased stance duration in the hind legs and increased swing duration in the front legs. Finally, we present data from calcium imaging using a genetically encoded calcium indicator expressed in fCO neuron subsets with precise movements of the tibia, demonstrating different response properties of these groups of neurons. Our findings add to the understanding of the functional structure of the dipteran fCO and the role of proprioceptive feedback encoding various movement parameters in leg movements and coordination during walking.

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Characterization of campaniform sensilla on the legs of *Drosophila melanogaster*

Gesa F. Dinges[1], Alexander Blanke[1], Sasha N. Zill[2], Ansgar Büschges[1], Till Bockemühl[1]

[1]University of Cologne, Zoological Institute, Zülpicher Straße 47b, 50674 Köln , Germany

[2]Marschall University, Biomedical Institute, 1600 Medical Center Drive,Huntington, WV 25701 , USA

Within the wide spectrum of locomotive means in animals, walking is one of the most common forms of terrestrial propulsion. To produce efficient walking, a tight network of interacting neurons, sensory organs, muscles, and other tissues of the body needs to exist. Sensory organs are able to provide feedback to this network of what the body is doing (motor output and proprioception) as well as what the environment is doing (sensory perception). In insects, one of these sensory systems is that of campaniform sensilla (CS), which detect deformations of the exoskeleton. This detection is possible through the external cuticular cap that suspends over a cuticular hole, under which a bipolar sensory neuron is located. During walking, active forces cause mechanical deformations of the elastic CS caps when movement is resisted by ground contact or external perturbations (Zill et al., 2012a; Zill et al., 2013). CS activity can represent both the rate and magnitude of forces applied to the limb (Ridgel et al., 2000). Changes in load can enhance the magnitude (Pearson, 1972) and alter the timing (Graham, 1985; Tang et al., 1986) of muscle contractions.

CS in insects, such as the stick insect, have been characterized functionally and morphologically through electrophysiological and anatomical approaches, which has shown their directional sensitivity and modulatory influence on motor output and locomotor networks. However, analysis of the functional input of CS on the locomotor system has only be performed in reduced preparations; advantages, therefore, may lie in *Drosophila melanogaster* and its extensive genetic toolkit; this might allow for noninvasive manipulation of CS during otherwise normal walking behavior. In *D. melanogaster*, however, the exact morphological distribution of CS on the legs and their morphology remain unclear and the lack of genetic driver lines labeling CS has prevented experimentation. To address this issue, we used several methods, including micro-computer tomography (µCT), scanning electron microscopy (SEM), and genetics tools, to investigate and anatomically describe CS in *D. melanogaster*. Based on µCT scans we reconstructed a 3D model of one of the legs, that can be used as a detailed anatomical reference frame. In addition, we explored the setup, location, and shape of CS on all three of *D. melanogaster*'s legs using SEM. During an extensive search we were also able to identify several GAL4 driver lines that specifically label different CS on the legs. Finally, we characterized the projection patterns of these driver lines in the ventral nerve system of the fly.

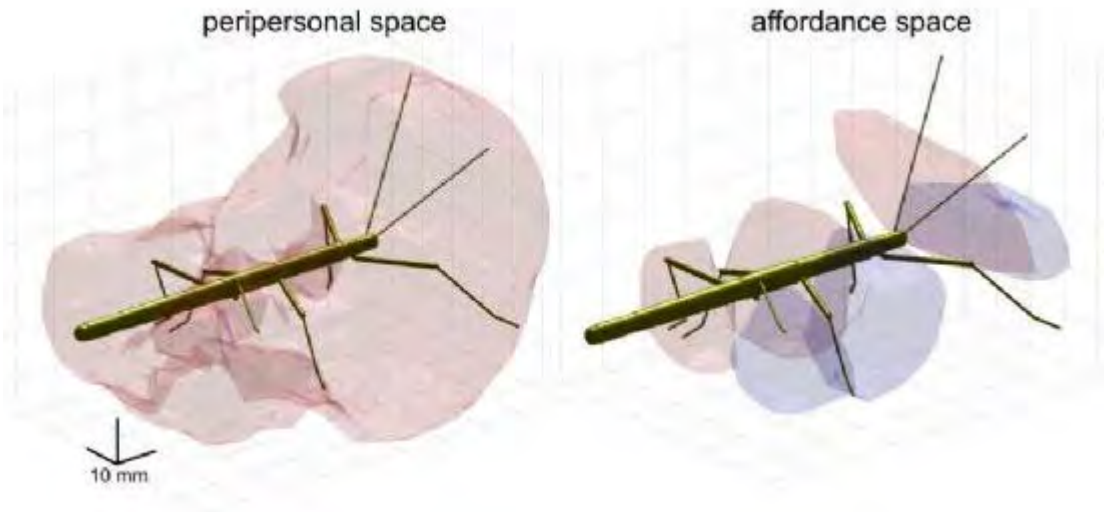
Knowledge about the outer structure and localization of CS on *D. melanogaster*'s legs will help to inform about the function of the individual sensory structures during behavior, more specifically walking. Knowing which CS shapes are present at specific joints or areas of torsion will allow more in-depth study of the cuticle's influence on sensory modulation. This knowledge is fundamental for more precise studies of both sensory modulation as well as functional analysis through the experimental manipulation of individual CS in largely intact animals and during natural behavior. Once the morphological structure is fully understood, activation or inhibition studies using, for example, Channelrhodopsins (Zhang et al., 2007) will be able to show the influence of sensory information provided by CS on the locomotor networks.

Touch-induced affordances, spatial coordination of limbs and the definition of peripersonal space in insects

Volker Dürr[1], Malte Schilling[2]

[1]Bielefeld University, Biological Cybernetics, Universitätsstr. 25, D-33615 Bielefeld, Germany
[2]Bielefeld University, Cognitive Interaction Technology - Center of Excellence , Inspiration 1, D-33615 Bielefeld, Germany

Internal representation of far-range space in insects is well established, as it is necessary for navigation behaviour. Although it is likely that insects also have an internal representation of near-range space, the behavioural evidence for the latter is much less evident. Here, we estimate the size and shape of the spatial equivalent of a near-range representation that is constituted by somatosensory sampling events. To do so, we use a large set of experimental whole-body motion capture data on unrestrained walking, climbing and searching behaviour in stick insects of the species *Carausius morosus* to delineate 'action volumes' and 'contact volumes' for both antennae and all six legs. As these volumes are derived from recorded sampling events, they comprise a volume equivalent to a representation of coinciding somatosensory and motor activity. Accordingly, we define this volume as the *peripersonal space* of an insect. It is of immediate behavioural relevance, because it comprises all potential external object locations within the action range of the body.
In a next step, we introduce the notion of an *affordance space* as that part of peripersonal space in which contact-induced spatial estimates lie within the action ranges of more than one limb. Because the action volumes of limbs overlap in this affordance space, spatial information from one limb can be used to control the movement of another limb. Thus, it gives rise to an affordance as known for contact-induced reaching movements and spatial coordination of footfall patterns in stick insects.
Finally, we probe the computational properties of the experimentally derived affordance space for pairs of neighbouring legs. This is done by use of artificial neural networks (ANN) that map the posture of one leg into a target posture of another leg with identical foot position. We find that a simple feed-forward ANNs with a single small hidden layer can explain a very accurate limb posture mapping within each affordance volume. The number of hidden layer neurons necessary for high accuracy is in the range of known premotor interneurons in the thoracic ganglia of stick insects.



Correlative light and electron microscopy (CLEM) for investigating keratinocyte-nerve fiber contact zones in human skin

Christoph Erbacher[1], Philine Dinkel[1], Alexandra Gentschev[1], Sebastian Britz[2], Christian Stigloher[2], Nurcan Üçeyler[1]

[1]Department of Neurology, University of Würzburg, Josef-Schneider-Str. 11, 97080 Würzburg, Germany
[2]Biocenter, Imaging Core Facility, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

Background: Traditionally, peripheral sensory neurons were assumed the only detectors and transducers of external stimuli from the periphery toward the central nervous system. Afferent fibers originating from dorsal root ganglion (DRG) neurons terminate within the epidermis, which is the outer barrier of the body and is built up mainly by keratinocytes. These intraepidermal nerve fibers (IENF) conduct thermal stimuli, pain, itch, and pleasant touch, and also have autonomic functions. Several studies showed nocifensive behavior and action potential generation in DRG neurons of experimental animals without direct activation of IENF, but via stimulation of keratinocytes. Also, innocuous and noxious touch is partially mediated by these cells, which express a range of sensory receptors and produce inflammatory mediators and even neurotransmitters like ATP and acetylcholine. Therefore, the question arises if and how sensory stimuli are perceived by and transduced to IENF via keratinocytes. Here, we show a correlative light and electron microscope (CLEM) approach to investigate proteins potentially involved in cell-nerve communication between keratinocytes and IENF. CLEM allows integration of data on specific protein localization and the cellular context of the tissue.
Methods: 2-mm skin punch biopsies were taken from the upper back of healthy controls. Tissue was immediately high-pressure frozen and embedded in London Resin (LR-white) after freeze substitution. Ultrathin sections of 100 nm were cut in consecutive series. Target proteins were labelled via indirect immunofluorescence (IF) and imaging was performed via the Structured Illumination Microscope Elyra System (Zeiss). Antibodies against protein product 9.5 (PGP9.5), vesicular acetylcholine transporter (VAChT), synaptosomal-associated protein 23 (SNAP23), and syntaxin 1 were used. Cell core signal was obtained by 4′,6-diamidino-2-phenylindole (DAPI) staining. For electron microscopy (EM), uranyl acetate and lead citrate were used as contrasting agents, followed by carbon coating. The scanning electron microscope JSM-7500F (JEOL) with a LABE detector was used to generate electron microscopic images. To align different magnifications of the same tissue area, multi-layer mosaic alignment from the Fiji plugin trakem2, with similarity as expected transformation, was applied. Alignment of images from consecutive slices required the program etomo. EM and IF images were correlated via the vector program inkscape, using cell core localization as an unbiased correlation signal. The overlay of channels was achieved with GIMP2 software.
Results: We successfully embedded human epidermal tissue after high-pressure freezing and freeze substitution. At IF level, all tested antibodies revealed detectable signals. Specific antibody binding was verified by observation of the signal pattern of the same region on consecutive slices and by primary antibody omission. EM imaging showed sufficient tissue preservation and enabled correlation via DAPI signal. We present ultrastructural correlations of the vesicular transport proteins VAChT, SNAP23, and syntaxin1 within keratinocytes and tracking of IENF within the human epidermis.
Conclusion: The CLEM approach represents an excellent method for the investigation of close contact areas between keratinocytes and IENF in human skin. We visualized and located vesicle associated proteins, potentially involved in signaling between keratinocytes and IENF.

Wisdom of the crowd vs. power of the few An electrophysiological assessment of the impact of individual neurons on local networks

Beate Knauer[1], Maik C. Stüttgen[1,2]

[1]University Medical Centre of the Johannes Gutenberg University Mainz, Pathophysiology, Duesbergweg 6, 55128 Mainz, Germany

[2]Focus Program Translational Neurosciences Mainz, Langenbeckstr. 1, 55131 Mainz

Synchrony of large neuronal ensembles is thought to rule in cortical signal processing. Nonetheless, recent studies showed that manipulating the activity of even a single neuron can have marked impact on brain state, motor output and sensation. However, it is still unclear how an individual neuron can determine an output at the system level in the presence of numerous other cells generating a notoriously noisy background. We aim to elucidate how these system effects may be conveyed by the local network.

Using the barrel cortex of an anesthetized rat as model system, we applied juxtacellular current stimulation (200 ms, 1-20 nA) to individual neurons and simultaneously recorded the local network activity at a distance of a few hundred micrometers with 16 contact sites each on two shanks of a silicone probe which was inserted perpendicular to the cortical surface and spanned all cortical layers.

Juxtacellular stimulation could effectively drive single neurons for several hundreds of trials to a peak firing frequency of 49 Hz, averaged across the cells sampled thus far (10 ms bins, range: 3 to 180 Hz, median 50 Hz). The high number of trials was realized in expectance of small effect sizes. Preliminary data showed that 14% of the local network units significantly changed their average firing rate during the concomitant juxtacellular stimulation of an individual cell when compared to their average firing rate during an interval of 200 ms prior to stimulation. Thereby, significantly more local network units were affected by the changed activity of an individual neuron than expected ($\alpha = 0.05$; X^2 test, $p = 0.007$). Of the significantly affected units, 32% increased and 68% decreased their average firing rate during the stimulation period. Yet, the overall network activity (measured as firing rate change between a 200 ms pre-stimulus interval and the stimulus period) across all network units, was unperturbed (n.s.) by the juxtacellular stimulation because 1) significantly affected cells constituted only a small subset of the local network, 2) effects sizes were small, and 3) firing rate increases and decreases appeared to cancel each other out.

Cross-modal adaptation in a descending interneuron of the indian stick insect *Carausius morosus*

Gaetan Lepreux[1,2], Stephan Suichi Haupt[1], Volker Dürr[1,2]

[1]Bielefeld University, Department of Biological cybernetics, Universitätstrasse 25, 33165 Bielefeld, Germany
[2]Cognitive Interaction Technology — Center of Excellence, Bielefeld University, Inspiration 1, 33615 Bielefeld, Germany.

Sensory systems adjust their sensitivity through adaptation, i.e., a history-dependent shift of the stimulus-response characteristic of neurons. This mechanism is found in many, if not all, neural sensory systems as it can improve coding efficiency, discrimination of stimulus intensities. For example, in motion-encoding proprioceptors, adaptation to constant stimulation during continuous active movement could ensure high responsiveness to changes in movement speed. In the stick insect *Carausius morosus*, continuous active movement occurs during active tactile exploration movements of the antennae such that an obstacle on the path can be detected. Indeed, when an antenna touches an obstacle as the animal walks, it often reacts with a rapid reaching movement of the front leg. The information transfer from the head to the legs should then be affected by adaptation of neurons of the antennal-mechanosensory pathway. Here, we measure adaptation in an identified descending interneuron that conveys antennal proprioceptive information to the thoracic ganglia: The contralateral ON-type velocity-sensitive (cONv) interneuron. This interneuron is located in the gnathal ganglion (GnG) and encodes antennal-joint velocity as well as substrate vibration (Ache et al., 2015, J.Neurosci.). Preliminary results showed that cONv reduces its response to repeated antennal deflection suggesting adaptation. Since cONv receive multimodal inputs, we ask whether bimodal activation of cONv can lead to a change in response intensity of the neuron when compared to unimodal stimulation. Using extracellular whole-nerve neck-connective recordings with hook-electrodes, we examined the responses of the bilateral pair of cONv interneurons to antennal ramp-and-hold deflections at one of two ramp velocities. We found that cONv reached the same level of adaptation after 20 ramps irrespective of the velocity imposed, implying faster adaptation at higher velocity. Imposed movements of the opposite antenna did not affect the level of adaptation of cONv, while substrate vibration delivered at a rate comparable to footfall during walking set both cONv into the adapted state, suggesting cross-modal adaptation.

J. M. Ache, S. S. Haupt, and V. Dürr. A direct descending pathway informing locomotor networks about tactile sensor movement. J.Neurosci. 35 (9):4081-4091, 2015.

Repeated stimulation causes hyperpolarization and increased spike counts in leech touch cells

Sonja Meiser[1], Go Ashida[1,2], Birte Groos[1], Jutta Kretzberg[1,2]

[1]Computational Neuroscience, Department of Neuroscience, Faculty VI, Carl von Ossietzky University, 26129 Oldenburg, Germany

[2]Cluster of Excellence Hearing4all, Department of Neuroscience, Faculty VI, Carl von Ossietzky University, 26129 Oldenburg, Germany

High frequency spiking in leech touch mechanoreceptors (T cells) induces a long term afterhyperpolarization (AHP), arising from the activation of the Na+/K+-pump and Ca2+-dependent K+-current (Jansen and Nicholls, 1973). Many reports have described the involvement of Na+/K+-pump in activity-dependent synaptic plasticity in both vertebrates (Wyse et al., 2004) and invertebrates (Pinsker and Kandel, 1969; Scuri et al., 2007). We provide for the first-time evidence that the Na+/K+-pump is also involved in activity-dependent non-synaptic cellular plasticity in leech sensory neurons. Assuming that T cells have two distinct spike-initiation zones (Burgin and Szczupak, 2003; Kretzberg et al., 2007), a peripheral one to signal touch stimuli and a central one to process synaptic inputs within the ganglion, we study how repeated somatic and/or peripheral stimulation change the response properties of the stimulated T cell and its postsynaptic partner.

The main finding of this study is that T cells, stimulated by repeated somatic current pulse injection, hyperpolarize their resting membrane potentials, while at the same time increasing their spike count. By combining experimental electrophysiological approaches with biological neuron modelling we investigated the physiological mechanisms underlying this unexpected behavior. Our Hodgkin-Huxley type neuron model, adjusted to physiological T cell properties, suggests that action potential discharge leads to increased Na+/K+-pump activity, which hyperpolarizes the resting membrane potential. This might result in a decrease of a slow, non-inactivating current, which is presumably mediated by voltage-dependent, low-threshold potassium channels (Benda and Herz, 2003). This m-type current deactivates when the membrane potential hyperpolarizes, causes tonic firing and thereby affects the T cell spiking.

Furthermore, we investigated if and how peripheral T cell spike initiation could be influenced by repeated skin indentation. Contrary to the findings of repeated somatic current injection, the analysis revealed that spikes induced in the periphery led to a form of short-term depression within one trial of repeated tactile stimuli. Additionally, we examine if both initiation zones influence each other by stimulating the soma and the periphery alternately.

Finally, we evaluated whether the increased spike count after repeated somatic stimulation could affect the synaptic transmission to another T cell on the same side of a segmental ganglion. We analyzed the magnitude of postsynaptic responses after eliciting repeatedly either a specific number of single action potentials in the presynaptic T cell or after injecting current pulses to induce the increase in spike count presynaptically. Our results demonstrate that the postsynaptic responses increase with increasing presynaptic spike counts.

This study provides evidence that repeated mechanoreceptor stimulation causes two different forms of non-synaptic activity-dependent neuronal plasticity and supports the hypothesis of independent processing of sensory and synaptic inputs in one cell. Especially in invertebrates like leeches, which crucially depend low energy consumption for survival, the number of neurons is limited to a minimum, but neurons often perform several computational tasks simultaneously. Multiple spike initiation sites, which can change their properties independently from each other, could further improve this multi-tasking ability.

Influence of cooling on C-fibres activation to rectangular and sinusoidal electrical stimulation

Julius Pakalniskis[1], Martin Schmelz[1], Richard W. Carr[1]

[1]Department of Experimental Pain Research, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1–3, 68167 Mannheim, Germany

Patients with neuropathic pain have spontaneous and unpredictable attacks, but often colder temperatures can induce pain (“cold hyperalgesia”). We investigated if activation thresholds for unmyelinated C-fibres are reduced by cooling using i) slowly depolarizing sine waves and ii) conventional rectangular electrical stimuli.

We used compound action potential recordings from isolated mouse n. suralis. Nerves were stimulated with up to 50 µA, by either 4 Hz sine waves or 1 ms rectangular pulses at three different temperatures: 20^{o}C, 26^{o}C, 32^{o}C. Activation thresholds were analyzed by a sigmoidal fit of the stimulus-response function for each temperature. Cooling had opposite effects on electrical threshold for sine vs. rectangular stimulus: while thresholds increased for rectangular stimulation at lower temperatures (one-way ANOVA $p<0.01$, post hoc Bonferroni $p<0.01$ 20-26^{o}C and 20-32^{o}C, $p=0.65$ 26-32^{o}C) they decreased for sine wave stimulation (one-way ANOVA $p<0.01$, post hoc Bonferroni $p<0.01$ 20-26^{o}C, 26-32^{o}C and 20-32^{o}C). As activation of the cold sensitive receptor Trpm8 might be interfere with electrical thresholds we tested Trpm8 agonists (10 µM icilin or 1 mM menthol) for activation threshold changes. However, no differences between treated and control were observed for sine wave (two-way ANOVA for menthol $p=0.55$, for icilin $p=0.11$) or rectangular stimulation (two-way ANOVA for menthol $p=0.24$, for icilin $p=0.35$). Thus, activation of cold receptors cannot explain the observed threshold changes. We therefore focused on passive membrane characteristics and analyzed how much charge needs to be delivered to activate the neurons calculated as time * current for sine waves of different intensities. Lower intensity sine waves required a longer time to excite the axon such that the charge needed to activate axons did not significantly change for different sine wave amplitudes. However, we found temperature to modulate the charge required for activation: cooling increased the charge thresholds for both, sine wave (one-way ANOVA $p=0.02$, post hoc Bonferroni $p=0.36$ 20-26^{o}C, $p=0.02$ 20-32^{o}C, $p=0.33$ 26-32^{o}C) and rectangular stimulation (one-way ANOVA $p<0.01$, post hoc Bonferroni $p<0.01$ 20-26^{o}C and 20-32^{o}C, $p=0.65$ 26-32^{o}C). No significant correlation was found between sine wave amplitude and amplitude compound action potential for the sine wave stimulation. In contrast, the expected sigmoidal stimulus/response function confirmed for rectangular stimuli.

In conclusion, cooling unexpectedly decreased activation thresholds of C-fibers for slow depolarizing stimuli while applied charge increased. Thus, under cold conditions stimuli of shallower slope can induce action potentials which might be related to increased membrane resistance following closure two-pore domain potassium channels. Such mechanisms could also contribute to cold-induced pain in neuropathic patients.

Central amygdala modulates nociception: optogenetic manipulation of network dynamics

Pinelopi Pliota[1], Isabel Wank[2], Sylvia Badurek[3], Klaus Kraitsy[3], Joanna Kaczanowska[1], Silke Kreitz[2], Andreas Hess[2], Wulf Haubensak[1]

[1]Research Institute of Molecular Pathology (IMP), Vienna Biocenter , Campus Vienna Biocenter 1, 1030 Vienna, Austria
[2]Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander University Erlangen-Nuremberg, Fahrstrasse 17, 91054 Erlangen, Germany
[3]Preclinical Phenotyping, Vienna Biocenter (VBC), Dr. Bohr Gasse 3, 1030, Vienna, Austria

Recent advances in circuit genetics have resolved local circuit mechanisms of key brain functions. However, how the brain translates stimuli into meaningful information at the level of neuronal circuits and how these local circuits interact with brain wide networks to shape certain brain functions remains a challenge. Here we tackle the assembly of such brain wide networks in the context of nociception by combining deep sequencing, optogenetic BOLD fMRI (ofMRI) and nociception related behavioral tasks. We specifically investigate the role of central amygdala (CE) network interactions in nociceptive behavioral decisions. Based on the genetic differences of the two major CE antagonistic populations, PKCδ; and SST, we build a hypothesis about their antagonistic relations in nociception, and fusing optogenetics with BOLD fMRI and functional network analysis, we then explore their role as part of the global nociception-related brain network. Using pharmacogenetics to specifically control PKCδ; and SST, we reveal their active involvement and opposing functions in nociception-related paradigms. We find that (i) distinct brain wide functional subnets map to specific nociception-related behavioral features and (ii) that local CE circuitry drives antagonistic behavioral nociceptive responses.

The cortical signature of acute pain in rodent thalamus and somatosensory system

Jiaojiao Zhang[1], Simon Ponsel[1], Evgueni Ianovskii[1], Jurij Brankack[1], Andreas Draguhn[1]

[1]Heidelberg University, Institute of Physiology and Pathophysiology, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany

For a long time, the standard method to measure pain is the oral report by the subject, which is subjective and not accurate enough for diagnosis. In recent years, it was reported that it's possible to use fMRI to assess pain elicited by noxious heat in healthy persons. (Wager T et al., N Engl J Med, 2013). Rat models of acute and neuropathic pain manifest increased power in primary somatosensory cortex (S1) recorded by both electrocorticography (ECoG) and EEG. (LeBlanc B et al., pain, 2016).

Hereby, we investigated the signature of acute pain induced by the capsaicin injection in the hind paw of mice. Local field potentials (LFPs) were recorded in the thalamus and somatosensory system including primary somatosensory cortex (S1), anterior cingulate cortex (ACC), insular cortex (Ins), ventroposterior lateral thalamus (VPL), parietal cortex (PaC) and olfactory bulb (OB). We found a significant increased power spectrum density of 30-80 Hz in Ins and OB after capsaicin injections compared to saline injections. And for all recorded regions, the increase of power lasted for even one hour after the injections, although the pain caused by capsaicin only lasted for about ten minutes. Furthermore, there were increased cross-frequency coupling (CFC) between gamma2 (80-120 Hz) and low frequencies (1-15 Hz) under capsaicin induced pain, which indicates an increased information process of brain networks.

Our study reveals an increased cross-frequency coupling as well as a long-time effect of power spectrum increase under acute pain, suggesting the involvement neural network interactions and more than "acute" effect of acute pain. These may shed light on the mechanisms of acute pain, contributing to the objective diagnose of pain.

Poster Topic

T21: Motor Systems

reference frame during BCI learning
Enrico Ferrea, Pierre Morel, Alexander Gail

T21-2B Object location and size influence parietal and premotor reference frames during object-oriented reach planning
Ole Fortmann, Bahareh Taghizadeh, Alexander Gail

T21-3B Processing of Load Signals in the Leg Muscle Control System of Insects
Corinna Gebehart, Joachim Schmidt, Ansgar Büschges

T21-4B Slope-dependent modulation of muscle co-contraction in freely walking stick insects (*Carausius morosus*)
Yannick Günzel, Chris J. Dallmann, Josef Schmitz, Volker Dürr

T21-5B Optogenetic inhibition of premotor cortex projections to parietal reach region modifies rule-based sensorimotor transformation in non-human primates
Hao Guo, Michal Fortuna, Janina Hueer, Jens Gruber, Stefan Treue, Hansjoerg Scherberger, Alexander Gail

T21-6B Parallel cortico-cerebellar pathways through a pretectal cerebellar relay nucleus in birds.
Cristian Gutierrez-Ibanez, Douglas R Wylie

T21-7B The role of chordotonal organs for local pattern-generating activity of a leg stump during walking in *Drosophila*
Moritz Haustein, Ansgar Büschges, Till Bockemühl

T21-8B Survival of the anucleated giant Mauthner axon is required for high-performance escape responses
Alexander Hecker, Wolfram Schulze, Jakob Oster, Stefan Schuster

T21-9B Comprehensive disynaptic mossy fibre pathways from sensory and motor-related regions of the cerebral cortex to the cerebellum
Julia Henschke, Janelle M.P. Pakan

T21-1C Duets in the wild: Inter-individually coordinated motor control enables cooperative behavior
Susanne Hoffmann, Lisa Trost, Cornelia Voigt, Stefan Leitner, Alena Lemazina, Hannes Sagunsky, Markus Abels, Sandra Kollmansperger, Andries Ter Maat, Manfred Gahr

T21-2C Glycine transporter 2-deficient mice show an altered development of the ultrasonic vocalization-associated breathing
Swen Hülsmann, Yoshihiko Oke, Guillaume Mesuret, A. Tobias Latal, Michal G. Fortuna, Marcus Niebet, Johannes Hirrlinger, Julia Fischer, Kurt Hammerschmidt

T21-3C Construction principles of miniaturized neural circuits on the example of the flight motor network for asynchronous flight
Silvan Hürkey, Carsten Duch

T21-4C Anatomical and physiological specializations for high spike time precision in *Drosophila* flight steering motoneurons
Nina Kramer, Dario Music, Carsten Duch

T21-5C Platform-induced vertical vestibular ocular reflex in humans
Dieter F. Kutz, Florian P. Kolb, Stefan Glasauer, Hans Straka

T21-6C The neural basis of amplitude adjustments during vocal interactions
Alena Lemazina, Susanne Hoffmann, Lisa Trost, Manfred Gahr

T21-7C Is Common Inhibitor Action Mediated by a Dual Control via Fast Synaptic Transmission and Neuromodulation?
Sander Liessem, Reinhard Predel, Ansgar Büschges

T21-8C The temporal dynamics of action-effect prediction: An EEG study
Elisabeth Lindner, Álvaro Darriba, Qing Yang, Alexander Jones, Florian Waszak, Andrea Desantis

T21-9C The aimed limb movements of a hemimetabolous insect are compensated for allometric wing growth
Tom Matheson, Alexandra J. Patel

T21-10C Motor phenotype and Cognitive testing of PCLO Knockout (Pclogt/gt) rats measured in an operator-independent motor analysis system (OptiMan) connected with an Operant Touchscreen Chamber
Humaira Munawar, Marion Rivalan, Katharina Stumpenhorst, Christian Jung, Claus Reimertz, Craig Garner, York Winter

T21-2D Encoding of movement force in the fronto-parietal reach network in primates
Julia Wanda Nowak, Pierre Morel, Alexander Gail

T21-3D Inhibition of HSP90 increases individual variability of behaviour in the desert locust
Swidbert Roger Ott, Tom Matheson, Ben Cooper

T21-4D Motion Hacking: A Method for Interference with Neural Control of Walking, Based on External Muscle Stimulation in Stick Insects
Dai Owaki, Volker Dürr, Josef Schmitz

T21-5D Optical Inactivation of Leg Proprioceptors in the Stick Insect *Carausius morosus*
Burak Özbagci, Anna Haberkorn, Ansgar Büschges, Matthias Gruhn

T21-6D Female zebra finches use their song control system for call-based communication
Lisa Trost, Andries Ter Maat, Manfred Gahr

T21-7D Spatial averaging and inference of decision time in go-before-you-know tasks depends on motor control demands and movement time constraints
Philipp Ulbrich, Alexander Gail

T21-8D Leg coordination and gait choice in poly-pedal locomotion – numerical models and experiments
Tom Weihmann

T21-9D Motor skill learning and execution in a distributed brain network

Steffen Benjamin Eggert Wolff, Ashesh Dhawale, Raymond Ko, Bence Ölveczky

Studying Changes of Walking Direction and Kinematic Parameters in Response to Unilateral Tactile Stimuli in the Stick Insect *Carausius morosus*

Volker Berendes[1], Florian Paul Schmidt[1], Florian Hofmann[1], Volker Dürr[1]

[1]University Bielefeld, Biological Cybernetics, Universitätsstrasse 25, 33615 Bielefeld, Germany

Insects navigate through rough and uneven terrain constantly searching for new foothold and coordinating their six legs, accordingly. In addition to their sense of sight, many insects use their antennae as tactile probes to explore their environment during walking. It has been shown for example in cockroaches that they turn in response to unilateral antennal contact [3], [4]. The antennae of the Indian stick insect *Carausius morosus* have approximately the same length as their front legs, which allows them, for example, to find suitable foothold within reach of the front legs [1], [2]. In order to study the stick insects' responses to tactile stimuli we combine several previously existing techniques to a new setup that allows us to record the kinematics of front leg and antennal movements along with rotational and translational velocities of locomotion, while presenting tactile stimuli at arbitrary locations.
The setup consists of 4 Vicon MX 10 motion capture cameras surrounding an air-cushioned, spherical treadmill of 20 cm in diameter. The treadmill allows three degrees of freedom rotation and consist of a light-weight (6 g), hollow Styrofoam sphere. The animals are tethered above the center of the treadmill in a way that they still have to carry their own body weight. Yaw, pitch and roll movement of the spherical treadmill are recorded by 2 optical mouse sensors, using similar hardware as presented by Dahmen and collegues [5]. The hard- and software of the treadmill tracking system was modified to be synchronized to the Vicon system using a TTL frame trigger signal delivered by the Vicon hardware. A custom written Matlab function allows us to calculate the walking trajectory of the animals from the movement of the sphere.
Tactile cues are presented by positioning a rod with a two-axis linear robot. The robot is controlled by G Code commands sent to an Arduino board that runs Grbl v.1.1, an open source software usually used to control CNC mills.
Kinematics are calculated from marker-based motion capture of front legs, prothorax, head and antennae, achieving a spatial resolution of 0.2 mm and a temporal resolution of 200 Hz.
Combining all components described above allows us to study stimulus-induced changes of temporal and spatial coordination of antennae and legs in a controlled and repeatable manner. Here, we present preliminary results on touch-induced changes in walking direction, antennal beating field and front leg movements.

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Functional heterogeneity of neurons within a midbrain nucleus driving locomotion in adult zebrafish

Eva M. Berg[1], Abdeljabbar El Manira[1]

[1]Karolinska Institute, Neuroscience Dept., 17177 Stockholm, Sweden

Animals navigate their environment with great versatility to find food, escape predators, or find mating partners. While the basic rhythmic locomotor pattern can be generated by circuits in the spinal cord, these circuits are driven by descending projections from the brain. How this descending drive is organized is only beginning to be understood.

In the zebrafish, neurons of the nucleus of the medial longitudinal fasciculus (nMLF) in the midbrain are the most rostral cells projecting to the spinal cord. In the larvae, the nMLF has been implicated in controlling swimming behavior in various ways, e.g. by providing a general excitatory drive or by governing steering movements. Thus far, however, most of these studies have regarded the nMLF as a uniform entity and have examined its role on swimming behavior using imaging or overall ablation and activation. Here we have taken a single-neuron approach to assess the functional heterogeneity of nMLF neurons. For this, we have used single or dual patch-clamp recordings from identified nMLF neurons during spontaneous swimming using the ex-vivo adult zebrafish preparation.

First, we show that electrical stimulation of the nMLF region induces swimming activity and that nMLF neurons display cell-specific projection profiles along the rostro-caudal level of the spinal cord. Second, we show that nMLF neurons can be subdivided into several subgroups based on their firing properties with individually identified nMLF neurons consistently displaying either a strong spike frequency adaption, tonic firing, or a delayed firing onset with increasing frequency. These neuron types are predominantly located in different regions of the nMLF. Finally, we show that nMLF neurons exhibit different activity patterns during spontaneous swimming. While the activity of all neurons was strongly correlated with swimming activity, they show appreciable differences. Some nMLF neurons displayed a rather sustained activity pattern for the duration of a swimming episode, while in others the activity was tightly linked to the vigor of swimming. These two neuronal types seem to occupy different regions along the medio-lateral aspect of the nMLF. Thus, our results reveal a large degree of heterogeneity in the nMLF that could endow this nucleus with the necessary versatility to control different aspects of swimming behavior.

Static stability is a good predictor of interleg coordination during walking in *Drosophila*

Till Bockemühl[1], Nicholas S. Szczecinski[2], Alexander S. Chockley[1], Ansgar Büschges[1]

[1]Department of Animal Physiology, Zoological Institute, University of Cologne, Cologne, Germany
[2]Department of Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, Ohio, USA

In insect walking, several speed-dependent interleg coordination patterns have been described: tetrapod-like coordination at slower walking speeds and tripod-like coordination at faster walking speeds. While these coordination patterns can be readily observed in various insect species, transitions between them and specifically their speed dependence have not received much attention in the past. Furthermore, it is not directly evident why insects should change interleg coordination in this speed-dependent manner at all. Tripod coordination would also be usable at lower walking speeds. In spite of this, insects typically exhibit tetrapod-like coordination at lower walking speeds, which implies that it has advantages over tripod.

We created a kinematic model to investigate whether static stability might play a role in the speed-dependent shifts in interleg coordination during insect locomotion. The model considers several basic parameters known from the walking fruit fly *Drosophila melanogaster*:

1. The duration of stance phase and each leg's stepping frequency depends on walking speed.
2. The duration of swing phase and the stance amplitude do not depend on walking speed.
3. The phase relationships between each pair of ipsilateral legs are identical. The same is true for contralateral legs.

We then compiled a large data set of current and previous experimental data of walking flies and compared the predictions of the model against them. The model correctly predicts several aspects of our experimental data, as well as aspects of walking in other insects:

1. The model suggests that walking insects should change interleg coordination from tripod-like patterns at high walking speeds to more tetrapod-like and wave gait-like patterns at lower and very low walking speeds, respectively. This change should be continuous and should also produce stable patterns that are in-between ideal tripod and tetrapod patterns.
2. The model predicts that the classical tetrapod coordination, in which ipsilateral and contralateral phase relationships are 1/3, is not optimal with regard to static stability; instead, it predicts that a contralateral phase relationship of 1/2 is more stable.
3. The model suggests an explanation for the anteriorly-directed swing phase progression observed in insects: transitions from swing to stance phase in front and hind legs have a much lower influence on instantaneous stability if the ipsilateral middle leg is close to the transitioning leg. This is the case for the anteriorly-directed swing phase progression, but not the posteriorly-directed one.

While *Drosophila* does not seem to exactly follow the predicted ipsilateral phase relationships, this observation can be explained by an additional measure of how robust a particular coordination pattern is: the ipsilateral phase relationships that are predicted to be most stable are also not very robust. This seems to be taken into account by walking flies and the resulting coordination pattern is a compromise between static stability and robustness. All of these findings suggest that the static stability of an insect's body during walking is a good predictor of which interleg coordination pattern is utilized.

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Multimodal object recognition in the primate brain during a delayed-grasp task

Daniela Buchwald[1,2], Hans Scherberger[1,2]

[1]German Primate Center, Neurobiology Laboratory, Göttingen, Germany
[2]Faculty of Biology and Psychology, Georg-August-Universität Göttingen, Germany

The ability to interpret their surroundings is important for all animals in order to plan meaningful actions, such as object grasping. Many brain areas are involved in the sensorimotor transformation process in control of hand grasping, ranging from object recognition to grasp preparation and execution. However, the interaction between these different cortical areas and how grasp planning activity depends on the senses that were used to perceive the object has not been extensively studied.

In this project, we trained two rhesus macaques (*macaca mulatta*) to perform a delayed-grasping task, in which they lifted objects of different size and shapes that they had perceived beforehand either visually or tactually. While the animal performed this task, we recorded the activity of four relevant cortical areas: anterior intraparietal area (AIP), premotor area F5, primary motor area (M1), and the primary somatosensory area (S1).

Preliminary data of the first animal revealed significant differences in the cortical activity between visual and tactile trials. These differences were most prevalent during the preparatory period of the task, after the object was seen or touched but before the animal was instructed to grasp and lift it. During visual trials, we found strong, object-selective preparatory activity, as expected, especially in the grasp-related areas F5 and M1. However, during tactile trials, this effect became much weaker, hinting towards a sensory modality-specific representation of objects and action intentions in these areas. In AIP, we found only a week memory effect during visual trials, which disappeared almost completely during tactile trials. In S1, there was no memory effect when the object was not touched (visual trials) and only a weak memory effect in tactile trials.

Overall, these results show that preparatory object information is processed differently, depending on how the object was previously perceived.

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Load feedback in the stick insect is channeled towards leg motor neuron specificity by means of synaptic inhibition

Ansgar Büschges[1], Victoria Schuckel[1], Stephan Dodt[1], Nicole Jüngermann[1], Jacqueline Kasemir[1], Angelina Ruthe[1], Matthias Gruhn[1], Joachim Schmidt[1]

[1]University of Cologne, Dept. Animal Physiology, Zülpicher Strasse 47b, 50674 Cologne, Germany

Coordinated movements of legs require constant adaptation to the environment. For this purpose basic motor activity is modified through sensory feedback such as load, which in insects is detected by leg campaniform sensilla (CS; e.g. Zill et al., 2017, Arth. Struc. Dev.46:564). Depending on the behavioral context, a given motor neuron (MN) may respond differently to the same load stimulus. For example, in forward walking, the posterior directed loading of the trochanter leads to activation of retractor coxae (RetCx) and inactivation of protractor coxae (ProCX) MNs, while the reverse influence is exerted during backward walking (e.g. Akay et al. 2007, J. Neurosci. 27:325). The mechanisms by which such task-dependent changes in influence are mediated are not well understood (Büschges, 2017, J. Physiol. 595:625), neither for the underlying local mechanisms nor for the contribution of descending inputs from the brain.

The present study investigated 1) the influence of load stimuli on coxal MNs of ipsilateral legs locally and in neighboring thoracic segments, including the contralateral side of the same segment. 2) We tested, by applying the GABAA blocker Picrotoxin, the hypothesis that the specificity of load stimuli-evoked reflex-like responses in given MN pools is mediated through synaptic inhibition of CS input. 3) Through removal of the brain ganglia we tested the influence of descending inputs from the brain on the effects of local load stimuli on ProCX and RetCx MNs.

For these purposes, we stimulated trochanteral and femoral middle leg CS by horizontally deflecting an immobilized leg stump, and simultaneously recorded the activity of RetCx and ProCx MNs in different thoracic segments extracellularly in the quiescent animal (Schmitz, 1993). Neither of the contralateral coxal MN pools responded to stimulation, as was the case for the same MN pools of the prothoracic segment, which did not show a response to ipsilateral mesothoracic load stimuli. Only the metathoracic protractor MNs showed a weak increase in tonic activity upon load stimuli in the mesothorax. Interestingly, removal of GABAAergic synaptic inhibition by application of picrotoxin unmasked excitatory influences from CS onto all investigated MN pools locally as well as intersegmentally. Horizontal load stimuli led to simultaneous phasic-tonic responses in RetCx and ProCx MN pools on the side of the stimulus, in the contralateral hemiganglion, and in the ipsilateral metathoracic ganglion. Removal of the brain ganglia modified the mesothoracic responses to local load stimuli in that responses of ProCx MNs to anteriorly directed deflections were attenuated. In contrast, after brain removal, posteriorly directed load stimuli triggered alternating bursting activity in re- and protractor MNs.

Our findings suggest that CS on the middle leg are topologically connected to all ipsilateral and contralateral RetCx and ProCx MNs via excitatory pathways. It is quite conceivable that task-dependent inhibition of a selection of these pathways is a mechanism contributing to the generation of specific response patterns observed in MNs, e.g. in the resting animal. It is tempting to assume that this mechanism is also responsible for reflex modulation and reversal. Our findings also suggest that reflex responses in pro- and retractor MNs evoked by stimulations of different groups of CS are differentially modified by removal of the brain ganglia.

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Characterization of resting state activity in macaque motor cortex

Paulina Anna Dabrowska[1], Nicole Voges[1], Michael von Papen[1], Alexa Riehle[2,1], Thomas Brochier[2], Sonja Grün[1,3]

[1]Forschungszentrum Jülich, Institute of Neuroscience and Medicine (INM-6) and Institute for Advanced Simulation (IAS-6) and JARA-Institute Brain Structure-Function Relationships (INM-10), Jülich, Germany

[2]Institut de Neurosciences de la Timone (INT), CNRS - Aix-Marseille Université, Marseille, France

[3]Theoretical Systems Neurobiology, RWTH Aachen University, Aachen, Germany

The resting state activity is typically studied in large scale measurements such as fMRI or M/EEG with the aim to understand spontaneous brain activity as opposed to task- or stimulus-evoked. To get an insight in network activity on the level of multiple single units, we recorded the spiking activity and LFP from macaque (pre-)motor cortex during rest (i.e. without any task), using a chronically implanted 4x4 mm^2 100 electrode Utah Array (Blackrock Microsystems). Based on video recordings of the monkeys, we defined epochs of resting (RS), sleepiness (RSS), spontaneous body movements (M) and contra-lateral arm movements (AM). Each recording session lasted 15-20 min and yielded approximately 140 simultaneously recorded single units, which we separated into putative inhibitory (INH) and excitatory (EXC) neurons according to their wave shape (Dehghani et al. 2016). We characterized the recorded data by their firing rates, the regularity of the firing activity (CV (global measure), LV (local measure)), the pairwise fine temporal correlations (~2 ms), rate covariances (~200 ms), and the power spectra of the LFP.

We find that INH units fire faster and more regularly compared to EXC in all states, although the distributions of the LV are relatively similar. The CV, that does not account for non-stationarities, yields highest values during RSS and AM, indicating highly varying firing rates. Focusing on the characteristics of individual neurons, we find that the change of firing rates with respect to behavioral state is most prominent in inhibitory units. The firing rates of excitatory units are much less correlated to behavior. The distributions of the fine temporal correlations and the rate covariances are broader and reach higher absolute values during AM, M and RSS compared to RS. INH-INH correlations are higher than for EXC-EXC, independently of the behavioral states. Power spectra show different dominant frequencies with respect to behavioral state and monkey with increased power in alpha-beta range (7-30 Hz) during RS and RSS, and in high-gamma (60-200 Hz) during M and AM.
Thus, resting state activity is characterized by less correlated and more regular spike trains compared to other states. However, there remains a significant overlap between state-resolved statistical distributions.

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Orthogonal population dynamics and functional connectivity in the macaque fronto-parietal grasping network

Benjamin Dann[1], Hans Scherberger[1,2]

[1]German Primate Center, Neurobiology Laboratory, Goettingen, Germany
[2]Department of Biology and Psychology, University of Goettingen, Germany

Recently developed multi-electrode arrays and corresponding recording systems enabled analyses of how neuronal populations perform cognitive and behaviorally relevant computations. Several studies on monkeys and mice performing perceptual choice and delayed movement tasks revealed that neuronal population activity in prefrontal, parietal, and temporal cortex could be well understood as a dynamical process in a low-dimensional space with far less dimensions than neurons. It has been hypothesized that the low-dimensional population structure is tied to the underlying network connectivity, but since it is impossible to measure the structural connectivity of the corresponding neuronal population, this relationship has not been studied directly. However, noise correlations capturing the trial-to-trial co-fluctuations estimated for the same specific task conditions and with high temporal precision can be assumed to reflect structural connectivity as an approximation. This allows a direct comparison of the noise correlation network structure with the low-dimensional population response to provide a first glimpse of their relationship.

We used parallel recordings from about 48-90 neurons in the fronto-parietal grasping network while two monkeys performed a free-choice or instructed delayed grasping task. The low-dimensional single trial population structure was extracted using a customized dimensionality reduction method based on linear discriminate analysis. We found that seven dimensions captured more than 80 percent of all task-related single trial population activity. The fine-scale noise correlations network structure was extracted using pairwise cross-correlations that were corrected for correlations induced by task-related activity. For this cross-correlated surrogate activity with the same low-dimensional population structure was simulated and subsequently subtracted. Intriguingly, the contributions to the seven dimensions of population activity and the number of significant noise correlations per neuron were heavy-tailed distributed showing a high degree of heterogeneity of neuronal contributions. However, the number of significant noise correlations per neuron was uncorrelated with the contributions to any of the population activity dimensions (mean $R^2 < 10E-2$).

Based on these results we hypothesize a continuum in the population, in which some neurons strongly encode task relevant information but contribute little to the network communication (‘information hubs’), whereas other neurons hardly encode task-related information but are crucial for network coordination (‘coordinator hubs’).

Tyramine action on motoneuron excitability and adaptable tyramine/octopamine ratios adjust Drosophila locomotion to nutritional state

Carsten Duch[1], Natalie Schuetzler[1], Chantal Girwert[1], Isabell Huegli[1], Stefanie Ryglewski[1]

[1]JGU Mainz, Institute of Developmental Biology and Neurobiology, Col KLeinmann Weg 2, 55099 Mainz, Germany

Biogenic amines can act as neuromodulators to render animal behavior adaptive to changing external and internal conditions. For example the invertebrate counterpart of norepinephrine, octopamine, and its biological precursor and functional antagonist, tyramine, adjust multiple motor behaviors to different nutritional states. In Drosophila larvae, food deprivation increases locomotor speed via octopamine mediated structural plasticity of neuromuscular synapses, whereas tyramine reduces locomotor speed, but the underlying cellular and molecular mechanisms remain unknown. We show that tyramine is released into the CNS to reduce motoneuron intrinsic excitability and responses to excitatory cholinergic input, both by tyramine^{honoka} receptor activation and downstream decrease of L-type calcium current. This central effect of tyramine on motoneurons is required for the adaptive reduction of locomotor activity after feeding. Similarly, peripheral octopamine action on motoneurons has been reported to be required for increasing locomotion upon starvation. We further show that the level of tyramine-â-hydroxylase (TBH), the enzyme that converts tyramine into octopamine in aminergic neurons, is increased by food deprivation, thus selecting between antagonistic amine actions on motoneurons. Therefore, octopamine and tyramine provide global but distinctly different mechanisms to regulate motoneuron excitability and behavioral plasticity, and their antagonistic actions are balanced within a dynamic range by nutritional effects on TBH.

A cell-type specific driver line library targeting motoneurons and interneurons in the wing neuropil of the ventral nerve cord of *Drosophila melanogaster*

Erica Ehrhardt[1,2], Sam Whitehead[3], Shigehiro Namiki[2], Igor Siwanowicz[2], Hideo Otsuna[2], David Stern[2], Michael Dickinson[4], Kei Ito[1], Jim Truman[2], Itai Cohen[3], Wyatt Korff[2], Gwyneth M Card[2]

[1]University of Cologne, Institute of Zoology, Zülpicher Str 47b, 50674 Cologne, Germany

[2]Howard Hughes Medical Institute, 19700 Helix Dr, Ashburn VA 20174, USA

[3]Cornell University, Department of Physics, 271 Clark Hall, Ithaca NY 14853, USA

[4]Caltech, 1200 E California Blvd, Pasadena, CA 91125, USA

The ventral nerve cord (VNC) is an important command center for fruit fly sensorimotor control. Fly behavior is often driven by command signals carried by descending neurons from the brain, but once this information reaches the thorax, neural circuits in the ventral nerve cord are responsible for the generation and fine control of motor activity. Motor circuits in the wing and haltere neuropils of the ventral nerve cord enable flies to perform extremely fast, agile aerial maneuvers or to sing a precise, species-specific courtship song. At the same time, these neuropils receive somatosensory input which is critical for flight stabilization and steering. This sensory information must be processed, integrated into the neural circuits controlling movement, and some of these signals must be transmitted to decision-making centers in the brain via ascending interneurons. In order to enable the systematic analysis of the neural circuits of the VNC, we created cell-type specific driver lines targeting cells in the wing and haltere neuropils using the split-GAL4 system. In split-GAL4 lines, two different transgenes (or “regulatory sequences”) each drive the expression of one of the two domains of the GAL4 transcription factor. Only cells that express both domains produce a functional GAL4 protein that can drive the expression of the gene of interest. Well-chosen combinations of split-GAL4 transgenes can generate fly lines with such sparse expression patterns that the functional GAL4 protein only exists in one or a few specific cells. We used a FIJI plugin to draw masks around neurites of cells of interest and then automatically search over 14,000 brightness-adjusted maximum intensity projections of expression patterns in Janelia Generation 1 GAL4 lines to identify candidate lines for split intersections which had expression in the same or similar neurites. We screened over 1,500 split intersections and selected 230 for stabilization, including 29 wing control motoneuron lines, 19 power motoneuron lines, 11 haltere motoneuron lines, 4 mesothoracic ventral unpaired median neuron lines, 77 local interneuron lines, and 80 ascending interneuron lines. We activated wing control motoneurons in tethered flight using UAS-CsChrimson and found that activation of the hg2 motoneuron increases amplitude and wingbeat frequency, while activation of the i2 motoneuron has an opposite effect.

Enriched environment accelerates action potentials

Abdelmoneim Eshra[1], Petra Hirrlinger[2], Stefan Hallermann[1]

[1]Carl-Ludwig-Institute for Physiology, Medical Faculty, University of Leipzig, Liebigstr. 27, 04103 Leipzig, Germany
[2]Medizinisch-Experimentelles Zentrum, Medical Faculty, University of Leipzig, Liebigstr. 26a, 04103 Leipzig, Germany

Environmental enrichment for rodents enhances motor performance skills. Structural and molecular changes have been reported to be coupled with enriched environment, but functional alterations of single neurons remain elusive. Here, we compare mice grown up under control conditions and enriched environment. We tested the motor performance on a Rota-rod and subsequently preformed whole cell patch clamp recordings from granule cells of lobule IX of the cerebellum, an area of the brain known to be involved in motor coordination. We show that neurons undergo a certain functional adaptation to enriched environment, manifested in faster action potentials and higher maximal frequency of action potential firing. These data show that enriched environment causes specific alterations in the biophysical properties of neurons. Furthermore, we speculate that the ability of granule cells in lobule IX of the cerebellum to generate higher firing frequencies improves motor performance.

Posterior parietal cortex reflects a forward model driving sensorimotor control in a motor reference frame during BCI learning

Enrico Ferrea[1], Pierre Morel[1], Alexander Gail[1,2,3,4]

[1]Cognitive Neuroscience Laboratory, German Primate Center – Leibniz Institute for Primate Research, Kellnerweg 4, 37077, Goettingen, Germany

[2]Bernstein Center for Computational Neuroscience, Am Fassberg 17, 37077, Goettingen, Germany

[3]Georg-Elias-Müller-Institute of Psychology, University of Goettingen, Gosslerstrasse 14, 37073, Germany

[4]Leibniz ScienceCampus Primate Cognition, Kellnerweg 4, 37077, Goettingen, Germany

It has been hypothesized that the motor system uses an internal model of an effector motion to successfully online control movements despite sensory delays. It has also been shown that posterior parietal cortex (PPC) neurons in monkeys correlate better with the immediate position of a joystick controlled cursor rather than its preceding position [1]. Since the current real state of motion would not be available in this area because of visual delays, these findings are in line with PPC operating as a forward internal model for on-line motor control. However, in the previously used settings, this forward estimation correlates with both the expected sensory feedback (visual/proprioceptive) and its motor representation (i.e. an efference copy). We here ask which of these two aspects of the movement state is reflected in PPC neural encoding. Moreover, we ask if this forward estimate correlates with acquired performance and hence can play a role for movement error correction during motor adaptation.

To answer these questions, we leverage a brain-computer interface (BCI) reach task in a Rhesus monkey. BCI allows us to study adaptive motor control such that the mapping between the neural activity and the motor output (cursor motion) is experimentally controlled and that proprioceptive feedback is constant. We apply a perturbation of the visual cursor feedback (visuomotor rotation) to disentangle the visual signal from its motor representation. We record single unit signals from three sensorimotor areas from chronically implanted electrode arrays. In different sessions the BCI decoder was linked to different populations of neurons (‘decoding units’), i.e. varying subsets of primary motor (M1) and dorsal premotor cortex (PMd) units. The complementary subset of M1 and PMd units, as well as all units from the parietal reach region (PRR) were not linked to the decoder (‘non-decoding units’), i.e. could not affect the brain controlled cursor movements. Results show that neurons in all three areas, including non-decoding units, coherently shifted their tuning to counteract the applied visuomotor rotation. This suggests that BCI visuomotor adaptation is associated with widespread neural adaptation. Based on these changes, we can test which aspect of the cursor movement PRR units are reflecting. First, we offline trained a decoder to reconstruct either the actual movement trajectories of the cursor, or the “intended” trajectories (vector from current position to movement endpoint) based on PRR activity which was recorded during unperturbed BCI trials. We found that PRR units better decode the intended trajectories rather than the actual cursor movements. Second, we applied the same decoder during visuomotor rotation trials. We found that PRR neurons during rotation, consistently encode the motor rather than the visual signal despite not being part of the BCI control. Finally, to test whether this signal can be used for movement corrections across trials, we quantified trial-to-trial updating of decoded trajectories from PRR during rotation trials. Similarly to the behavioral adaptation of the animal, the decoded PRR trajectories correlate with the behavioral improvements. In conclusion, our findings support the hypothesis that PRR reflect a forward model for online control and error correction of movements in a motor but not visual reference frame.

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Object location and size influence parietal and premotor reference frames during object-oriented reach planning

Ole Fortmann[1], Bahareh Taghizadeh[2], Alexander Gail[1,3,4,5]

[1]Cognitive Neuroscience Laboratory, German Primate Center – Leibniz Institute for Primate Research, Göttingen, Germany
[2]Brain Engineering Center and School of Cognitive Sciences, Institute for Research in Fundamental Sciences, Tehran, Iran
[3]Bernstein Center for Computational Neuroscience, Göttingen, Germany
[4]Georg-Elias-Müller-Institute of Psychology, University of Göttingen, Göttingen, Germany
[5]Leibniz ScienceCampus Primate Cognition, Göttingen, Germany

During visually guided reach planning, neurons in monkey parietal reach region (PRR) and dorsal premotor cortex (PMd) encode task-relevant spatial parameters of the upcoming reach movement in different ego-centric reference frames [1]. When reaches are directed towards objects, then spatial parameters for motor planning are partly constrained by features of the object, such as size and location. Behavioral studies suggest that, when reach goals need to be localized relative to other objects, humans use both ego-centric and allo-centric spatial information for localizing targets [2]. Our previous analysis suggests that, in early stages of planning reaches towards different positions on the object, spatial selectivity of a subset of neurons is consistent with an object-centered reference frame: neural responses depend on the position on the object but not on the location or size of the object. Neurons in both areas encode a spectrum from object-centric to ego-centric reference frames. Here, we investigate the effect of this diverse encoding on the population level using a transconditional decoding approach.

Two rhesus monkeys were trained to conduct an object-based reach task, while we recorded extracellularly with microelectrodes from single neurons of areas PMd and PRR. The monkeys first saw a horizontally extended visual object (reference object) at one of two locations on a screen. Then, they had to memorize a briefly flashed peripheral visual cue which could occur at one of five positions relative to the reference object. After a first delay period, another object (decision object) appeared at one of the two locations, i.e. either congruent to the reference object, or laterally offset to it. After a second delay period, the monkey had to reach to the target position on the decision object which corresponded to the cued location on the reference object (object-based reach). There were two versions of the task: In experiment 1, the size of the reference and decision objects was the same, but the location of both was randomly varied between congruent and incongruent. In experiment 2, size and location of the reference object varied, but the decision object was fixed.

We trained a linear SVM classifier with short-term spike-rate data from one condition, defined by size and location of the object, and tested the decoding performance on data from another condition. Accurate decoding indicates object-centric encoding while a systematic offset in the decoded labels suggests an ego-centric reference frame. Considering only trials where the target can be represented in either reference frame, we quantified the dominance of each representation in shifting 300ms windows over the course of the trial.

The results of our population analysis expand on our previous findings on the single cell level: Despite large variability in single-cell tuning, we show that, during the first delay period, both PMd and PRR encode the target predominantly in an object-centered reference frame, independent of not only the location but also the size of the object. Only after the decision object is shown, the representation shifts gradually towards ego-centricity. This demonstrates flexible encoding in these areas suitable for transforming between object- and ego-centric reference frames when behavior demands interaction with objects.

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Processing of Load Signals in the Leg Muscle Control System of Insects

Corinna Gebehart[1], Joachim Schmidt[1], Ansgar Büschges[1]

[1]University of Cologne, Dept. Animal Physiology, Institute of Zoology, Zülpicher Straße 47B, 50674 Cologne, Germany

Neural circuits coordinating locomotor movements such as walking need to process movement and load feedback signals to generate a functional motor output for leg stepping (Edwards & Prilutsky 2017). In insect motor systems, local nonspiking interneurons (NSI) are important premotor elements determining the activity of postsynaptic leg motor neurons (MN) by graded transmitter release (Pearson & Fourtner, 1975). NSIs integrate synaptic inputs from sense organs on the leg (Burrows, 1996), from local central pattern generating networks (e.g. Büschges, 1995), but also inter-, and intrasegmental information (e.g. Ludwar et al. 2005). A previous study has shown that movement and load feedback signals can converge on premotor NSIs in the generation of local reflexes (Schmitz & Stein, 2000), giving rise to the question whether both signals share common pathways of processing in the generation of motor output for leg stepping.

To address this question, the responses of NSIs to force and load signals from campaniform sensilla (CS) located on the stick insect leg were examined. In a single leg preparation, the trochanterofemur (TF) of the right middle leg was immobilized and bent in the horizontal or vertical axis to activate specific groups of CS located on the TF. Simultaneously, NSIs were recorded intracellularly with a sharp microelectrode. The activity of MNs supplying the extensor tibiae or the retractor coxae muscles was recorded with extracellular hook electrodes on segmental motor nerves and confirmed by intracellular recording. NSIs were classified by their effect on postsynaptic MN pools and their morphology.

MNs were in general activated by bending stimuli in the posterior or ventral direction. NSIs were also directionally sensitive, but expressed more diverse response patterns, i.e. either phasic-tonic or purely phasic responses signaling on- and offset of the stimulus. Sign and pattern of the responses differed between NSIs, stimulus direction, and load increase or decrease. Responses were consistent for a given NSI in the resting animal.

In summary, changes in membrane potential of NSIs upon load signals could support or oppose the responses of other NSIs affecting the same postsynaptic MNs, as well as the stimulus-related activity of those MNs. When compared with the existing literature on the processing of movement signals from the femoral chordotonal organ (fCO, Bässler & Büschges 1998), and considering that the recorded NSIs also responded to stimulation of the fCO, the results suggest that feedback signals about leg loading and movement are processed in a parallel and distributed fashion along at least partially the same pathways in the local premotor circuit.

In a next step, stimulation of the fCO and CS was combined to identify integrative mechanisms and reciprocal dependencies in the processing of both sensory modalities. The fCO was stimulated by pulling on the receptor apodeme while load was imposed on tibial CS. Responses were monitored in NSIs known to be involved in the femur-tibia control loop, thereby concentrating the experimental design on a well-known neuronal network. Future questions will address how the processing of load and movement signals from the leg via premotor NSIs contributes to shaping the motor output of the leg in different behavioral contexts.

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Slope-dependent modulation of muscle co-contraction in freely walking stick insects (*Carausius morosus*)

Yannick Günzel[1], Chris J. Dallmann[1,2], Josef Schmitz[1,2], Volker Dürr[1,2]

[1]Bielefeld University, Department of Biological Cybernetics, Universitätsstraße 25, 33615 Bielefeld, Germany
[2]Cognitive Interaction Technology - Center of Excellence, Bielefeld University, Inspiration 1, 33615 Bielefeld, Germany

Natural locomotor behaviour in a variable and unpredictable environment requires animals to adjust their limb movements appropriately. For example, during vertical climbing or when running on inclines, direction and magnitude of the forces generated by each leg must be adjusted in a flexible manner. During walking, each leg of an insect undergoes rhythmically alternating swing and stance phases. It has been suggested that stance phases can be divided into an early support and a later propulsive part (Graham, 1983, J.Exp.Biol.). That being the case, the maximum propulsive force is determined by the number of legs in stance. Further, the timing of each step phase transition must ensure stability at any time. Consequently, appropriate coordination of all legs is crucial to guarantee their synergistic action. One option for flexible control of motor output during stance is the adjustment of co-contraction of antagonistic muscles, thus modulating the stiffness of a joint. In locusts, for instance, the degree of co-contraction of hind leg extensor and flexor tibiae muscles allows the control of joint torque over a large range with comparably small changes in muscle activity (Zakotnik et al., 2006, J.Neurosci.). With the present study, we evaluate the behavioural adjustments of unrestrained walking stick insects (*Carausius morosus*) in response to a step change in slope of the walkway (±45°). As the animal stepped from the horizontal part to the slanted part of the walkway, we examined the effects of step-by-step changes in load on kinematics and protractor/retractor coxae muscle activity of a hind leg. We focused on the hind leg since it is particularly important for both propulsion and posture control due to its close vicinity to the animal's centre of mass (Cruse, 1976, J.Comp.Physiol.A). Overall, we observed only small kinematic changes, despite the fact that a hind leg experienced changes in load with every leg stepping onto the slope. At the same time, however, the muscle activities of retractor and protractor coxae changed considerably. Our results show an early load-dependent activation of both muscles with delayed maximum retractor activity in case of only little load (downward walking), corroborating the idea of a biphasic power stroke. The transition from level to slope walking was accompanied by a shift in co-activation of the two muscles: With each leg stepping onto an upward slope, maximum retractor activity increased whereas maximum protractor activity decreased. With each leg stepping onto a downward slope, maximum protractor activity increased whereas maximum retractor activity decreased. Given the long activation time constants of insect leg muscles, this step-by-step change in co-activation causes stepwise decrements in co-contraction, equivalent to a change in joint stiffness. Based on these results, a neuromechanical model, transforming measured protractor and retractor motoneuron spikes into force, can now be used to analyse occurring joint torques in more detail.

Optogenetic inhibition of premotor cortex projections to parietal reach region modifies rule-based sensorimotor transformation in non-human primates

Hao Guo[1,3], Michal Fortuna[1], Janina Hueer[1], Jens Gruber[1], Stefan Treue[1,3,4], Hansjoerg Scherberger[1,3,4], Alexander Gail[1,2,3,4]

[1]German Primate Center - Leibniz Institute for Primate Research, Goettingen, Germany
[2]Bernstein Center for Computational Neuroscience, Goettingen, Germany
[3]Faculty of Biology and Psychology, University of Göttingen, Goettingen, Germany
[4]Leibniz Science Campus Primate Cognition, Goettingen, Germany

Planning movements during rule-based reaching task involve several areas in frontal and parietal cortex that are reciprocally connected. Simultaneous recording from the dorsal premotor area (PMd) in frontal cortex and the parietal reach region (PRR) in parietal cortex revealed coordinative activity between these areas [1]. The coordinative effects are more prominent for freely made movement choices than when following instructions. In addition, this study suggested that PMd is activated before PRR during free search and the latency is specific to making a decision. Furthermore, other studies investigated the frontal-parietal interaction using rule-based center-out reach tasks, where the monkey was required to map a spatial cue onto one of two reach goals, either at the location of the cue (pro) or opposite to it (anti), based on the instructed rule. The spatial transformation rule was instructed with a colored rule cue. The selectivity for motor goal occurs earlier in PMd than PRR when spatial remapping is required [2]. These findings support the possibility that dynamic reorganization of network activity in PRR is contingent on frontal-parietal projections from PMd, but not vice versa. Nevertheless, recent approaches based on Granger-causality analyses of LFPs provide evidence against the hypothesis, as no functional frontal-parietal interaction was found in the beta (12–32 Hz) and low-frequency (≤10 Hz) band [3]. The previous analyses are not capable of revealing the causal role of frontal-parietal interaction in movement planning. To determine the causal dependency of PRR on PMd direct axonal inputs more directly, we used optogenetics to transiently inhibit the presynaptic terminals of PMd projections to PRR. If PMd projections are causal for motor-goal selection in PRR, optogenetic inhibition of PMd axon terminals should modify PRR neural responses. If motor-goal selection in PRR is independent on PMd, then we should expect unchanged response profiles despite optogenetic stimulation. We infused a viral vector with inhibitory opsin (rAAV5/CamKIIα-eArchT3.0-eYFP) into the left PMd of a rhesus macaque. After three months of opsin expression, recording electrodes and the optical fiber (inter-tip linear distance: 350-850µm) were placed in the left PRR. Single-neuron activities were recorded while the monkey was performing a pro/anti reach task. A continuous laser pulse (532nm, 12-16mW at the tip of optical fiber) was applied simultaneously to the visual display of the spatial cue and context cue on the screen. Trials with and without optical stimulation were randomly interleaved and not indicated to the animal.

We observed changed neural activity of PRR at the single-neuron level. In the laser treatment conditions, 52% of motor goal tuned neurons changed their directional selectivity profiles during the memory period following the cue/laser stimulation, predominantly in the pro reach condition. Our results reveal that the direct projections from PMd to PRR play a causal role in PRR neural responses to a cue during reach planning. This study shows that top-down signals from PMd influence motor goal planning in the frontal–parietal circuit.

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Parallel cortico-cerebellar pathways through a pretectal cerebellar relay nucleus in birds.

Cristian Gutierrez-Ibanez[1], Douglas R Wylie[1]

[1]University of Alberta, Neuroscience and Mental Health Institute, Biological science building, p-217, T6G 2e9 Edmonton, Canada

Birds, like mammals, have relatively large telencephala and cerebella. In mammals, one of the largest circuits in the brain involves a projection from the cerebral cortex to the cerebellum through the pontine nuclei. Inputs to the pontine nuclei arise from all parts of the cortex, and the organization of these inputs are organized in discrete, non-overlapping areas. The telencephalon of birds also sends descending projections to several nuclei that in turn project to the cerebellum, but the organization of these efferents has not been studied extensively. Birds have two nuclei at the base of the brainstem thought to be homologous to the pontine nuclei of mammals, however, birds are unique in that they have a pretectal nucleus: the medial spiriform nucleus (SpM). The SpM, like the pontine nuclei, receives projections from the telencephalon and projects to the cerebellum. We have recently shown that the SpM is enlarge in parrots compared to other birds, which suggests SpM has an important role in motor skill and cognitive tasks, but very little is known about how inputs and outputs are organized in SpM. Here we used injections of anterograde tracers in the two main output regions of the pallium of birds, the wulst and the arcopallium combined with injections of retrograde tracers in different zones of the cerebellum of the pigeon to study organization of this telencephalon-cerebellar pathway. We found that the anterior somatomotor and more posterior visual wulst project upon two separate and non-overlapping regions of the medial SpM, while the arcopallium projects to more lateral regions of the SpM. Subsequently, we found that these two regions of SpM project to different zones of folia VI-VIII, where the lateral, arcopallial receiving zone of SpM, projects to the more medial zones of the cerebellum, while the more medial, wulst receiving area of SpM projects to a more lateral zone. Our results suggest that the organization of telencephalic inputs to SpM in birds parallels that of the projection to the pontine nuclei in mammals, and supports the notion that SpM can be considered a “displaced” pontine nuclei. Our results further suggest that the two main outputs of the forebrain in birds, the arcopallium and the wulst, are kept separated in the cerebellum. This results are an important step to understand cortico-cerebelar interactions in birds, and the role they may play in motor and cognitive task.

The role of chordotonal organs for local pattern-generating activity of a leg stump during walking in *Drosophila*

Moritz Haustein[1], Ansgar Büschges[1], Till Bockemühl[1]

[1]Zoological Institute, Department of Animal Physiology, Zülpicher Straße 47b , 50674 Cologne, Germany

During walking, insects must coordinate their six legs to generate propulsion of the body. While central pattern generators (CPGs) produce basic rhythmicity, their activity needs to be strongly modulated by sensory feedback for adaptation to the current state of the motor system and environmental conditions to generate a functional stepping motor output[1]. Moreover, sensory feedback is not only crucial for intra-leg concertation of the joints within a single leg, but also contributes to the coordination between legs by inter-segmental signaling[2]. Thus, the distinction between intra- and inter-leg influences represents an inherent problem for studying walking-regulating sensory structures.A method to reduce intra-leg sensory feedback is leg amputation[3]. The remaining stump is mechanically decoupled from the ground and sensory structures distally to the lesion are removed. Insects can still walk coordinately after the loss of a single leg and, as previously shown in *Drosophila*, the stump remains rhythmically active. Thus, emerging oscillatory stump movements could serve as proxy to study inter-segmental signals coming from the intact legs. In line with this, previous experiments in *Drosophila* showed that stump movements are indeed temporally coordinated with lift-off events from ipsilateral legs. In insect legs, chordotonal organs (COs) monitor position, velocity, and acceleration of leg segments[4]. Here, we investigated the inter-segmental influences of COs in intact legs on a middle leg stump in *D. melanogaster*. For optogenetic inhibition of COs, hyperpolarizing GtACR1 was expressed in CO neurons using the GAL4/UAS system. Walking sequences were recorded in the dark (COs active) and during exposure to green light (COs inactive) with a high speed camera. To reveal intra-leg influences of COs, kinematic parameters of the ipsilateral front (R1) and hind (R3) legs were analyzed. Latencies between lift-off events in intact legs and the dorsal and ventral extreme positions (DEP and VEP, respectively) of the stump served as measure of temporal coordination. While CO inhibition did not affect stump kinematics, it prolonged swing durations in all intact legs (132%, n=350). Additionally, the spatial positioning of R1 and R3 was impaired when the legs were maximally flexed. The coordination between intact legs and the stump was clearly observable when COs were active: as shown previously, VEPs occurred shortly after lift-off in R1 and R3 and the stump reached its DEP approx. 50 ms later. Strikingly, CO inhibition markedly affected the coordination between the stump and R1, while the temporal relationship to R3 was largely maintained. Although the temporal occurrence of VEPs remained unaltered, the latency between R1 lift-off events and DEPs was more variable when COs were inhibited (quartile CV: COs active: 0.21, n=325 vs. COs inactive: 0.38, n=224). These findings suggest that CO signaling has inter-segmental influences that play a role for the coordination between legs, in addition to its role for intra-leg coordination during walking in *Drosophila*. Moreover, these influences seem to be directionally stronger from anterior to posterior for ipsilateral legs. We hypothesize that these effects are the result of interaction between pro- and retractors between the examined legs, similar to previous findings in the stick insect[5].

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Survival of the anucleated giant Mauthner axon is required for high-performance escape responses

Alexander Hecker[1], Wolfram Schulze[1], Jakob Oster[1], Stefan Schuster[1]

[1]University of Bayreuth, Department of Animal Physiology, Universitätsstraße 30, 95440 Bayreuth, Germany

The escape circuitry of many animals from flies to mammals contains uniquely identifiable neurons with 50-100 µm diameter axons. These giant axons arise from cell bodies located in the brain and extend over the full length of the CNS. In fish, the paired Mauthner (M-) cell exceeds any other neuron in size. Its axon is capable of remaining fully functional even months after separation from its nucleus. Presently, these remarkable features are puzzling, given that laser-ablation studies suggested that M-cells are not required for high-performance escapes. Here we laser-ablated the M-cell soma and monitored the subsequent full course of Wallerian degeneration of the axon while simultaneously probing escape performance. This required monitoring individual larvae over the course of two full days. After M-soma ablation top escape performance still occurred if and only if the M-axon was still present and its initial segment (AIS) at least partly intact. In absence of the AIS, active M-cell homologs were unable to rescue performance, and survival of predator attacks dropped drastically. Most strikingly, larvae with one Mauthner axon removed showed compromised escapes to the contralateral side even after 18 months of growth to adulthood. Our results clearly show that the well-documented capacity of anucleate M-cell axons to survive for months is required to secure vital escape behavior.

Comprehensive disynaptic mossy fibre pathways from sensory and motor-related regions of the cerebral cortex to the cerebellum

Julia Henschke[1], Janelle M.P. Pakan[1,2]

[1]Institute of Cognitive Neurology and Dementia Research (IKND) Otto-von-Guericke-University Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany

[2]Center for Behavioral Brain Sciences, Universitätsplatz 2, 39120 Magdeburg, Germany

To successfully interact with the environment, an organism must gather surrounding sensory information, integrate these signals with current internal and external states, and then update ongoing motor programs. The process of comparing active sensory information with motor output occurs at various locations in the brain, but higher-level processing for fine-tuned sensorimotor integration has been described in both the cerebral cortex as well as the cerebellum. Prominent models of cerebellar processing implicated Purkinje cells as the primary site of sensory and motor system convergence, however, recent research suggests that sensorimotor integration already occurs earlier in cerebellar circuitry, via mossy fibre afferent inputs to granule cells. A main source of mossy fibre input to the cerebellum originates from the cerebral cortex and is relayed by select brainstem nuclei; the majority travelling via the pontine nuclei. However, the precise organization of these disynaptic pathways, and the function of this cortico-cerebellar communication in general, is widely understudied. For example, it is largely unclear how sensory, motor, and association cortico-cerebellar projections are related to each other or to the highly modularized organization of the cerebellum. Until now it has only been feasible to study these pathways indirectly by mapping either the cortico-pontine projections or the ponto-cerebellar pathways, but not continuous cortico-cerebellar pathways in a functionally precise way.

Thus, we investigated the cortico-cerebellar pathways originating from various sensory (visual, somatosensory, auditory), motor, and association areas in mice using an anterograde transsynaptic AAV virus in order to directly link functionally confined cortical regions with the mossy fiber afferent organization in the cerebellum, including its parasagittal pattern as delineated by the molecular marker zebrin II (aldolase C). Our results show the largest overlap between cortical sensory and motor afferents in the paramedian lobule followed by crus I, crus II and the simple lobule; highlighting these regions of the cerebellar hemispheres as hubs for cortico-cerebellar sensorimotor integration. While the bias of cortical pathway terminals to the cerebellar hemispheres was expected, we also found convergence from primary somatosensory and primary motor cortical pathways in lateral regions of the vermis (namely lobules IV/V and VI). Additionally, we found a heterogeneous pattern of coherence with zebrin II parasagittal stripes that differed across cerebellar regions, such that functionally specific cortical mossy fibre terminals were biased to either zebrin+ or zebrin- stripes, or showed no clear bias. While the patterns were highly consistent across animals, there is no single, fundamental organizational principle governing the relationship of this intrinsic biochemical map to mossy fibre afferents across cerebellar lobules and functional cortical pathways.

Duets in the wild: Inter-individually coordinated motor control enables cooperative behavior

Susanne Hoffmann[1], Lisa Trost[1], Cornelia Voigt[1,2], Stefan Leitner[1], Alena Lemazina[1,3], Hannes Sagunsky[1], Markus Abels[1], Sandra Kollmansperger[1,4], Andries Ter Maat[1], Manfred Gahr[1]

[1]Department of Behavioural Neurobiology, Max-Planck-Institute for Ornithology, Seewiesen, Germany
[2]Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa
[3]Graduate School of Systemic Neurosciences, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany
[4]Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany

Investigating the neural mechanisms that underlie an animal's natural behavior is the fundamental aim of neuroethology. Neurophysiological experiments, however, are usually performed in animals that are kept alone in cages inside laboratories, which clearly limits their ability to behave naturally. Here we present the first extracellular neural data that has been synchronously recorded from socially interacting animals while they ranged completely freely in their natural habitat. We exploited a newly developed radio-telemetric recording technique to investigate the neural basis of a rhythmic cooperative behavior: duet singing. Especially in birds, duet singing is a widespread phenomenon and duet songs show a high diversity in the precision of coordination between the partners' vocal emissions. While the spectrotemporal structure and ecological function of duet singing has already been described for several bird species, the neural mechanisms that mediate the precise inter-individual coordination of vocal activity during duetting is so far not known. To elucidate the neural basis underlying vocal duets, we simultaneously recorded individual vocalizations and multiunit vocal premotor activity in wild and free-living duetting songbirds (*Plocepasser mahali*) in the South African Kalahari. In HVC, the highest level of the descending motor pathway that controls birdsong, we found that the onset of the partner's contribution to the duet triggered a change in rhythm in the periodic neural discharges of the bird initiating the duet. The resulting inter-individually synchronized pattern of neural activity elicited vocalizations that perfectly alternated between partners in the ongoing song. We conclude that rhythmic cooperative behavior requires exact inter-individual coordination of premotor neural activity, which can be achieved by integration of sensory information originating from the interaction partner.

Glycine transporter 2-deficient mice show an altered development of the ultrasonic vocalization-associated breathing

Swen Hülsmann[1,2], Yoshihiko Oke[3], Guillaume Mesuret[1,2], A. Tobias Latal[1,2], Michal G. Fortuna[1,2], Marcus Niebet[2], Johannes Hirrlinger[4,5], Julia Fischer[6], Kurt Hammerschmidt[6]

[1]Universitätsmedizin Göttingen, Klink für Anästhesiologie, Humboldtallee 23, 37073 Göttingen, Germany
[2]Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany.
[3]Division of Physiome, Department of Physiology, Hyogo College of Medicine, Nishinomiya, Japan
[4] Carl-Ludwig-Institute for Physiology, Faculty of Medicine, University of Leipzig, Leipzig, Germany
[5] Max Planck Institute of Experimental Medicine, Department of Neurogenetics, Göttingen, Germany
[6] German Primate Center – Leibniz Institute for Primate Research, Cognitive Ethology Laboratory, 37077 Göttingen, Germany

The neuronal glycine transporter (GlyT2) is responsible for efficient loading of synaptic vesicles in glycinergic neurons. Mice lacking GlyT2 develop a hyperekplexia phenotype and do not survive the second postnatal week. Here we show that despite the strong impairment of glycinergic inhibition, GlyT2-knock out mice can produce sufficient expiratory airflow to produce ultrasonic vocalization. However, GlyT2-knock out mice do not acquire adult ultrasonic vocalization-associated breathing patterns. Moreover, we show that the USV-related breathing pattern of mice undergoes a fundamental shift from active expiration to a post-inspiratory control of passive expiration during the first postnatal week. In summary, our data not only help to understand the unclear role of glycinergic neurons in the control of breathing, but also shines new light on USV generation in general and on its postnatal development.

Construction principles of miniaturized neural circuits on the example of the flight motor network for asynchronous flight

Silvan Hürkey[1], Carsten Duch[1]

[1]Institute of Developmental Biology and Neurobiology, AG Duch, Hans-Dieter-Hüsch-Weg 15, 55128 Mainz, Germany

Miniaturization and flight are thought to be two of the major factors contributing to the great evolutionary success of insects. The small fruit fly is capable of complex flight maneuvers easily outperforming all existing technical solutions. Small insect flight relies on high wing beat frequencies to compensate for lower aerodynamic forces compared to their body mass. Therefore, insects developed “asynchronous” indirect flight muscles (A-IFM) that are specialized to generate high mechanical power at fast contraction frequencies (~200 Hz). A-IFM are not activated and deactivated in concert with neurogenically controlled cycling of myoplasmic calcium but rather are driven myogenically by oscillatory stretch activation. The muscle power output is regulated indirectly by A-IFM motoneuron firing rates, which tune the intracellular muscle calcium concentration within the muscle by firing every ~20th wingbeat. In Drosophila melanogaster the wing depressor muscle (DLM) consists of 6 muscle fibers, each innervated by one motoneuron. During flight the DLM motoneurons fire at the same frequencies, but spiking occurs not simultaneously but in a splayed state at a preferred sequence. We combine Drosophila genetics with electro- and optophysiological tools to unravel both, the neural mechanism that generate this motor pattern and the resulting function for flight behavior.
We find that all motoneurons receive common but unpatterned, tonic, excitatory, cholinergic input, which can be adjusted to different power demands. The splayed state DLM motoneuron firing patterns are generated by synaptic interactions between the motoneurons, thus bypassing the necessity of pattern generation by premotor interneurons. These synaptic interactions comprise of inhibition through the rdl GABAA receptor and the glutamate gated chloride channel, which fine tune motoneuron phase relationships and overall drive to the muscle. In addition weak electrical coupling between motoneurons is required to establish splayed state firing and to stabilize the preferred firing sequence. Therefore, in this motor circuits gap junctions serve the de-synchronization of electrically coupled neurons. The circuit design principle builds on the computation power of a motoneuron network, and thus, robust and adaptive motor output can be achieved with only on a minimal set of neurons. We currently further characterize the cellular and molecular features of this highly specialized circuit and test functional consequences of these features by genetic manipulation and in vivo experiments during tethered flight behavior.

Anatomical and physiological specializations for high spike time precision in *Drosophila* flight steering motoneurons

Nina Kramer[1], Dario Music[1], Carsten Duch[1]

[1]Johannes Gutenberg University - Institute of Developmental Biology and Neurobiology, Hanns-Dieter-Hüsch-Weg 15, 55128 Mainz, Germany

The flight muscles of *Drosophila* can be subdivided into two anatomically and physiologically distinct classes. Flight power is generated by large asynchronous stretch activated indirect power muscles specialized to maximize power output. In contrast the smaller steering muscles insert directly onto the sclerites of the wing hinge and conduct conformational changes in the arrangement of the wing hinges, therefore adjusting the patency of the wing. This enables rapid changes of wing stroke amplitude during turning manoeuvers within milliseconds. Given that flies can alter wing stroke amplitude and angle from wingbeat to wingbeat, this likely requires sub-millisecond spike time precision in some of the steering motoneurons. We use the b1 flight steering motoneuron to unravel neural mechanisms for high spike time precision.

Temporal precision of the b1 motoneuron was characterized during tethered flight by simultaneous extracellular recordings of the b1 muscle, high-speed video recordings, and laser based detection of the wing beat frequency. This revealed a spike time precision below 0.75 ms, which resembles 1/6 of the total wing beat cycle. Anatomically, low time constant is reflected in particularly short and stubby dendritic branches that merge onto a thick primary neurite. B1 axon terminals are two times larger and contain thrice as many active zones as compared to flight power motoneurons.

In an effort to make the b1 motoneuron accessible for patch clamp analysis by GFP expression and to manipulate its function by selective transgene expression during flight behaviour, a split GAL4 line was generated. This strategy effectively reduced expression but, unfortunately, still includes other neurons of the flight circuitry. We currently employ *Drosophila* genetics to generate a single cell driver line for the b1 motoneuron. This will allow dissecting its specific function during flight by optogenetic manipulation.

Platform-induced vertical vestibular ocular reflex in humans

Dieter F. Kutz[1], Florian P. Kolb[2], Stefan Glasauer[3], Hans Straka[4]

[1] Chemnitz University of Technology,
Faculty of Behavioral and Social Sciences, Institute of Human Movement Science and Health, Thüringer Weg 11, 09126 Chemnitz, Germany

[2] Institute of Physiology, Ludwig-Maximilians-University Munich, Munich, Germany

[3] Institute of Medical Technology, Brandenburg University of Technology Cottbus-Senftenberg, Cottbus, Germany

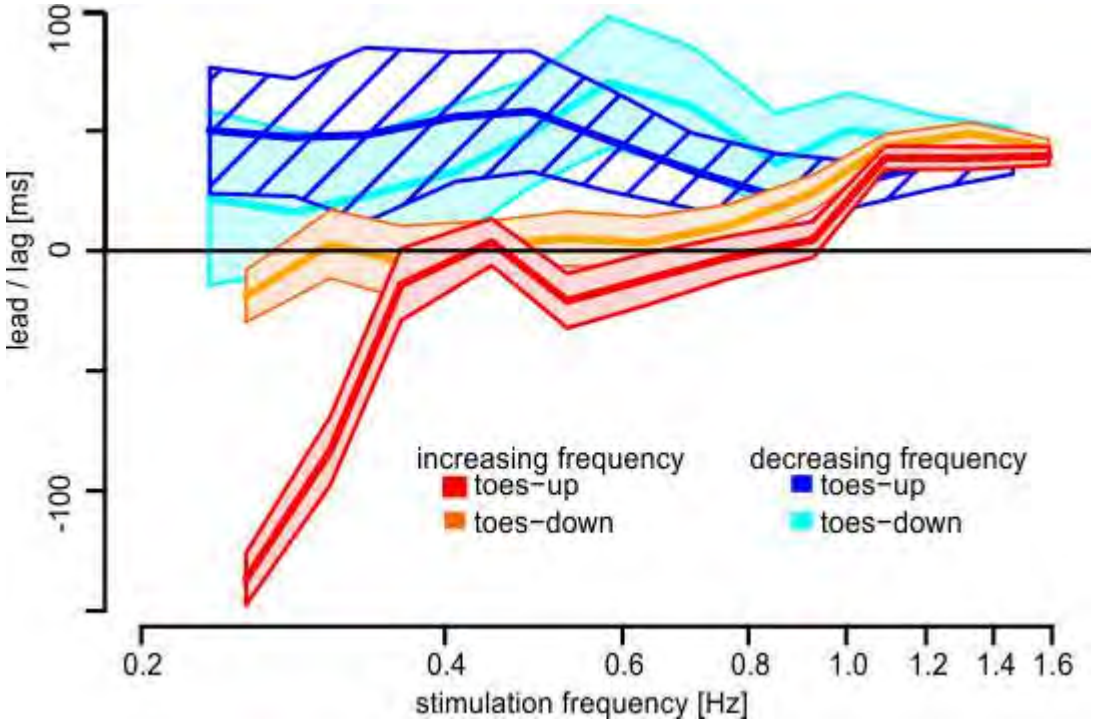
[4] Department of Biology, Ludwig-Maximilians-University Munich, Munich, Germany

Objectives: The vestibulo-ocular reflex (VOR) consists of two parts: the angular VOR (aVOR)-the classical VOR-elicited by semicircular canal signals and the translational VOR (tVOR) elicited by otolith signals. tVOR is divided in a horizontal and a vertical component. The aim of the study is to systematically analyse the latency between eye-movements elicited by whole-body vertical translations in a natural standing position.

Methods: Twenty (13 females) healthy, young subjects (age 25.2years ± 0.6, mean ± SEM) participated in the study. Subjects stood quietly at the end of a computer-controlled motor-driven platform (Stopper, Burladingen, Germany) described elsewhere (Baldinotti et al., 2010) fixating a LED 50cm in front at the height of the eyes of them. Subjects were exposed to a stimulation of 22 up and down movements of the platform with continuously changing frequency at each change of the movement direction. Each trial was separated in two halves. During the first half stimulation frequency increased (range: 0.25Hz-1.59Hz) whereas during the second half it decreased. The movement amplitude of the platform was ± 6° from horizontal position and induced a vertical translation of 2.6cm of subject's body that is similar to vertical translation during self-paced walking. Eye movements from five trials starting with toes in upwards position and the five trials with toes downwards were recorded using a video-oculography setup (EyeSeeCam, EyeSeeTec GmbH, Munich, Germany). Time differences between eye- and platform-movement were analysed using the R-package "WaveletComp, version 1.1" (Roesch & Schmidbauer, 2018).

Results: The latencies differed depending on whether the stimulation frequency continuously increased or decreased. The results are shown in the figure (mean: thick line, +/-SEM: shaded area) separated for data recorded during increasing frequencies (red and orange lines) and during decreasing frequencies (blue and cyan lines). During increasing frequencies eye movements were first leading the platform movements or were in time at frequencies up to 0.76Hz. During frequencies above that range eye movements lagged behind (at 1.59Hz: 40ms ± 4, mean ± SEM). During decreasing frequencies eye movements lagged always the platform movements with a mean latency of 41ms ± 11. For further analysis, trials were subdivided by start position (toes-up vs toes-down). Mean latencies differed in dependence of the starting position and trial-half. During increasing frequencies up to 0.93Hz the latencies of trials starting with toes-up (Fig. red line) were significantly shorter than latencies in trials starting with toes-down (Fig. orange line). For higher increasing frequencies as well as for decreasing frequencies no timing differences were found. In summary, significant differences were found for i) frequency, ii) trial-half (increasing, decreasing), iii) the interaction of frequency and trial-half), iv) and the interaction of frequency with trial-half and starting position (ANOVA with mixed design and multiple repetitions, all $P < 0.001$).

Conclusion: It is evident that during low frequencies eye-movements start earlier when the foot position starts with toes-up. This indicates that eye-movement is influenced by proprioceptive signals originating form the whole body. Hence, at least the vertical direction of the tVOR is influenced by somatosensory signals.


100
0
-100
lead / lag [ms]
increasing frequency
toes-up
toes-down
decreasing frequency
toes-up
toes-down
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
stimulation frequency [Hz]

The neural basis of amplitude adjustments during vocal interactions

Alena Lemazina[1,2], Susanne Hoffmann[1], Lisa Trost[1], Manfred Gahr[1]

[1]Max Planck Institute for Ornithology, Department of Behavioural Neurobiology, Eberhard-Gwinner-Str. house 6a, 82319 Seewiesen, Germany

[2]Graduate School of Systemic Neurosciences,LMU BioCenter,Department Biology II Neurobiology,Großhaderner Str. 2 D-82152 Planegg-Martinsried

Pairs of White-browed sparrow-weavers (Plocepasser mahali) produce duet songs that are used for within pair communication and territory defense. Duets in this songbird species consist of introductory syllables sung solo by either of the partners, which are followed by duet syllables that are emitted by the male and the female bird in a rapid and precisely coordinated alternating fashion. We assume that during duetting the partner's syllables likely create additional auditory input, which could mask auditory feedback in the focal bird. Now the question arises, how the birds cope with this masking to receive adequate auditory feedback, which is likely needed for the precise temporal coordination of vocalizations during duetting. To tackle this question we recorded individual vocal activity in pairs of duetting White-browed sparrow weavers in parallel. In ten male and ten female birds, syllables that occurred in both the introduction and duet part of the song were tested for differences in mean amplitude. The effect of the song part on syllable amplitude was analyzed with a linear mixed model. The analysis showed that the birds exhibited a significant increase of 3.7±0.5 dB in amplitude after the partner started to vocalize. There are two possible explanations for this finding: 1) The rise in amplitude may be an audio-vocal reflex to improve auditory feedback in a noisy environment, as observed during Lombard speech. 2) The birds intentionally increased the amplitude because the introductory and duet part have different behavioral functions. While the introduction may only be sung to motivate the partner to join the duet, the duet part is likely sung to signal commitment to the partner but also to signal territory boundaries to potential intruders. To investigate the neural mechanism that underlies the vocal adjustments during duetting, we recorded multiunit activity from HVC, a premotor nucleus controlling song in the brain of songbirds, in parallel with the birds' vocal activity in pairs of duetting White-browed sparrow weavers. We found that the increase in amplitude after the second bird's song onset is accompanied by an increase in vocal premotor activity. This effect is currently tested for significance.

Is Common Inhibitor Action Mediated by a Dual Control via Fast Synaptic Transmission and Neuromodulation?

Sander Liessem[1], Reinhard Predel[1], Ansgar Büschges[1]

[1]University of Cologne, Zoological Department, Biocenter Cologne, Büschges Lab/Predel Lab, Zülpicher Str. 47b, 50674 Cologne, Germany

In insects, considerable information on the operation and organization of neural networks in the thoracic ganglia is available that underlie the generation of the default locomotor patterns for jumping, flying and walking. One thoroughly studied insect species, with respect to terrestrial locomotor behavior, is the Indian stick insect *Carausius morosus* (Bidaye *et al.*, 2018). In the recent years, many studies revealed detailed information on neuronal mechanisms, which are involved in the control of walking and contributing to behavioral flexibility such as changing walking direction, speed of walking or turning (Borgmann & Büschges, 2015). However, only sparse knowledge exists on the set of neuroactive substances like neuropeptides (e.g. Büschges *et al.*, 1993) and protein hormones involved in the generation and modulation of these motor activities.
To address this subject, the neuropeptidome of the central nervous system was extensively studied via transcriptomic and neuropeptidomic analysis (Liessem *et al.*, 2018). Briefly, 159 novel and likely bioactive neuropeptides and protein hormones were found. In the current study, this information was used to perform MALDI-TOF single cell profiling of motorneurons (e.g. Goldammer *et al.*, 2012) to restrict the number of putative neuropeptide candidates involved in locomotion. Our analysis revealed the processing of several neuropeptides including Myoinhibitory Peptides (MIP) e.g. in dorsal unpaired median neurons (DUM) and Common Inhibitor motor neurons (CI). CIs serve inactivation of slow muscle fibers in leg muscles contributing to the generation fast movements. Currently pharmacological experiments are conducted (i) to investigate the potential influence of MIP on contraction properties, contraction dynamics as well as other biomechanical properties of leg muscle fibers using the tibial extensor muscle and (ii) to elucidate possible interactions with the inhibitory neurotransmitter of CI, i.e. GABA, in the control of muscle fiber operation. Under the assumption that MIP is released at the neuromuscular junction and influences force generation this would mean that insect CIs affect muscle fibers both by fast synaptic transmission and by neuromodulation.

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The temporal dynamics of action-effect prediction: An EEG study

Elisabeth Lindner[1,2,3], Álvaro Darriba[1,2], Qing Yang[1,2], Alexander Jones[4], Florian Waszak[1,2], Andrea Desantis[1,2,5]

[1]Université Paris Descartes, Sorbonne Paris Cité, Paris, France
[2]CNRS (Laboratoire Psychologie de la Perception, UMR 8158), Paris, France
[3]Cognitive Neuroscience Laboratory, German Primate Center – Leibniz Institute for Primate Research, Goettingen, Germany
[4] Middlesex University London, United Kingdom
[5] Office National d'Études et de Recherches Aérospatiales (ONERA), Salon-de-Provence, France

The effects of self-initiated voluntary actions are widely found to be attenuated in their phenomenology and their cortical response. This phenomenon of sensory attenuation as observed across different sensory modalities as for auditory, tactile or visual stimuli, has been assigned to motor-based sensory prediction. However, it remains unclear when action-effect prediction is generated by the brain and whether it is related to late motor preparatory stages and motor execution (schema theory) or to early motor preparation (ideomotor theory). The present study investigates at what time action-effect prediction, as reflected in sensory attenuation, emerges and if it is already at place even before the actual action is executed. We hypothesized to observe a difference in brain-evoked potentials during late motor preparatory stages as suggested by the schema theory, such that expected events are attenuated in the auditory N1 event-related potential compared to unexpected events, even when auditory effects are presented before action execution (see Desantis, Roussel, & Waszak, 2014). Therefore, during a learning phase, participants acquired an association between two self-initiated key presses and their respective sounds. In a second phase, those sounds were presented between a cue (an arrow indicating whether to perform a left or a right action) and the self-initiated action and either respected (congruent sound) or violated (incongruent sound) the association acquired in the learning phase. Congruent and incongruent sounds were presented at different time points before action execution. Factorial analysis revealed a significant interaction between the congruency of the sound and time of sound presentation before action onset for N1 and P2 event-related potentials. Amplitudes for congruent sound presentation significantly increased for N1 the closer the sound was presented towards movement onset compared to the amplitudes for incongruent sounds, which indicates that congruent sounds are attenuated compared to incongruent sounds at time points closest to the action (around 235ms before action). Those findings suggest that action-effects are already represented before movement onset, at late motor preparatory stages, in line with the schema theory.

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The aimed limb movements of a hemimetabolous insect are compensated for allometric wing growth

Tom Matheson[1], Alexandra J. Patel[2]

[1]University of Leicester, Neuroscience, Psychology and Behaviour, University Road, LE1 7RH Leicester, United Kingdom
[2]University of Leicester, Leicester Learning Institute, 103–105 Princess Road East, Leicester LE1 7LG, United Kingdom

If aimed limb movements are to remain functional they must adapt to developmental changes in body morphology and sensory-motor systems. The hemimetabolous development of the locust *Schistocerca gregaria* provides an opportunity to investigate such compensation. In adults, touching a wing leads to aimed movements of the ipsilateral hind limb that cross the target site. In juvenile animals the wing buds are not yet fully developed, and aimed scratching has not previously been investigated. When juveniles moult into adulthood, there is dramatic allometric growth and reorientation of the wings. The tips of the wings are located more posteriorly in adults than in juveniles. We show that: (1) both juvenile and adult locusts make scratching movements in which (2) the distal end of the tibia is aimed at different targets on the wings and abdomen. (3) Initial movement trajectories of scratches elicited by touch of the wing tip differ in juveniles and adults. (4) The cyclical components of scratches aimed at the wing tip also differ in juveniles and adults. And finally (5) hind leg joint angles at the point of closest approach to the wing tip target also differ in juveniles and adults, but for both, the angles at the closest point of approach to each target fall on a common continuum. We thus show that despite marked allometric growth and radical changes in wing morphology during locust development, scratches aimed at the wings remain targeted to appropriate locations, and the characteristic form of the movements remains the same.

Motor phenotype and Cognitive testing of PCLO Knockout (Pclogt/gt) rats measured in an operator-independent motor analysis system (OptiMan) connected with an Operant Touchscreen Chamber

Humaira Munawar[1], Marion Rivalan[2], Katharina Stumpenhorst[3], Christian Jung[4], Claus Reimertz[5], Craig Garner[6], York Winter[7]

[1]Humboldt University Berlin, Germany, Cognitive Neurobiology
[2]Humboldt University Berlin, Germany, Cognitive Neurobiology
[3]Humboldt University Berlin, Germany, Cognitive Neurobiology
[4]Humboldt University Berlin, Germany, Cognitive Neurobiology
[5]Sanofi-Aventis Deutschland GmbH, Germany
[6]DZNE, Germany
[7]Humboldt University Berlin, Germany, Cognitive Neurobiology

Automated operator-independent analysis tools of animal's behavior present the great potential to elucidate complex phenotypes as previously seen in studies involving neurodegenerative diseases. We thus aim to explore the advantages of automated, operator-independent analysis to obtain a detailed description of the behavioral effects caused by an inactivation of the PCLO gene in rats. Piccolo is a major part of presynaptic cytomatrix. It is thought to be involved in stabilizing and organizing the synapse and to be required for hippocampus-dependent learning and memory function. We thus expect Pclo knockout rats to not only display symptoms such as passivity and impaired motor performance but also impaired spatial learning when compared to wild-type rats (Pclowt/wt).

We used the OptiMan system for behavioral assessment of rats in a fully operator-independent setup. It consisted of two connected home cages resting on RFID sensor arrays that automatically tracked the movement of each RFID-tagged rat. The home cages were connected via automated animal sorters with two different operant chambers one containing a touch screen and other an isometric pull task.

Results from our initial studies indicated that the performance of Pclo knockout rats in isometric pull-task would be significantly lower than in Pclo wildtype rats. This impairment was even more pronounced when a reaching-out movement of the paw was not required. Such reduction in reaching-grasping ability indicated an impairment in motor coordination.

Additional behavioral tests evaluating learning and memory will be carried out in a battery of different touchscreen tasks to assess whether PCLO plays a significant role in learning and memory.

Encoding of movement force in the fronto-parietal reach network in primates

Julia Wanda Nowak[1], Pierre Morel[1], Alexander Gail[1,2,3,4]

[1]German Primate Center, Leibniz Institute for Primate Research, Sensorimotor Group, Goettingen, Germany

[2]Bernstein Center for Computational Neuroscience, Göttingen, Germany

[3]Georg-Elias-Müller-Institute of Psychology, University of Göttingen, Göttingen, Germany

[4]Leibniz ScienceCampus Primate Cognition, Göttingen, Germany

When choosing among multiple movements, the brain, according to the affordance competition hypothesis, generates competing neural representations of these movements, which are biased by the desirability of each action outcome [1]. Studies in reach movements show that neuronal activity in the fronto-parietal reach network in primates co-encodes the directions of two reach targets in parallel, before a reach movement is chosen [2]. This direction-related neuronal activity can be biased by action benefits, e.g. reward value [3]. When performing a reach movement, the physical effort associated with that movement can be seen as action cost. This leads to the question whether direction-related neuronal activity in the fronto-parietal reach network can be biased by costs associated with a reach movement, similar to benefits [3]. We address this question by investigating the effects of physical effort, here movement force, on the direction-related neuronal activity in the fronto-parietal reach network in primates. We trained two rhesus macaques to perform a memory-guided center-out reach tasks with a planar haptic manipulandum. Reaches had to be conducted against noticeable resistive forces (high-force condition; 4.5N or 6N) or negligible resistive forces (low-force condition; 0N). One animal performed a task were high-force and low-force condition were randomly interspersed and visually cued trial-by-trial. The other animal performed a task were force conditions were “blocked” i.e. high-force and low-force conditions alternated after every 32 successful trials. We recorded simultaneously from neuronal populations in dorsal premotor cortex (PMd) and parietal reach region (PRR). Additionally we recorded from primary motor cortex (M1) in one of the animals. To decompose direction-related and force-related neuronal activity, we used demixed principle component analysis (dPCA) [4]. Our results show that neurons in PMd and PRR encode reach target direction during movement planning and movement execution. Movement force was encoded during movement execution in both animals, but when high-force and low-force conditions were blocked, all three areas encoded movement force also during movement planning, even though the force was not yet physically present. Preliminary data suggest that force encoding in PMd and PRR depended on movement direction during movement execution, but was statistically independent from movement direction during planning. Our results indicate that the fronto-parietal reach network encodes information about the movement force of planned and performed reach movements. Direction-independent force-encoding during planning could hint to a more abstract representation that is not coupled to direct motor output, while force-direction encoding during movement might be more related to direct motor output or different proprioceptive feedback in force-loaded reaches.

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Inhibition of HSP90 increases individual variability of behaviour in the desert locust

Swidbert Roger Ott[1], Tom Matheson[1], Ben Cooper[1]

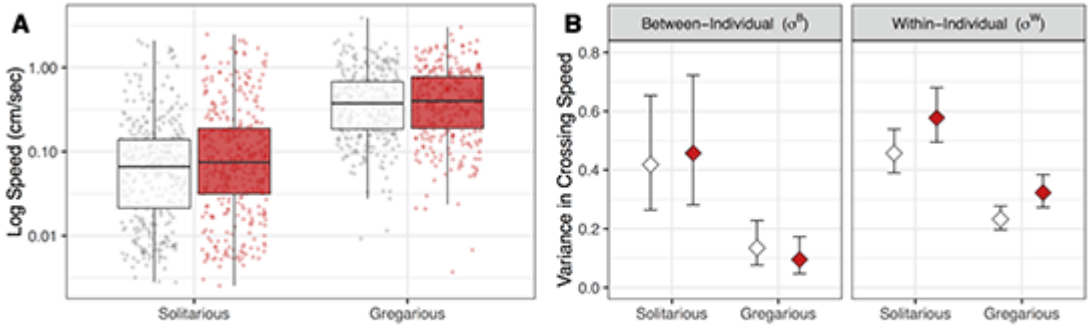
[1]University of Leicester, Neuroscience, Psychology and Behaviour, University Road, LE1 7RH Leicester, United Kingdom

All animals can tailor their phenotype to changing environmental conditions (phenotypic plasticity), yet are also robust to environmental perturbations (canalisation). Heat shock proteins (HSPs) are molecular chaperones that are thought to have a central role in shaping the genotype-phenotype map; HSP90 in particular has been proposed as a key mediator of robustness against genetic and environmental perturbations. Previous research has focussed on HSP90 canalising the intracellular machinery in yeast and the development of morphological traits in animals. Although very recent research implicates HSP90 in synaptic function, its role in behaviour is almost entirely unexplored.

Here we investigated the role of HSP90 in behavioural consistency in the desert locust, *Schistocerca gregaria,* a grasshopper (family Acrididae) with a capacity for extreme phenotypic plasticity. In response to crowding or isolation, locusts transform between a shy, cryptic and relatively inactive 'solitarious phase' that avoids other locusts and a brightly coloured and highly mobile 'gregarious phase' that is attracted to conspecifics. Locusts therefore provide a powerful model for investigating mechanisms that modulate phenotypic plasticity of behaviour in a clearly defined ecological context.

We analysed the effect of pharmacological inhibition of HSP90 on behavioural consistency in a simple locomotor hesitation assay. Fifth-instar nymphs were starved for 24 h to control motivation; injected with the selective HSP90 inhibitor tanespimycin (17-N-allylamino-17-demethoxygeldanamycin, 17-AAG) or a vehicle control; and then walked on a horizontal wooden beam in a longitudinal arena upwind towards a visually concealed food odour source (wheatgrass). Approach behaviour was analysed from video footage, and total approach time was expressed as a nominal speed (1/time). Each individual locust was assayed twice daily over 4 days, with daily injection. Bayesian Markov Chain Monte Carlo mixed-effects models were used to obtain separate estimates of between- and within-individual variance components and associated Bayesian credible intervals.

Solitarious locusts approached food much more hesitantly than gregarious locusts (Fig. A). Solitarious locusts also showed considerably greater between- and within-individual variability in locomotor hesitation (Fig. B). 17-AAG had no effect on mean approach speeds in either phase (Fig. A), but caused a clear increase in within-individual variances in both phases without affecting between-individual variances (Fig. B). Our data provide first evidence for a role for HSP90 in canalising behaviour towards greater individual consistency. We are currently using RNAi knock-down to investigate whether individual differences in behaviour (between-individual variability) also increase following down-regulation of HSP90 over longer timescales.



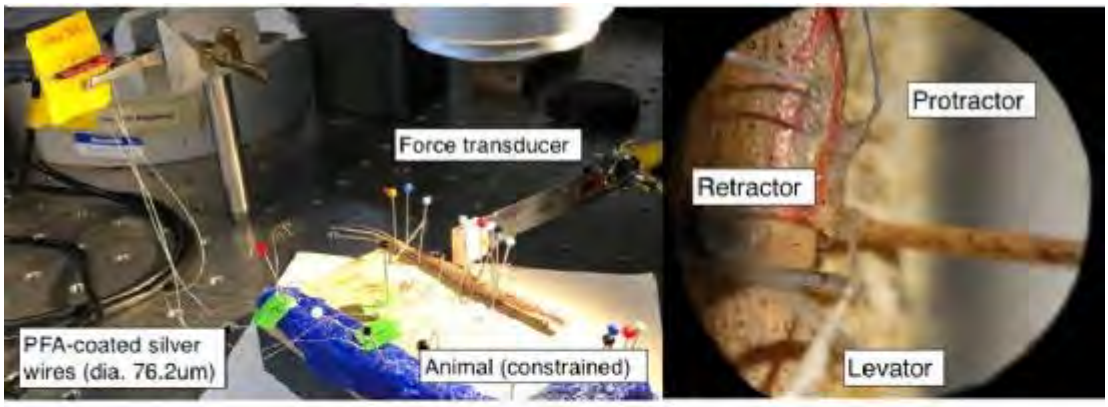
Effect of 17-AAG (red) or vehicle control (white) on locomotor hesitation (expressed as 1/time = speed).
A: 17-AAG does not affect average speeds in either phase. **B:** 17-AAG causes a significant increase in within- but not between-individual variances in both phases: Bayesian estimates (points) and 95% credible intervals (bars).

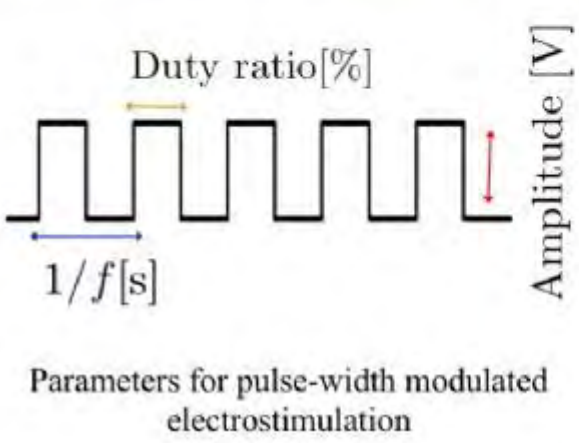
Motion Hacking: A Method for Interference with Neural Control of Walking, Based on External Muscle Stimulation in Stick Insects

Dai Owaki[1], Volker Dürr[2], Josef Schmitz[2]

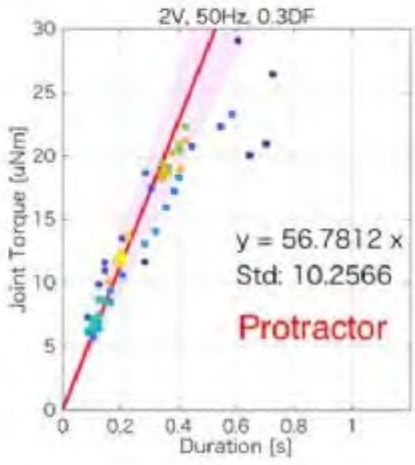
[1]Tohoku University, Dept. of Robotics, 6-6-01 Aoba, Aramaki, Aoba-ku, 9808579 Sendai, Japan
[2]Bielefeld University, Dept. of Biological Cybernetics, Faculty of Biology, Universitätsstr. 25, 33615 Bielefeld, Germany

Insects adapt their locomotion behavior in response to changes in environment and context by altering both inter- and intra-leg coordination. To elucidate the mechanisms underlying adaptive coordination, we apply an external and precise method of interference with neural motion control, based on electrostimulation of leg muscles. Here, we propose this method as "Motion Hacking", based on engineering techniques. As a first step, we investigated externally induced joint torques that were generated by stimulating one out of three leg muscles in the stick insect *Carausius morosus*. For a given parameter set of pulse-width modulated electrostimulation, we found that a linear relationship between burst duration and generated joint torque. Linearity holds for a burst duration range between 100 and 500 ms, corresponding to typical values of stance and swing durations of walking stick insects. The result suggests that we can control joint torques of a single leg so as to "what extent" and "when" we want it to move. This is a necessary prerequisite for hacking the motion of a leg via external muscle stimulation in free walking insects.





Parameters for pulse-width modulated electrostimulation



Optical Inactivation of Leg Proprioceptors in the Stick Insect *Carausius morosus*

Burak Özbagci[1], Anna Haberkorn[1], Ansgar Büschges[1], Matthias Gruhn[1]

[1]University of Cologne, Dept. Animal Physiology, Institute of Zoology, Zülpicher Str. 47b, 50674 Köln, Germany

The generation of a functional motor output for walking heavily depends on sensory feedback from the limbs with feedback about movement and load or force playing a prime role. *Campaniform sensilla* (CS) are mechanoreceptors, which monitor load as mechanical strain to resisted movements on the arthropod cuticle (e.g. Zill et al. 2017). Individual sensilla are usually arranged in groups that are located on all leg segments, except for the coxa (Bässler, 1977; Zill et al. 2011). Their specific role in locomotor pattern generation is traditionally investigated by means of mechanical ablation that can lead to injury discharges, changes in the structural integrity of the cuticle, and is challenging during ongoing recordings. Here, we present a new method to silence CS, allowing to temporarily deprive the nervous system of load feedback by optical inactivation of selected CS. Using blue light we tested, whether the sensory activity of individual CS groups can be deactivated during ongoing recordings. For this purpose, we recorded extracellularly from the main leg nerve carrying sensory information, while applying load stimuli to the middle leg by deflecting the tibia upwards (50µm). Illumination of tibial CS Gr6a and 6b for 3 minutes was sufficient to stop the sensory response to load stimuli (N = 5). This effect was temporary, as the response of the CS upon stimuli reappeared after a recovery time of less than 5 minutes. To test, if the temporal knockout of CS can have an effect on motor activity, we illuminated the surface of the anteriorly located trochanteral CS Gr2, which, upon stimulation, elicits a motor response in *protractor coxae* (ProCx) motor neurons (MNs) (Schmitz, 1993). Simultaneously, we recorded extracellularly from ProCx MNs and from *retractor coxae* (RetCx) MNs, while load stimuli were applied on the femur in anterior direction. Again, upon light application the response to stimuli in the ProCx MNs gradually decreased and typically ceased between 2 and 3 minutes after light onset (N = 4). Deactivation of the MN response to CS stimulation was limited to the illuminated CS group, as RetCx MN responses to stimulation of other trochanteral CS, were not affected throughout the illumination and thereafter. Deactivation was also temporary and typically recovered within 10 to 30 minutes after ending laser light illumination. To demonstrate that blue light induced temporary ablation of CS can be used during behavioral experiments, we illuminated the femoral CS (Gr5) while the intact middle leg was placed on a treadmill and recorded *flexor tibiae* (FlxTi) muscle activity with EMG electrodes during the extension and flexion of the femuro-tibial joint. Illumination of CS Gr5 decreased FlxTi activity upon leg extension as already shown using mechanical ablation of this CS group (Akay et al. 2001). The mechanism of this deactivation of CS by blue light application is not fully understood, but appears to be temperature independent, as application of light using an infrared laser (808 nm) at the same intensity and with similar temperature generation inside the targeted area tissue did not affect the CS response(N = 2). Future experiments will be performed to systematically test the effect of single CS temporal knockout on walking movements. We will also study, if the illumination can be used to temporarily knock down other proprioceptors such as hair plates or the femoral chordotonal organ. Supported by DFG grant Bu857/14.

Female zebra finches use their song control system for call-based communication

Lisa Trost[1], Andries Ter Maat[1], Manfred Gahr[1]

[1]Max Planck Institute for Ornithology, Behavioural Neurobiology, Eberhard-Gwinner-Straße, 82319 Seewiesen, Germany

The zebra finch male uses a dedicated brain network, the song control system, to learn and maintain his song. Conspecific females don't sing. However, female brains also possess a song control system, which is less developed.
In an earlier study we found that parts of the male song control system are active when using unlearned calls. Therefore we speculated that the male specific song control system evolved from a more basic system controlling calls. One necessary step to understand the neuronal mechanisms of call usage is the comparison between male and female song control system. We implanted electrodes in RA (premotor song control nucleus) of female zebra finches and recorded the animals while they behaved freely in their groups. We found that female RA is indeed active when calling. Like in males, females do not need RA to physically produce the calls, but probably have to master requirements when using calls in communication with mate and group members, like call recognition, precise timing in the course of antiphonal call exchange and so on. These tasks all have a learning component that requires neural control by higher brain centers of innate call interaction.

Spatial averaging and inference of decision time in go-before-you-know tasks depends on motor control demands and movement time constraints

Philipp Ulbrich[1,2], Alexander Gail[1,2,3,4]

[1]Cognitive Neuroscience Laboratory - Sensorimotor Group, Göttingen, Germany
[2]Faculty of Biology and Psychology, Georg August University, Göttingen, Germany
[3]Bernstein Center for Computational Neuroscience, Göttingen, Germany
[4]Leibniz ScienceCampus Primate Cognition, Göttingen, Germany

Movement trajectories in go-before-you-know action selection paradigms provide a continuous marker for ongoing decision processes. When tight time constraints require subjects to initiate reaching movements before knowing which one of multiple potential targets to choose ('go-before-you-know'), reach trajectories are initially often aimed at the spatial midpoint of the potential targets ('spatial averaging'). The time course at which the trajectory is corrected towards the ultimately chosen target serves as marker for the decision time. These effects are thought to require rapid movements, usually achieved with tapping a touchscreen under tight time constraints. It is unclear if spatial averaging depends on movements with such reduced need for motor control or if slower and more controlled movements can reveal underlying decision processes equivalently.
To test this, we asked subjects (N = 30) to reach towards two potential targets with different reward value using a haptic manipulandum and cursor feedback in 3D space. Different to a previous, touchscreen-based rapid-reach version (325ms reaction time limit and 425ms movement time limit, 40cm distance to target) of this task [1], movements here covered 18cm distance within a 325ms reaction time limit and 700, 900 or 1200ms movement time limit. Since subjects did not receive haptic feedback on target acquisition and had to enter a spherical target zone in 3D, movements required more visuomotor control than tapping on a screen. We additionally manipulated motor control demands by varying the target diameter depending on the movement time limit to create low and high motor control demands conditions relative to each respective movement time limit (700ms/60mm, 900ms/30mm, 1200ms/15mm: low control demands; 700ms/30mm, 900ms/15mm: high control demands).
Subjects showed spatial averaging in all but the 700ms low control demands condition, but often guessed and performed direct reaches to one of the two targets in the 700ms low control and all high control demands conditions (guessing rates: on average 28% and 32% for 900ms/30mm and 1200ms/15mm vs. 58%, 56% and 52% for 700ms/60mm, 700ms/30mm, and 900ms/15mm). This pattern was largely congruent with the combined effect that movement time limit and target size had on optimal choice rates (on average 93% and 94% for 900ms/30mm and 1200ms/15mm vs. 75%, 72%, and 70% for 700ms/60mm, 700ms/30mm, and 900ms/15mm). Target acquisition rates, instead, were largely unaffected by the combined movement time and target size, except for the 900ms/15mm condition (on average 58% vs. 71-79% in all other conditions).
Our findings suggest that spatial averaging is not restricted to rapid movements, but that there is a minimum movement time needed to elicit these behavioral effects, set by the level of control the movements require. Thus, movements with higher control demand still allow using go-before-you-know tasks to determine 'online' decision latencies, as long as the movement time windows are adequately large. This allows studying decision processes in a wider range of applications, such as effort- based choice tasks, where effort can be operationalized via movement-resistive forces on the manipulandum.

[1] Chapman, S, Gallivan, JP, Wong, JD, Wispinski, NJ, & Enns, JT (2015). Journal of Experimental Psychology: General, 144, 4.

Leg coordination and gait choice in poly-pedal locomotion – numerical models and experiments

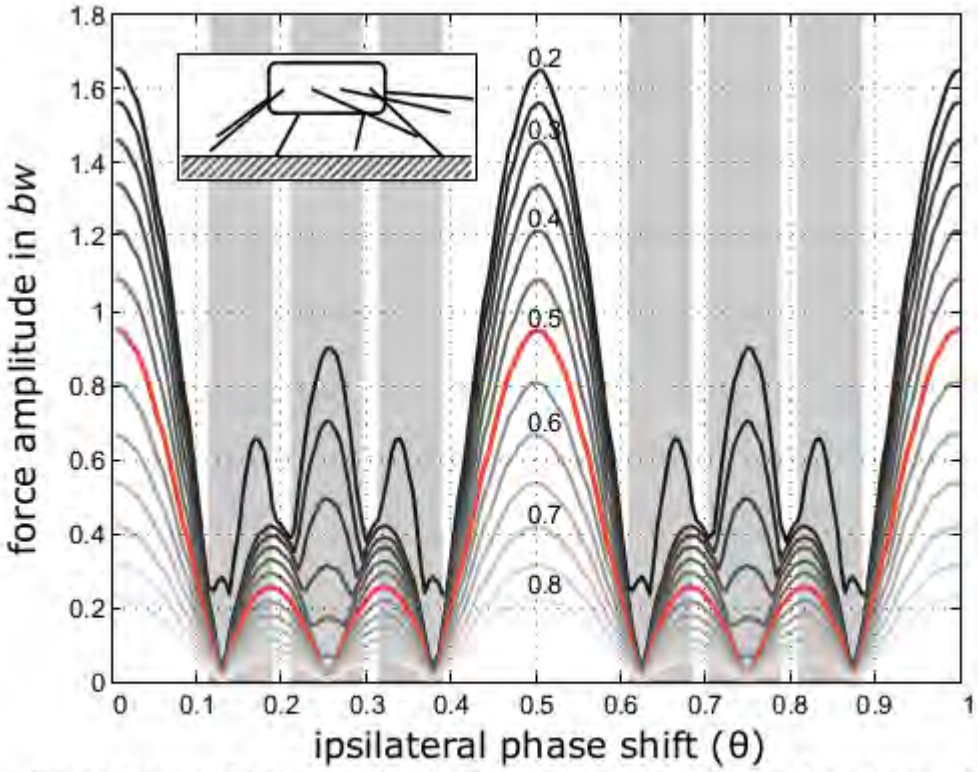
Tom Weihmann[1]

[1]University of Cologne, Department of Animal Physiology; Inst. of Zoology, Zülpicher Str.47b, 50674 Cologne, Germany

Leg coordination and gait choice in poly-pedal locomotion – numerical models and experiments

In terrestrial locomotion, CPGs provide the basis for the rhythmicity of the legs. However, typical temporal patterns of leg force application only emerge in interaction with the environment. Examinations of gait patterns and gait-changes have been the focus of movement physiology since the very beginning of the field. While most studies focussed on bipedal and quadrupedal designs, many small species have more than 4 pairs of legs. Nevertheless, overarching theory for arbitrary numbers of legs is largely missing; even experimental examinations of gait-changes in poly-pedal organisms, such as arthropods, are rare.
Crucial measures determining the application of the legs' forces onto the ground are the mean phase shift between ipsilateral legs, the duty factor and the number of propulsive legs. Ipsilateral phase relations determine the temporal relation of the legs' touch downs, whereas the duty factor, i.e. the quotient of stance duration and stride duration, determines the interval of time in which forces can be transmitted to the ground. In animals with high numbers of legs like centipedes, the temporal coordination of the legs can be described as a metachronal wave over the entire speed range, that is the phase relation between ipsilateral adjacent legs always deviates from 0.5.
An ipsilateral phase ratio of 0.5, in turn, is determining for alternating sets of synchronized legs as found in trotting quadrupeds and the tripodal coordination scheme of insects. In slow moving animals with few legs (2 to 4 pairs of legs) the typical coordination scheme is also metachronal. However, in animals capable of higher running speeds elastic properties of the walking legs often enable storage and recovery of significant proportions of movement energy. Thus, elastic structures like tendons store energy in the initial stance phase and release this energy in the final part of the stance phase, which helps to economise locomotion. To exploit elastic structures, animals with more than 3 pairs of walking legs require much higher degrees of leg synchronization then those with fewer as even small temporal deviations result in significantly reduced total vertical ground force amplitudes [1]. Since in animal locomotion vertical force components are always the largest, they are most decisive for the ability of loading elastic structures. Accordingly, animals with more than 3 pairs of propulsive legs do never exhibit elastic structures in their walking legs. However, in animals with 2 to 3 pairs of walking legs with implemented elastic structures, the stiffness and natural frequencies of the legs has to be matched to each other in order to provide concerted loading and unloading rates. Such a tuning provides the basis for a mechanical attractor causing the synchronisation of legs in alternating sets over considerable speed ranges. In the vicinity of the attractor, spring-mass dynamics of the locomotor system prevail, whereas at speeds too low or too high for the effective use of the legs natural frequencies, or when the substrate prevents efficient energy recovery, gait patterns with temporally dissociated sets of legs occur [2].

1. Weihmann (2018) Science Advances, 4:eaat3721.
2. Weihmann et al. (2017) Front Zool, 14:54.



Dependency of total vertical leg force amplitudes on the phase shift of ipsilateral adjacent legs and duty factor for locomotor apparatuses with four pairs of legs [Weihmann (2018) Science Advances 4(9), eaat3721].

Motor skill learning and execution in a distributed brain network

Steffen Benjamin Eggert Wolff[1], Ashesh Dhawale[1], Raymond Ko[1], Bence Ölveczky[1]

[1]Harvard University, Center for Brain Science, 52 Oxford Street, 02138 Cambridge, USA

The remarkable capacity of the brain to acquire and execute motor skills depends on a distributed motor network. While many individual components have been identified, less is known about their specific roles and how they interact during learning and execution of motor skills.
We are addressing these questions by training rats in a timed lever-pressing task that produces complex and highly stereotyped movement sequences. We probe the contributions of individual brain regions and their inter-connections by recording and manipulating their activities. Our previous finding that motor cortex is necessary for learning, but not for execution of this motor skill, suggests that motor cortex may act as a tutor for subcortical motor circuits during learning. A main candidate to receive this tutoring is the dorsolateral striatum, a major target of motor cortical projection neurons. In line with this hypothesis, I will show that the dorsolateral striatum is indeed necessary for acquisition and execution of the motor skills we train. Additional findings in fact place the dorsolateral striatum at the center of the distributed motor network, integrating not only cortical, but also thalamic inputs. Our results suggest a circuit level model in which motor cortical inputs to the striatum guide plasticity during learning at thalamo-striatal synapses, which in turn play a crucial role during skill execution.

Poster Topic

T22: Homeostatic and Neuriendocrine Systems, Stress Response

T22-1A Dopamine blocks homeostatic excitatory synaptic plasticity in immature dentate granule cells of entorhino-hippocampal tissue cultures
Sonja Dähn, Prof. Dr. Uli Müller

T22-2A Inflammatory Stress-Induced c-Fos Expression in the Nesfatin-1 Neurons in the Supraoptic Nucleus
Gulcin Ekizceli, Kiymet Zulal Halk, Ilker M. Kafa, Zehra Minbay, Ozhan Eyigor

T22-3A Nesfatin-1 Neurons Express Glucocorticoid Receptors in the Paraventricular and Arcuate Nuclei of the Hypothalamus
Ozhan Eyigor, Gulcin Ekizceli, Zehra Minbay

T22-1B Electrophysiological and morphological characterization of PVN neurons in mice
Debora Fuscà, Andreas Klein, Peter Kloppenburg

T22-2B Neuroanatomical mapping of hypothalamic core areas involved in the regulation of spontaneous daily torpor in the Djungarian hamster *(Phodopus sungorus)*
Elena Haugg, Dr. Victoria Diedrich, Prof. Dr. Annika Herwig

T22-3B Do central stress responses contribute to inner hair cell synaptopathy?
Philine Marchetta, Philipp Exert, Marie Manthey, Lukas Rüttiger, Wibke Singer, Marlies Knipper

T22-1C The impact of lipid homeostasis on glucose transport in neuroblastoma cells
Janine Mett, Uli Müller

T22-2C Nesfatin-1 Neurons Express c-Fos Following Restraint or Forced Swimming Stress
Zehra Minbay, Gulcin Ekizceli, Kiymet Zulal Halk, Ozhan Eyigor

T22-1D Amino acid dependent regulation of neuronal energy metabolism
Sandra Muehlenbacher, Uli Müller

T22-2D Early life growth retardation in *Tph2*$^{-/-}$ deficient rats
Polina Peeva, Daniel Beis, Mihail Todiras, Elena Popova, Michael Bader, Natalia Alenina

T22-3D Immune Response and Behavior modulation in *Drosophila melanogaster*
Thomas Dieter Riemensperger, Fabienne Reh, Kei Ito

Energy metabolism in honey bees is affected by the neonicotinoid thiamethoxam

Sonja Dähn[1], Prof. Dr. Uli Müller[1]

[1]Universität des Saarlandes, Zoologie/Physiologie, Campus Gebäude A2.4 Raum 3.40, 66123 Saarbrücken, Germany

Neonicotinoids are pesticides that act agonistically on insect nicotinic acetylcholine receptors leading to paralysis and death. Although neonicotinoids have been designed to bind specifically to insect receptors, chronic exposure to neonicotinoids is also connected to metabolic dysfunctions in mammals. This poses the hypothesis that neonicotinoids affect the regulation of glucose metabolism, which, in turn affects neuronal and cognitive functions. To address this question I investigate the effects of Thiamethoxam on systemic and molecular aspects of glucose metabolism in the honeybee as well as in human cell lines.
First results in honeybees demonstrate an effect of Thiamethoxam on the consumption of sugar and water. In accordance Thiamethoxam causes changes of the hemolymph glucose level. Using a fluorescent glucose we further investigate whether this effect is caused by dysfunction of the digestive tract. Test on human cell lines treated with Thiamethoxam will show whether the neonicotinic effect shown in honey bees is mechanistically conserved. The latter is of special importance since it will shed new light on the observed effects of neonicotinoids in humans.

Inflammatory Stress-Induced c-Fos Expression in the Nesfatin-1 Neurons in the Supraoptic Nucleus

Gulcin Ekizceli[1], Kiymet Zulal Halk[1], Ilker M. Kafa[2], Zehra Minbay[3], Ozhan Eyigor[3]

[1]Bursa Uludag University Institute of Health Science, Department of Histology and Embryology, Bursa, Turkey
[2]Bursa Uludag University Faculty of Medicine, Department of Anatomy, Bursa, Turkey
[3]Bursa Uludag University Faculty of Medicine, Department of Histology and Embryology, Bursa, Turkey

Introduction: Nesfatin-1 is an anorexigenic peptide regulating the food intake in the organism. Nesfatin-1 neurons are localized in distinct hypothalamic nuclei including the paraventricular (PVN), supraoptic (SON) and arcuate nuclei. These neurons are shown to respond to many different physiological and/or pathological challenges in terms of neuronal activation. In this study, we aimed to investigate the activation of nesfatin-1 neurons following lipopolysaccharide (LPS)-induced inflammatory stress. For this aim, we employed dual immunohistochemistry for nesfatin-1 and c-Fos proteins. The transient expression of c-Fos was used as the marker of neuronal activation.

Materials and Methods: In this study, young adult male (n=6; LPS n=3, control n=3) Sprague-Dawley rats were used. Rats were treated with LPS (i.p., 500 µg/kg) and sacrificed after 90 minutes by perfusion fixation. c-Fos and nesfatin-1 dual-immunohistochemistry was carried out on free-floating, 40-micrometer-thick coronal vibratome sections. The number of nesfatin-1-positive neurons as well as the number of c-Fos-expressing nesfatin-1 neurons was counted in the SON. Then the ratio of c-Fos-positive nesfatin-1 neurons to all nesfatin-1 neurons was calculated. The data was statistically analyzed to compare the experimental group to the control group.

Results: Activated nesfatin-1 neurons mainly localized in the SON and PVN while the neurons in the arcuate nucleus showed no c-Fos immunoreactivity. In the SON, the percentage of the activated nesfatin-1 neurons was about 86% in male rats in the LPS- injected group. The ratio of the dual-labeled nesfatin-1 neurons in the control group were found as about 12%. Statistical analyses revealed that the number of c-Fos-expressing nesfatin-1 neurons in the male SON was significantly increased following LPS inflammatory stress application. Although similar increase was seen in the PVN, the data collection is not completed yet.

Discussion and Conclusion: In this study, the analyses of SON revealed that LPS, an endotoxin, significantly increased the activation of nesfatin-1 neurons in the experimental animals. Both SON and PVN are reported to be involved in the regulation of food intake. Accordingly the results of this study suggest that the inflammatory challenge may cause eating disorders through the anorexigenic effects of nesfatin-1 in the SON. In conclusion it is suggested that LPS administration which is defined as an inflammatory stress modeling, can repress food intake by activating neurons in the SON.

This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) (Project No: 116S748).

Nesfatin-1 Neurons Express Glucocorticoid Receptors in the Paraventricular and Arcuate Nuclei of the Hypothalamus

Ozhan Eyigor[1], Gulcin Ekizceli[2], Zehra Minbay[1]

[1]Bursa Uludag University Faculty of Medicine, Department of Histology and Embryology, Bursa, Turkey
[2]Bursa Uludag University Institute of Health Science, Department of Histology and Embryology, Bursa, Turkey

Introduction: Central nervous system regulates food intake through a large number of neuropeptides. Nesfatin-1 is a novel peptide which controls the eating behavior and decreases the food intake. Stress can cause eating disorders and females are more affected by stress when compared to males. Glucocorticoids, also known as the stress hormones, show their effects through their nuclear receptors (GR). In study, we aimed to determine if the nesfatin-1 neurons express the appropriate receptors in order to directly receive stress signals. For this aim we used dual-immunohistochemistry for glucocorticoid receptors and nesfatin-1 peptide.

Materials and Methods: In this study, young adult female (n=7) Sprague-Dawley rats were used. After perfusion fixation, 40-micrometer-thick coronal vibratome sections were collected and stained using antibodies against nesfatin-1 and GR. The immuno-positive cells were localized in paraventricular and arcuate nuclei and the number of nesfatin-1-positive cells as well as the number of GR-expressing nesfatin-1 neurons were determined in these areas. The ratio of GR-positive nesfatin-1 neurons to all nesfatin-1 neurons were calculated separately for each hypothalamic area.

Results: GR immunoreactivity was determined in nesfatin-1 neurons which mainly are localized in paraventricular and arcuate nuclei of the hypothalamus. In the paraventricular nuclei, the percentage of nesfatin-1 neurons expressing GR protein was about 28% in female rats. In the arcuate nucleus of hypothalamus the percentage of nesfatin-1 neurons expressing GR protein was about 35% in female rats. GR and nesfatin-1 co-localization was not detected in the supraoptic nucleus and lateral hypothalamic area.

Discussion and Conclusion: In this study, the analyses of the aforementioned hypothalamic areas revealed that nearly one-third of nesfatin-1 neurons possess glucocorticoid receptors, as one of the binding receptors for stress molecules and that these neurons can be stimulated by peripheral glucocorticoid signals. In conclusion it is suggested that stress can repress food intake, in part, through the GR receptors expressed in nesfatin-1 neurons.

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Electrophysiological and morphological characterization of PVN neurons in mice

Debora Fuscà[1], Andreas Klein[1], Peter Kloppenburg[1]

[1]Biocenter, Institute for Zoology and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Zülpicher Straße 47b, 50674 Cologne, Germany

The Paraventricular Nucleus of the Hypothalamus (PVN) is an important hypothalamic integration site for both neuroendocrine and autonomous pathways. It contains a heterogeneous population of neuron types that play an important role in regulating autonomic renal and cardiovascular functions, stress responses, and is crucial for controlling energy balance.

Within the PVN, three main types of neurons have previously been identified in rats: Magnocellular neuroendocrine neurons (MC), Parvocellular neurosecretory (NS) and preautonomic (PA) neurons. These neuron types can be identified by a number of anatomical features, molecular markers, and intrinsic electrophysiological properties. While the cell bodies of MC neurons are relatively big and mainly located in the medial PVN, cell bodies of NS and PA neurons are smaller and predominantly located in the anterior and posterior PVN respectively. Furthermore, MC and NS neurons extend their projections primarily to the median eminence where they regulate pituitary function, whereas PA neurons mainly project to hindbrain nuclei and the spinal cord.

Here we provide a comprehensive electrophysiological and morphological characterization of the three PVN neuron types in mice to establish a solid base for future experiments aiming to investigate the modulation of the PVN network under physiological and pathophysiological contexts. By performing perforated patch-clamp recordings in hypothalamic brain slices we found that the distinct neuron types can be identified by specific electrophysiological characteristics such as delayed action potential onset after hyperpolarization in MC neurons, generation of low threshold spikes in PA neurons, and typical spike frequency adaptation behavior in NS neurons. Additionally, single cell labeling and reconstruction of electrophysiologically identified cell types revealed distinct morphological characteristics.

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Neuroanatomical mapping of hypothalamic core areas involved in the regulation of spontaneous daily torpor in the Djungarian hamster *(Phodopus sungorus)*

Elena Haugg[1], Dr. Victoria Diedrich[1], Prof. Dr. Annika Herwig[1]

[1]Institut für Neurobiologie, Universität Ulm, AG Prof. Dr. Annika Herwig, Albert-Einstein-Allee 11, 89081 Ulm, Germany

The Djungarian hamster *(Phodopus sungorus)* inhabits the Central Asian steppes, characterized by a seasonally changing extreme environment. Especially survival of the very harsh and energy demanding winter conditions requires the establishment of comprehensive energy saving mechanisms. As soon as the shortening of daylight length to less than 13,5 hours announces winter, the nocturnal rodent starts an acclimatization period, which takes about 12 weeks. The hamsters voluntarily reduce their food intake and consequently their body weight and moult into a snow white, well insulating winter fur. Furthermore, their reproductive organs are regressed. These morphological and physiological changes serve as prerequisites for the expression of spontaneous daily torpor, a natural energy-saving mechanism, which can also be induced in our lab hamsters when kept at winter-like conditions of eight hours of light and sixteen hours of darkness per day. Interestingly, torpor occurs although the hamsters do not face the energetic challenges of cold and a decline of food availability as they would in their natural habitat.

Spontaneous daily torpor is expressed on average two to four times a week, lasts several hours during the resting phase of the hamster, and is characterized by a reduction of metabolism to 60 % of resting metabolic rate. This is accompanied by a decline of body temperature to about 15 °C. Up to now, the physiological mechanisms underlying proximate torpor induction are not known. Present research provides strong evidence for the hypothalamus as the major center of torpor regulation. The hypothalamus is responsible for blood pressure and osmolarity, sexuality and reproduction, homeostasis and metabolism as well as body temperature and circadian rhythm, parameters that are all related to the hamsters' winter acclimatization and energy saving mechanisms like torpor.

The present project compares different hypothalamic brain areas of torpid and non-torpid winter-acclimated Djungarian hamsters. To track their metabolic state in real-time, intraperitoneally implanted body temperature transmitters are used. Brain samples of torpid hamsters are taken at defined time points during the course of a torpor bout - namely at torpor entrance, mid torpor, arousal, and post torpor - and are compared to time-matched non-torpid hamsters.
Here, this comparison focusses on the transcriptional activity within different hypothalamic nuclei by immunohistochemical investigation of c-Fos expression on brain slices. The results shall provide important neuroanatomical insights into the role of distinct hypothalamic core areas in the regulation of spontaneous daily torpor in Djungarian hamsters. This neuroanatomical map may allow a more precise tissue sampling for next generation sequencing of mRNA, and thus the further investigation of molecular mechanisms underlying spontaneous daily torpor.

Do central stress responses contribute to inner hair cell synaptopathy?

Philine Marchetta[1], Philipp Exert[1], Marie Manthey[1], Lukas Rüttiger[1], Wibke Singer[1], Marlies Knipper[1]

[1]Department of Otorhinolaryngology, Hearing Research Centre Tübingen, Molecular Physiology of Hearing, Elfriede-Aulhorn-Str. 5., 72076 Tübingen, Germany

Systemic corticosteroids have been the mainstay of treatment for various hearing disorders for more than 30 years. Accordingly, numerous studies have described glucocorticoids (GCs) and stressors to be protective for the auditory organ during damage situations associated with a variety of health conditions, including noise exposure. Conversely, stressors are also predictive risk factors to promote hearing disorders. In this context we could demonstrate in a rat animal model that higher corticosterone levels during noise exposure were highly correlated with a decline of auditory fiber responses. This led to profoundly impaired auditory nerve processing and thereby influenced central auditory acuity.
How both of these contrasting stress actions are linked has remained elusive. The two different stress receptors mineralo- (MR) and glucocorticoid receptors (GR) were supposed to play a critical role. Whereas MRs are related to acute “positive” stress, GRs are connected to chronic “negative” stress. We studied the effect of both receptors by using KO mice with central deletion of either MR, GR or both receptors. An effect on sound-induced long-term changes in auditory nerve responses and central auditory processing could be observed.

The impact of lipid homeostasis on glucose transport in neuroblastoma cells

Janine Mett[1], Uli Müller[1]

[1]Universität des Saarlandes, Zoologie und Physiologie - Neurobiologie, Campus Gebäude A2.4, Raum 3.55, 66123 Saarbrücken, Germany[1]

Glucose homeostasis a fundamental aspect of neuronal life, among brain cells neurons expend 70-80% of the total energy and are highly dependent on glucose as major energy source. Most of the energy is required for neurotransmission and restoring ion gradients after action potentials explaining the reported link between a dysregulation of neuronal glucose homeostasis and neurological disorders. The movement of glucose across biological membranes is mainly facilitated by members of the facilitated superfamily glucose transporters GLUTs. All of them have a similar structure with 12 transmembrane domains, thus their activity might be strongly affected by changes in membrane lipid composition influencing its fluidity and thickness. Additionally, lipids and neurosteroids might affect the GLUTs by acting as intracellular signal molecules.

In this study we established methods allowing us to monitor the glucose transport into neuroblastoma cells by using the fluorescent-tagged glucose analogue 2-NBDG. These methods were used to analyze the impact of different lipids, steroids and other external factors like stress and toxins on glucose uptake in neuronal cells in the resting as well as in the activated state. The underlying mechanisms of the observed effects were further elucidated by analyzing the mRNA and protein level of the single transporters and their subcellular localization.

Nesfatin-1 Neurons Express c-Fos Following Restraint or Forced Swimming Stress

Zehra Minbay[1], Gulcin Ekizceli[2], Kiymet Zulal Halk[2], Ozhan Eyigor[1]

[1]Bursa Uludag University Faculty of Medicine, Department of Histology and Embryology, Bursa, Turkey.
[2]Bursa Uludag University Institute of Health Science, Department of Histology and Embryology, Bursa, Turkey.

Introduction: A wide variety of metabolic and behavioral changes occur in the organism when exposed to stress. In experimental animals nutrient intake significantly increases following acute stress. In humans stress affects the food intake in both directions as either increased or decreased eating. Nesfatin-1 was recently identified as an anorexigenic peptide, which also known as the saturation molecule. In this study, it was aimed to investigate whether the nesfatin-1 neurons are activated following acute restraint or forced swimming stress. For this purpose, the co-localization of nesfatin-1 and c-Fos protein in the neurons of the hypothalamic periventricular, paraventricular and arcuate nuclei was investigated using a dual immunohistochemistry method.

Materials and Methods: Adult Sprague-Dawley male and female rats were used. The study was carried out under 6 groups; restraint stress female (n=3) and male (n=3), forced swimming stress female (n=3) and male (n=3), stress control female (n=3) and male (n=3). The restraint stress was carried out in restrainer for 30 minutes and forced swimming stress was administrated in appropriate cylinders for 20 minutes. Rats were sacrificed by perfusion fixation, 40 micrometer coronal sections were taken and nesfatin-1 and c-Fos dual-immunohistochemistry was performed on floating sections. After determination of the localization of the immuno-positive cells in the paraventricular and arcuate nuclei, the nesfatin-1-containing neurons were counted and the number of dual-labeled neurons was determined. The ratio of c-Fos-positive nesfatin-1 neurons to all nesfatin-1-positive neurons was calculated separately for each hypothalamic region.

Results: In the experimental groups where restraint and forced swimming stress were applied, approximately one third of the nesfatin-1 neurons in the periventricular nucleus showed c-Fos positive immunoreactivity in female (22% restraint, 25% swimming) and male (19% restraint, 23% swimming) rats. In the PVN the percentage of the activated nesfatin-1 neurons after restraint or forced swimming stress was calculated as 39% and 35% in females and 29% and 32% in males, respectively. In control female rats the ratio of the dual-labeled neurons was calculated as 6% in periventricular nucleus and 4% in PVN. In the control male group the percentage of the activated nesfatin-1 neurons was determined as 8% in periventricular and 6% in PVN. Statistical evaluation revealed a significant increase in the number of activated nesfatin-1 neurons following acute stress in both periventricular and paraventricular nuclei. No significant gender differences were determined between groups. Recently we continue to collect data on the nesfatin-1 neurons in the arcuate nucleus.

Conclusion: In this study, it was found that an important number of nesfatin-1 neurons were activated following acute stress. This suggests that nesfatin-1 neurons may participate in the suppression of food intake during stress. Although it is known that there are gender-related differences in stress adaptation, our results showed no such differences in the case of nesfatin-1 neurons. In conclusion, it is suggested that the nesfatin-1 neurons may partly be involved in regulating the food intake behavior during stress and may have a role in the nutritional disorders that occur after stress.

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Amino acid dependent regulation of neuronal energy metabolism

Sandra Muehlenbacher[1], Uli Müller[2]

[1]Saarland University, Zoology/Physiology–Neurobiology, Campus A2.4, 66123 Saarbrücken, Germany
[2]Saarland University, Zoology/Physiology–Neurobiology, Campus B2.1, 66123 Saarbrücken, Germany

Cells without extensive energy storages such as neurons depend on a fast regulation of energy uptake by glucose transporters (GLUT) and monocarboxylate transporters (MCT). While it is well described that diabetes II or neurodegenerative diseases as Alzheimer are linked to changes in the energy regulation of neurons, it is not well understood how neurons cope with changes of the available energy sources.
In my project I address the question of how neurons regulate glucose and lactate uptake in respect to the availability of those components to handle changes in energy demand depending on neuronal activity. In addition, the impact of amino acids on the regulation of the transporters MCT and GLUT is tested. In some neuronal diseases changes in these components are relevant.
To cover a majority of potential processes involved in the function of the selected transporters, I investigate changes at the level of posttranslational modifications, membrane-cytosol translocations, and transcriptional regulation. The latter includes regulation by histone modifications that are known to be affected in metabolic and neurodegenerative diseases. The parallel investigation of GLUT and MCT provides insight of how neurons cope with fast but also long-lasting changes in the available energy sources.

Early life growth retardation in *Tph2*$^{-/-}$ deficient rats

Polina Peeva[1], Daniel Beis[1], Mihail Todiras[1], Elena Popova[1], Michael Bader[1], Natalia Alenina[1]

[1]Max Delbrueck Center for Molecular Medicine, Robert-Roessle Strasse, 10, 13125 Berlin, Germany

Serotonin is widely distributed throughout the body, produced mainly in the enterochromaffin cells of the gut and in the raphe nuclei of the brain stem. The rate limiting enzyme in the synthesis of serotonin is tryptophan hydroxylase (TPH), present in two different and independent isoforms. After the discovery of the brain specific isoform, TPH2, a rat with a genetic deletion in the Tph2 gene was generated (*Tph2*$^{-/-}$) using zinc-finger nuclease technology. The 11-bp mutation in the *Tph2*$^{-/-}$ gene led to a shift in the open reading frame and a premature stop codon, and, consequently to the nearly complete (99%) depletion of serotonin in the brain, leaving the peripheral levels unaltered. Serotonin has long been known as a major player in the pathophysiology of a broad spectrum of psychiatric disorders. However, one of the most intriguing features of *Tph2*$^{-/-}$ animals is a prominent growth retardation phenotype.

The aim of our study is to elucidate the link between central serotonin depletion and the growth retardation in *Tph2*$^{-/-}$ rats.

Although serotonin is an important maturation factor of some cerebral regions in the developing brain and of serotonergic neurons themselves, *Tph2*$^{-/-}$ animals have normal embryonic development and do not present any visible alterations in brain size and weight at birth. Only at 3-4 days after birth, a growth retardation phenotype becomes visible. The growth retardation persists throughout the first postnatal weeks being most prominent around 15 days of life. Body weight and size are however normalized later in life.

The appearance of lacteals, the lymphatic vessels responsible for fat absorption in the gut, is normal demonstrating unaltered milk fat absorption in *Tph2*$^{-/-}$ pups compared to wildtype littermates. Nevertheless, body composition analysis revealed that *Tph2*$^{-/-}$ pups are not only smaller, but also have a decrease in body fat content in comparison to wildtype animals of the same age, pointing to possible alterations in fat metabolism or metabolic rate of these animals.

A large body of literature points to growth and development alterations induced by oxygen deprivation in early life both in animal and clinical studies. Serotonin plays a major role in the control of respiration and therefore *Tph2*$^{-/-}$ pups show irregular breathing patterns which may lead to reduced oxygen supply to organs. The heart is particularly sensitive to hypoxia and reacts with hypertrophy. The increased heart/body weight ratio at two weeks of age, when the growth retardation phenotype is most prominent in *Tph2*$^{-/-}$ animals, supports our hypothesis of central serotonin deficiency being linked to a possible transient hypoxia phenotype.

We conclude that disturbed metabolic regulation, and alterations in the respiratory pattern, contributes to the growth retardation phenotype of *Tph2*$^{-/-}$ rats.

Immune Response and Behavior modulation in *Drosophila melanogaster*

Thomas Dieter Riemensperger[1], Fabienne Reh[1], Kei Ito[1]

[1]Universität Köln, Zoologisches Institut, Experimentelle Morphologie und Neuroanatomie, Zülpicher Straße 47b, 50674 Köln, Germany

Both, stimulus-oriented and internally generated behavior are influenced by intrinsic variables such as metabolic state or immune response. The acute homeostatic state of the animal is signaled to the brain where it is processed and evaluated. In the context of a health threat, the brain needs to trade the equilibration of the homeostatic state against the risk of bacterial infection. To lower the impact of infections, animals have, on the one hand developed direct immunological strategies to eliminate pathogens. On the other hand, animals show behavioral alterations reducing the risk of further infection. How the immune system communicates its state to the nervous system in response to bacterial infection is yet not understood. Our present study aims at investigating the neuronal networks underlying the integration of the information about the immune system's activity with the equation between homeostatic needs and the appropriate behavioral response of the animal.

Poster Topic

T23: Neural Networks and Rhythm Generators

T23-1A Computational modelling of the firing properties of morphologically distinct types of hippocampal pyramidal neurons
Matthias Klumpp, Beate Throm, Andreas Draguhn, Martin Both

T23-2A Non-canonical axon morphologies gate information flow in neuronal ensembles
Christian Thome, Martin Kaiser, Matthias Klumpp, Paul Pfeiffer, Andreas Draguhn, Maren Engelhardt, Martin Both

T23-3A Age- and NMDAR-dependent effects of hypoxia-ischemia on hippocampal function *in vitro*
Dimitri Hefter, Paul Grube, Alycia Lee, Dragos Inta, Peter Gass, Andreas Draguhn, Martin Both

T23-4A Glutamate attenuates and NMDA enhances synchronization of spontaneous locus coeruleus network bursting in newborn rat brain slices
Klaus Ballanyi, Vladimir Rancic, Bijal Rawal

T23-6A A genetically encoded system for modification of neuronal network activity patterns in vivo at cellular resolution
Sidney Cambridge, Firat Terzi, Johannes Knabbe

T23-7A Impact of physical activity on BDNF-signaling in the brain
Ina Eiffler, Jens Schepers, Matthias Voigt, Oliver von Bohlen und Halbach

T23-8A Effects of mild metabolic stress on ensemble formation of pyramidal cells during hippocampal gamma oscillations
Shehabeldin Elzoheiry, Jan-Oliver Hollnagel, Andrea Lewen, Oliver Kann

T23-9A Intersectional subpopulations of the dorsal raphe nucleus modulate sleep-wake behavior.
Mary Gazea, Lukas Oesch, Carolina Gutierrez Herrera, Antoine R. Adamantidis

T23-10A Laminar-restricted optogenetic manipulation in the mouse motor cortex for decomposition of the local field potentials.
Andreas J. Genewsky, Anton Sirota

T23-11A The singing-CPG in crickets shows a modular organisation along the abdominal ganglia
Berthold Hedwig, Pedro Jacob, Chu-Cheng Lin

T23-1B Abnormal entorhinal control of developing prefrontal-hippocampal circuits in a mouse model of mental illness
Marilena Hnida, Xiaxia Xu, Ileana Hanganu-Opatz

T23-2B Midbrain dopaminergic neurons' response to electrical stimulation of the LDTg across alternating brain states of urethane anaesthetised rat
Gabriela Izowit, Tomasz Blasiak, Magdalena Walczak

T23-3B Phase dependent afferent influence on a simple rhythmic behaviour
Reinhard Lakes-Harlan, Alberto Licona-Backenköhler, Ann-Sophie Sturzbecher

T23-4B Co-modulation effect of two antagonistic neuromodulators on rhythmic motor activity
Nils Laudenberg, Felix Clotten, Carmen Smarandache-Wellmann

T23-5B The distribution of inputs from several brain areas into the teleost Mauthner cell
Kathrin Leupolz, Peter Machnik, Wolfram Schulze, Stefan Schuster

T23-6B Electrophysiological characterization of human dopaminergic neurons derived from LUHMES cells
Dominik Loser, Timm Danker, Clemens Möller, Marcel Leist, Udo Kraushaar

T23-7B Recordings in an integrating central neuron provide a quick way for achieving appropriate anaesthetic use in fish
Peter Machnik, Elisabeth Schirmer, Laura Glück, Stefan Schuster

T23-8B Intersegmental CPG coupling in the deafferented walking system of the stick insect
Charalampos Mantziaris, Till Bockemühl , Ansgar Büschges

T23-9B retracted: Intracortical compensatory mechanisms for weakened thalamic input in the mislam somatosensory cortex of the reeler mutant.
Anouk JM Meeuwissen, Julien Guy, Martin Möck, Jochen F Staiger

T23-10B Infraslow Oscillations in the Mouse Accessory Olfactory Bulb
Julia Mohrhardt, Chryssanthi Tsitoura, Rudolf Degen, Minghong Ma, Marc Spehr

T23-1C Eslicarbazepine effects on hippocampal sharp wave-ripples in a mouse model of KCNQ2-related encephalopathy
Laura Monni, Pawel Fidzinski, Matthias Wawra, Martin Holtkamp

T23-2C A cellular basis for cross-frequency coupling?
Andreas Neef, Fred Wolf, Ricardo Martins Merino

T23-3C How versatile behaviors are flexibly supported by local circuits: Dissecting *Drosophila's* wing motor circuit
Nelson Christian Niemeyer, Jan-Hendrik Schleimer, Silvan Huerkey, Carsten Duch, Susanne Schreiber

T23-4C Reward rate during Upper Alpha Neurofeedback affects learning of Upper Alpha modulation
Mareike Nödler, Sjoerd Meijer, Mücahit Tasci, Lena Geiss, Marinus Breteler

T23-5C Searching for neural correlates that control sleep wake-cycles in the circadian clock of the Madeira cockroach
Jenny A. Plath, Pablo Rojas, Julia Gestrich, HongYing Wei, Martin E. Garcia, Bharath Ananthasubramaniam, Hanspeter Herzel

T23-6C Changes in hippocampal network oscillations and single cell properties of GAD65 KO mice-a model of reduced GABAergic synthesis
Evangelia Pollali, Gürsel Çaliskan, Thomas Munsch, Volkmar Lessmann, Oliver Stork

T23-7C Network-specific synchronization of delta oscillations gates sleep regulation in Drosophila
Davide Raccuglia, Sheng Huang, Anatoli Ender, Desiree Laber, Agustin Liotta, Stephan J Sigrist, Jörg R P Geiger, David Owald

T23-8C Rapid depth perception in hunting archerfish
Caroline Petra Reinel, Stefan Schuster

T23-9C Multiscale analysis of neuronal activity of the circadian clock of the Madeira cockroach
Pedro Pablo Rojas, Julia Gestrich, Jenny A. Plath, HongYing Wei, Martin E. Garcia, Bharath Anasthasubramaniam, Monika Stengl, Hanspeter Herzel

T23-10C Learning Central Pattern Generator models for the generation of rhythmic activity
Alessandro Salatiello, Martin A. Giese

T23-11C Connectomics of the rat brainstem
Oliver Schmitt, Peter Eipert, Sebastian Schwanke, Julia Beier

T23-1D Neuromodulation of circuit output variability and component variability
Anna C. Schneider, Dirk Bucher, Farzan Nadim

T23-2D An analysis of cultured hippocampal neuron activity in relation to the circadian rhythm
Sinem Meleknur Sertel, Silvio O. Rizzoli

T23-3D Abnormal hippocampal innervation of developing prefrontal cortex in a genetic-environmental mouse model of mental illness
Lingzhen Song, Xiaxia Xu, Peggy Putthoff, Ileana L. Hanganu-Opatz

T23-4D Gradients in the cerebellar cortex enable Fourier-like transformation and improve storing capacity
Isabelle Straub, Laurens Witter, Miriam Hoidis, Abdelmoneim Eshra, Niklas Byczkowicz, Sebastian Maaß, Igor Delvendahl, Kevin Drogans, Elise Savier, Ingo Bechmann, Jens Eilers, Martin Krüger, Philippe Isope, Stefan Hallermann

T23-5D Circadian clock connections to specific layers of lamina and medulla in the cockroach *Rhyparobia maderae*
Thordis Arnold, Sebastian Korek, Azar Massah, David Eschstruth, Monika Stengl

T23-6D Nucleus incertus is a pontine theta oscillator – electrophysiological *in vivo* studies on urethane anaesthetised rat
Aleksandra Trenk, Magdalena Walczak, Tomasz Blasiak

T23-7D Impact of network architecture on stimulus representations in vitro
Szabina Tudja, Samora Okujeni, Ulrich Egert

T23-8D Complex bursts of action potentials in dopaminergic neurons in response to cholinergic agonists administration - – in vivo electrophysiological and pharmacological studies on NR1DATCreERT2 mice
Magdalena Walczak, Lukasz Szumiec, Jan Rodriguez Parkitna, Tomasz Blasiak

T23-9D E/I ratio is maintained constant in neocortical cultured networks despite variation of the GABAergic neurons proportion
Wenxi Xing, Ana D. de Lima, Thomas Voigt

T23-10D Abnormal CA1 activity causes decreased neonatal prefrontal-hippocampal coupling in a gene-environment model of neuropsychiatric disorders
Xiaxia Xu, Lingzhen Song, Ileana L Hanganu-Opatz

Computational modelling of the firing properties of morphologically distinct types of hippocampal pyramidal neurons

Matthias Klumpp[1], Beate Throm[1], Andreas Draguhn[1], Martin Both[1]

[1]Heidelberg University, Institute of Physiology and Pathophysiology, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany

Cortical principal neurons receive multiple synaptic inputs via their dendrites, integrate them in their soma and once a certain threshold is reached, generate action potentials (AP) as output signal at the axon initial segment. Dendritic branches provide additional options for active modulation and integration of signals, depending on the temporal and spatial pattern of arriving inputs. We have recently shown that in about 50% of CA1 hippocampal pyramidal cells, the axon emerges from a basal dendrite instead from the soma. This special type of neuron morphology is called axon-connected dendrite cell, or AcD cell.
In these cells, input via axon-connected dendrites should be privileged compared to input from any other dendrite with respect to its likelihood to contribute to AP generation. Additionally, perisomatic inhibition should have a much stronger impact on input received via soma-connected dendrites compared to axon-connected dendrites, as their input partially circumvents the capacitive sink of the soma as well as perisomatic inhibition. This might result in axon-connected dendrites being a privileged input channel to the neuron.

To investigate the hypothesis made on axon-connected dendrite cells, we implemented a multi-compartment model of a hippocampal pyramidal cell using the NEURON simulation environment, and simulated a version with the axon connected to a dendrite and another version with the axon connected to the soma. We investigated input-output relations by systematically altering cell parameters and assessing action potential firing probability.
Varying excitation and inhibition levels as well as the length of the AcD stem segment, the simulation confirmed that the likeliness of action potentials increases with the length of the AcD stem segment. In particular, the level of perisomatic inhibition hardly affects an AcD cell with a long stem segment, whereas it provides an effective gain control for neurons with generic morphology and AcD cells with a very short stem segment.
Furthermore, varying the temporal distribution of synapse activation, the model suggests that for AcD cells with long stem segments this effect is more prominent if the synapses are active in synchrony.
In addition, we simulated rhythmic excitatory and inhibitory inputs resembling synaptic events during theta and gamma oscillations. Under these conditions AcD cells have longer windows of opportunity in which they can generate APs compared to neurons with axons emerging from the soma.

Non-canonical axon morphologies gate information flow in neuronal ensembles

Christian Thome[1], Martin Kaiser[1], Matthias Klumpp[1], Paul Pfeiffer[2], Andreas Draguhn[1], Maren Engelhardt[3], Martin Both[1]

[1]Institute of Physiology and Pathophysiology, AG Draguhn, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany

[2]Institute for Theoretical Biology, Humboldt University of Berlin, Germany

[3]Institute of Neuroanatomy, Medical Faculty Mannheim, CBTM, Heidelberg University, Germany

*equal contribution

Principal neurons receive multiple synaptic inputs at their dendrites, which integrate within the somato-dendritic compartment and generate action potentials (AP) once they reach threshold at the axon initial segment. We have recently shown that in about 50% of CA1 hippocampal pyramidal cells, the axon emerges from a basal dendrite rather than from the soma. These branches are intrinsically more excitable and might constitute a privileged input channel as excitatory currents partially circumvent the capacitive sink of the soma as well as perisomatic inhibition.

The question remains, whether this anatomical feature leads to a distinct recruitment of cells during certain network states. Furthermore, we investigate how non-canonical axon morphologies form during development and whether they respond to physiological and pathophysiological changes in local network activity in vivo.

We performed extracellular and sharp electrode recordings in acute hippocampal brain slices. These slices generate spontaneous network events called sharp wave ripple complexes, which coincide with strong perisomatic inhibition. We found that only cells with dendritic axon origin fired during sharp waves. In line with our previous data, APs generated during sharp waves resembled ectopically generated AP. However, multi-compartment computer modeling suggests that APs are ordinarily initiated at the distal part of the axon initial segment, but the somatic waveform is changed due to the perisomatic inhibition and slightly remote location of the axon origin. An intracellular block of GABAergic inhibition by picrotoxin changed AP waveforms from ectopic to classical shape and recruited more cells into SPW-R coupled firing.

In summary, cells with dendritic axon origin are selectively recruited into ensembles during sharp waves ripple events in vitro. Our findings suggest that perisomatic inhibition gates information flow from different dendrites to the axon and thus enables rapid modification of ensemble compositions.

Age- and NMDAR-dependent effects of hypoxia-ischemia on hippocampal function *in vitro*

Dimitri Hefter[1,2], Paul Grube[1], Alycia Lee[1], Dragos Inta[2,3], Peter Gass[2], Andreas Draguhn[1], Martin Both[1]

[1]Heidelberg University, Institute of Physiology and Pathophysiology, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany
[2]RG Animal Models in Psychiatry, Central Institute for Mental Health, J 5, 68159 Mannheim
[3]Universitäre Psychiatrische Kliniken Basel, Wilhelm Klein-Strasse 27 CH-4002 Basel

The brain´s vulnerability to hypoxia-ischemia (HI) is highly age-dependent. On the one hand, juvenile tissue is regarded as more resistant. On the other hand, however, HI insults can cause severe disturbances in excitatory-inhibitory balance in young brains and lead to seizures and psychiatric diseases in later life. The underlying processes on the local network and cellular levels remain elusive. Many of the detrimental effects of HI are mediated by NMDA receptors. Particularly, block of the GluN2B subunit has beneficial effects on neuronal survival in adult tissue. In mice, expression of NMDAR undergoes a shift from predominance of GluN2B to GluN2A subunit expression during the second postnatal week, implying that their role in HI also may change during development.

We employed a model of oxygen-glucose deprivation (OGD) in acute brain slices from postnatal, juvenile and adult mice (postnatal day (P) 7 – 8, P15 – 16 and P60 – 70, respectively). To investigate network, multiunit- and single-unit activity we performed local field potential (LFP) and tetrode recordings from the hippocampal CA3 and CA1 regions. In slices from adult mice, OGD led to a hypoxic spreading depression (HSD) within several minutes. In juvenile and postnatal tissue, the latencies to HSD were substantially longer. OGD in postnatal slices potentiated spontaneous LFP events corresponding to giant depolarizing potentials and led to ictal activity. Juvenile and adult tissue expressed spontaneous sharp wave-ripple activity. In both groups, they were rapidly abolished by OGD. Following reperfusion with oxygenated and glucose-containing medium after HSD, these network oscillations mostly recovered, retaining their typical waveforms. However, in contrast to slices from adult mice, interictal events occurred in most juvenile slices during OGD and in some slices during the recovery period. In tetrode recordings, firing rates of individual units were briefly abolished by HSD and then partially recovered. During OGD, spike waveforms became significantly wider, with a slower rising phase of the spike, but returned to baseline waveform during recovery. Furthermore, most units could regain their typical phase-locking to local field potential oscillations.

To investigate the role of NMDAR, we applied the GluN2B antagonist Ro25-6981 and NMDAR blocker MK-801 prior to OGD. Pre-treatment with Ro25-6981 dramatically increased the latency to HSD in postnatal slices, while having no effect in juvenile tissue and a mild protective effect in adult tissue, indicating a U-shaped response curve. Treatment with MK-801 prolonged latency to HSD in all groups. Thus, both drugs predominantly reduced the vulnerability to HI, however, without exerting clear effects on functional recovery.

In conclusion, we show age-specific consequences of acute HI at the level of local network activity and cellular functions. We further show an age-dependent differential role of the GluN2B NMDAR subunit in murine neuronal networks.

Glutamate attenuates and NMDA enhances synchronization of spontaneous locus coeruleus network bursting in newborn rat brain slices

Klaus Ballanyi[1], Vladimir Rancic[1], Bijal Rawal[1]

[1]University of Alberta, Physiology, MSB 7-50, T6G 2H7 Edmonton, Canada

Locus coeruleus (LC) neurons are controled by glutamatergic synaptic inputs. Here, we studied in brain slices of neonatal rats glutamate and NMDA effects on non-synchronous, phase-locked LC neuron spiking at ~1 Hz which summates to rhythmic ~0.2 s-lasting crescendo-decrescendo-shaped bursts.

Glutamate: 25-50 µM transformed the pattern of this local field potential (LFP) to irregular (ramp-like) multi-peak bursting due to attenuated network synchronization as verified by cross-correlation with 3.7-fold accelerated (irregular) spiking at concomitant 4-7 mV depolarization. 100 µM initially enhanced these effects before LFP amplitude and duration declined. 250-500 µM blocked rhythm after more pronounced and shorter initial discharge.

NMDA: 10 µM accelerated the LFP 1.7-fold and increased network synchronization as evident from burst-shortening by 30%. 25-50 µM accelerated rhythm 4-5-fold and separate bursts merged to 40-45% shorter oscillatory events indicating enhanced network synchronicity as verified by cross-correlation with 7-fold accelerated cellular spiking at concomitant 5-10 mV depolarization. After 4-6 min, oscillations became interrupted every 6 s by 1 s-lasting discharge arrest. During resulting decrescendo-shaped ‘oscillation trains’, LC neurons showed ramp-like depolarization by 15-30 mV and accelerated regular spiking which were both blocked during rhythmic LFP arrest. 100 µM blocked rhythm.

Both agents: evoked ‘post-agonist depression’ of LFP due to a 15-20 mV hyperpolarization lasting 1-6 min, also seen initially upon countering glutamate effects with kynurenic acid (0.1-2.5 mM).

The findings show that accelerated spiking during both glutamate and NMDA is associated, respectively, with attenuated and enhanced LC synchronization causing distinct LFP pattern transformations. Consequently, ionotropic glutamate receptors differentially shape LC population burst dynamics to potentially enable this possibly ‘modular’ network to fine-tune its influence on multiple brain functions.

A genetically encoded system for modification of neuronal network activity patterns in vivo at cellular resolution

Sidney Cambridge[1], Firat Terzi[1], Johannes Knabbe[1]

[1]Heidelberg University, Functional Neuroanatomy, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany

We combined an optimized version the inducible Tet system with the conditional, cell-type specific Cre recombinase system for genetic manipulation of tissues in vivo at cellular resolution based on adeno-associated viruses (AAV). Because Cre-mediated constitutive expression of a fluorescent reporter highlights cells prior to induction of the transgene via the Tet system, cells can be carefully characterized before, during, and after genetic manipulation. We use this optimized Cre-dependent TetOn system for acute genetic silencing of neurons in mouse cortex by expression of Kir2.1, a potassium channel that cell-autonomously hyperpolarizes membranes. Co-injection of an AAV with the GFP-based calcium indicator GCamP6 allowed in vivo 2-photon imaging the spontaneous activity of the same neurons over time in longitudinal experiments. Injection of doxycycline into the brain induced rapid, Kir2.1-dependent silencing of neurons within hours that lasted for at least 80 h. Using transgenic Parvalbumin-Cre mice, silencing of inhibitory Parvalbumin interneurons significantly increased the spontaneous activity of surrounding neurons in a distance dependent manner as nearby neurons were more affected. Finally, we tested if prolonged silencing influences dendritic morphology. Corroborating previously published in vitro results, we could show for the first time in vivo that silencing individual neurons for 75h did not change spine numbers. By manipulating and analyzing the same cells over time in vivo, this high-resolution, inducible interference and interval imaging of individual cells (high I5, “high five”) approach proved to be versatile, robust, and is readily implemented in common two-photon workflows.

Impact of physical activity on BDNF-signaling in the brain

Ina Eiffler[1], Jens Schepers[1], Matthias Voigt[1], Oliver von Bohlen und Halbach[1]

[1]University Medicine Greifswald, Institute of Anatomy and Cell Biology, Friedrich-Loeffler-Str. 23c, D-17489 Greifswald, Germany

Brain derived neurotrophic factor (BDNF) is an important growth factor in the brain contributing to neurogenesis and synaptic plasticity (Vilar & Mira 2016, Leal et al. 2017). Interestingly these processes are also affected by physical activity (Cassilhas et al. 2016, Hamilton et al. 2015) and several studies on humans and rodents have shown that serum BDNF levels increased after physical activity (meta analysis: Szuhany et al. 2015, Huang et al. 2014). Some studies also found increased brain levels of BDNF after exercise (Venezia et al. 2017, Seifert et al. 2010, Rasmussen et al. 2009).
Most of these studies examined changes in BDNF mRNA levels or total BDNF via ELISA. However, BDNF is expressed as long precursor protein (proBDNF) and then cleaved posttranslationaly into the small mature BDNF (mBDNF). Both forms of BDNF mediate different effects in the brain through their receptors TrkB or p75NTR, respectively (Hempstead 2015). Only two studies discriminated between mature BDNF (mBDNF) and its precursor protein proBDNF in rats, but do so in the context of hypertension or ischemia (Monnier et al. 2017 Quirié et al. 2012). Until now, it is not clear which form of BDNF is increased through physical activity and if the elevation of BDNF is sufficient to induce downstream signaling.
We therefore examined mBDNF and proBDNF levels as well as the activity related phosphorylation of their specific receptors in four brain regions (cortex, hippocampus, hypothalamus/thalamus, cerebellum) in wildtype (+/+) and heterozygous BDNF knockout (+/-) mice (C57Bl6/N background). Mice were either allowed to use a 4-inch running wheel voluntary (runners) or were kept in a cage without a running wheel (non-runners). Runners and non-runners where matched for genotype, gender, age and weight and activity was tracked for 2 weeks. After that, mice were killed and protein and mRNA levels were analyzed by western blotting and RT-qPCR, respectively.
To our knowledge, this is the first study to examine and discriminate between proBDNF and mBDNF levels in the brain of mice after physical activity. Our results show that physical exercise indeed is able to raise BDNF levels in the brain. Interestingly, baseline proBDNF levels were only marginally changed in BDNF knockout mice compared to their wildtype littermates, whereas mBDNF levels were depleted over 50%. We suggest that transcription of BDNF-mRNA is increased in these knock out mice to compensate the lack of BDNF but that they cannot convert proBDNF into mBDNF properly. Further research is needed to address this surprising issue.

Effects of mild metabolic stress on ensemble formation of pyramidal cells during hippocampal gamma oscillations

Shehabeldin Elzoheiry[1], Jan-Oliver Hollnagel[1], Andrea Lewen[1], Oliver Kann[1]

[1]Institute of Physiology and Pathophysiology and Interdisciplinary Center for Neurosciences, University of Heidelberg, Germany.

Gamma oscillations (30-100Hz) are associated with higher cognitive functions such as selective attention and sensory perception. They represent a synchronized interplay between excitation and inhibition. Gamma oscillations are known to exhibit high energy expenditure, which might partially explain the rapid loss of higher cognitive functions during ischemia.

Here, we explored ensemble formation of pyramidal cells during cholinergic gamma oscillations (bath application of carbachol) in the CA3 region of organotypic hippocampal slice cultures in the absence and presence of mild metabolic stress.

Metabolic stress was evoked using a low concentration of rotenone which inhibits complex I of the respiratory chain in mitochondria. To characterize pyramidal cell ensembles, we performed calcium imaging using the calcium sensor GCaMP6f under control of CaMKII promoter. Simultaneously, recordings of local field potentials (LFP) were performed.

With calcium imaging, we were able to simultaneously record activity from up to 155 cells per slice culture. We found that rotenone (0.1 µM) induced mild metabolic stress, which was reflected by the significant reduction in the power of gamma oscillations; the frequency of gamma oscillations was unchanged. No pathological activity was observed in any of the slice cultures during mild metabolic stress.

Mild metabolic stress also led to the significant reduction in percentage of pyramidal cells recruited in ensemble formation, as well as the percentage of recording time that exhibited synchronized activity. However, the overall activity of pyramidal cells showed no significant changes under control conditions and mild metabolic stress. In summary, mild metabolic stress leads to suppression of synchrony but not overall excitability of pyramidal cells.

Our current findings suggest that the energy expenditure for synchronization and provision of a temporal matrix for pyramidal cells activity is quite high.

Intersectional subpopulations of the dorsal raphe nucleus modulate sleep-wake behavior.

Mary Gazea[1,2], Lukas Oesch[1,2], Carolina Gutierrez Herrera[1,2], Antoine R. Adamantidis[1,2]

[1]Centre for Experimental Neurology(ZEN), Department of Neurology, Inselspital University Hospital, Bern, Switzerland.

[2]Department for Biomedical Research, University of Bern, Bern, Switzerland.

The dorsal raphe nucleus (DRN) influences many behaviors including mood, reward and the sleep-wake cycle. The exact role of serotonin, the major neurotransmitter of the DRN, in sleep-wake behavior is still controversial; depletion of central serotonin lead to transient insomnia, whereas juxtacellular recordings of serotonin neurons and pharmacological manipulations of their release highlighted a wake-promoting function of serotonin. Here, we hypothesize that intermingled neuronal subpopulations of the DRN that co-transmit several neurotransmitters might be responsible for the mixed outcomes on sleep-wake regulation.

To investigate the role of distinct DRN neuronal subpopulations in the regulation of sleep-wake behavior, we delivered intersectional viruses carrying channel-rhodopsin 2 (ChR2) to the DRN of Pet1-Flpe/Vglut3-iCre and Pet1-Flpe/Vgat-Cre transgenic mice. Sleep-state-dependent optogenetic stimulation of DRN cells was performed during the resting phase.

DRN neurons clustered into cells expressing only serotonin, Vgat or Vglut3, or both Pet1/Vglut3 (15 %) markers. In vivo, optogenetic activation of ChR2-expressing Pet1-OFF/Vglut3-ON induced a rapid arousal from NREM sleep. However, when stimulation of Pet1-OFF/Vglut3-ON neurons was initiated during REM sleep, the REM sleep episode duration was prolonged and the theta power peak was shifted to a higher frequency. In contrast, optogenetic activation of the complementary cell population, the Pet1-ON/Vglut3-OFF neurons, did not affect NREM or REM sleep episode duration. On the other hand, optogenetic activation Pet1-OFF/Vgat-ON neurons induced an arousal from both NREM and REM sleep.

Our data shows that glutamatergic and GABAergic cells in the DRN suppress NREM sleep and facilitate REM sleep or wakefulness. However, cells that express Pet1 (serotonin) without glutamatergic neurotransmission fail to induce any changes to NREM or REM sleep. Therefore, our data suggests that Vglut3- and Vgat-, rather than Pet1-expressing cells of the DRN are key regulators of sleep-wake behavior.

Laminar-restricted optogenetic manipulation in the mouse motor cortex for decomposition of the local field potentials.

Andreas J. Genewsky[1], Anton Sirota[1]

[1]Ludwig-Maximilians-Universität München, Department Biology II, RG Cognition and Neural Plasticity, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany

Among the various types of electrodes used for in-vivo recordings in the awake behaving animal, electrocorticogram (ECoG)-electrode arrays hold the greatest translational potential. Compared to penetrating electrodes, ECoG arrays inflict less damage to the recorded brain regions, which renders them ideal for the development of brain-machine interfaces (BMI) and neural prostheses for humans. However, one major limitation of cortical surface recording techniques such as ECoG, is the complex anatomical, biophysical and network dynamics which underlie the recorded surface potentials. Lack of a forward model linking laminar-specific dynamics to the ECoG signals have prevented detailed anatomically motivated analysis and interpretation of these signals so far.

In this study we use layer-specific optogenetic manipulations in the awake behaving but head-fixed animal, performing a simple repetitive motor behavioral task. Thereby, we are aiming to decompose specific contribution of distinct laminar sources to a total ECoG and intracranial signals. Applying this approach paves the way to identification of laminae-specific dynamics associated with internally generated motor program and testing our ability to recover this dynamics from the ECoG signals for future BMI applications.

The singing-CPG in crickets shows a modular organisation along the abdominal ganglia

Berthold Hedwig[1], Pedro Jacob[2], Chu-Cheng Lin[1]

[1]University of Cambridge, Zoology, Downing Street, CB2 3EJ Cambridge, United Kingdom

[2]Oxford University

Cricket singing behaviour is an iconic example of a species-specific fixed action pattern. Males sing by rhythmic opening-closing movements of their front wings, generating a sound pulse with every closing movement. During the calling song males of the bispotted cricket Gryllus bimaculatus generate sound pulses at a pulse-rate of about 30 Hz. Three to five pulses are grouped into chirps which are repeated about 3 times per second. The cricket therefore needs to control two rhythms for song pattern generation: a fast 30Hz timer for the pulse period and a slower 3 Hz timer for the chirp pattern.
As the front wings, singing muscles and corresponding motoneurons are located in the mesothoracic segment, it suggested that also the CPG for singing would be located in the meso-thoracic ganglion. Lesion experiments on the connectives supported this assumption (Huber 1962) and it became a textbook statement for quite some time. However, selective cooling experiments on the central nervous system (Pires and Hoy 1992), some further lesion experiments on the central nervous system (Kutsch and Otto 1972, Hennig and Otto 1996) and the results of intracellular experiments (Hennig 1990, Schöneich and Hedwig 2012) pointed towards a contribution of the abdominal ganglia.

Here we used systematic lesions of the abdominal ganglion chain, to analyse the acute effect on cricket singing performance. We compared the male calling song patterns before and within 10 nights after the lesions:

• When the connectives between the metathoracic ganglion complex T3 and the first free abdominal ganglion A3 are cut, we never observed any singing. Males are still able to raise their wings, but the rhythmic wing movements underlying sound production do no longer occur.
• Cutting the connectives between A3 and A4, still allows the generation of sound pulses. However, these pulses are no longer organised in chirps, the chirp pattern is abandoned.
• Lesioning the connectives between A4 and A5 substantially alters the chirp pattern: Chirps may contain up to 20 pulses and even more; subsequently the chirp duration and the chirp period are increased.
• Lesioning the A5-A6 connectives or the connectives between A6 and the terminal ganglion has only minor effects and increased the chirp period.

Lesion experiments in other species (G. assimilis, Teleogryllus commodus and T. oceanicus) gave results similar to the effects observed in G. bimaculatus.

The outcome of these experiments, supported by intracellular recordings, indicate that the abdominal ganglia contribute differently to the generation of the song pattern. Whereas the anterior ganglia (A3, A4) control the pulse pattern, the more posterior ganglia (A4, A5) seem to control the chirp pattern. No evidence supports the previous assumption, that the singing CPG is contained in the thoracic ganglia.

Abnormal entorhinal control of developing prefrontal-hippocampal circuits in a mouse model of mental illness

Marilena Hnida[1,2], Xiaxia Xu[1,2], Ileana Hanganu-Opatz[1]

[1]Developmental Neurophysiology, Institute of Neuroanatomy, University Medical Center Hamburg-Eppendorf
[2]Equal contribution

Oscillatory coupling between the adult prefrontal cortex (PFC) and hippocampus (HP) is critical for executive and mnemonic processing. This coupling emerges at early stages of postnatal development with hippocampal theta bursts directly driving the entrainment of local prefrontal circuits and discontinuous oscillatory activity in the lateral entorhinal cortex (LEC) controlling as a 'gatekeeper' both the PFC and HP. We previously showed that reduced prefrontal-hippocampal coupling during early development is present in mice mimicking the dual genetic (i.e. mutated Disrupted-in-Schizophrenia 1 (DISC1) gene) – environmental (i.e. with maternal immune activation) etiology (dual-hit GE mice) of mental disorders, such as schizophrenia. However, the contribution of LEC to the disease-related disrupted development of prefrontal-hippocampal networks is still unknown. To fill this knowledge gap, we performed recordings of local field potential and multi-unit activity simultaneously from the PFC, HP and LEC of neonatal (postnatal day (P) 8-10) and juvenile (P 20-26) mice *in vivo*. We show that the broadband (4-40 Hz) power of oscillatory activity is significantly decreased in the LEC of neonatal GE mice when compared to controls. Moreover, the coupling between LEC and HP as well as between LEC and PFC diminished in GE mice. The disrupted prefrontal-hippocampal-entorhinal coupling persisted at juvenile age and related to poorer behavioral performance in episodic-like and associative object-recognition memory tasks. Thus, LEC controls the disrupted entrainment of prefrontal-hippocampal network throughout neonatal and juvenile development.

Midbrain dopaminergic neurons' response to electrical stimulation of the LDTg across alternating brain states of urethane anaesthetised rat

Gabriela Izowit[1], Tomasz Blasiak[1], Magdalena Walczak[1]

[1]Institute of Zoology and Biomedical Research of Jagiellonian University, Department of Neurophysiology & Chronobiology, Gronostajowa 9, 30-387, Cracow, Poland

Ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) are the major sources of dopamine in the mammalian brain and constitute the centre of reward and motivation system. Midbrain dopaminergic neurons show patterns of electrical activity covering the continuum between tonic and bursting firing. Tonic, non-bursting electrical activity is involved in maintaining the basal level of dopamine in structures innervated by VTA/SNc, what results in basic level of motivation and motor control. In response to reward, reward-related cue or novelty, dopaminergic neurons can shift from tonic to the bursting pattern of firing, what leads to phasic increase of dopamine released in target structures. Laterodorsal tegmental nucleus (LDTg) and pedunculopontine tegmental nucleus (PPN) are one of the major inputs into VTA/SNc. These brain stem nuclei are mainly described as cholinergic, but they provide glutamatergic and GABAergic inputs as well. LDTg and PPN are known to contribute to maintenance of the tonic, as well as trigger the burst firing of dopaminergic neurons. Inactivation of LDTg attenuates bursting of VTA/SNc dopaminergic neurons, whereas electrical stimulation of LDTg and PPN in urethane anaesthetised rats results in increase of dopamine released in the striatum. Urethane is an anaesthetic widely used in neurophysiological studies. Due to the cyclic, sleep-like alternations of the brain state, observed in urethane anaesthetised animals, this type of preparation is proposed as a sleep model. The aim of the study was to determine differences in VTA/SNc dopaminergic neurons' response to stimulation of the lateral parts of the dorsal tegmentum across alternating brain states of urethane anaesthetised rats. For this purpose extracellular in vivo recordings of dopaminergic neurons' responses to electrical stimulation of LDTg were performed during spontaneously occurring states of cortical activation and slow wave activity (SWA) observed in electrocorticogram. Obtained results show that in case of majority (70%) of VTA/SNc dopaminergic neurons response to LDTg stimulation is biphasic (fast excitation followed by prolonged inhibition) and preserved in both brain states. The excitatory phase of these responses did not differ between brain states (amplitude, duration and latency). However, the inhibitory phase was significantly longer during SWA compared to the activated brain state. Interestingly, in the case of almost one third of dopaminergic neurons (30%), the nature of their response to LDTg stimulation varied depending on the state of the brain. This study shows that both the parameters and the nature of the midbrain dopaminergic neurons' response to LDTg stimulation are not constant and can vary depending on the general state of the brain. This broadens our knowledge about the heterogeneity of electrical activity of dopaminergic neurons with the variability of their responses to incoming stimuli.

Phase dependent afferent influence on a simple rhythmic behaviour

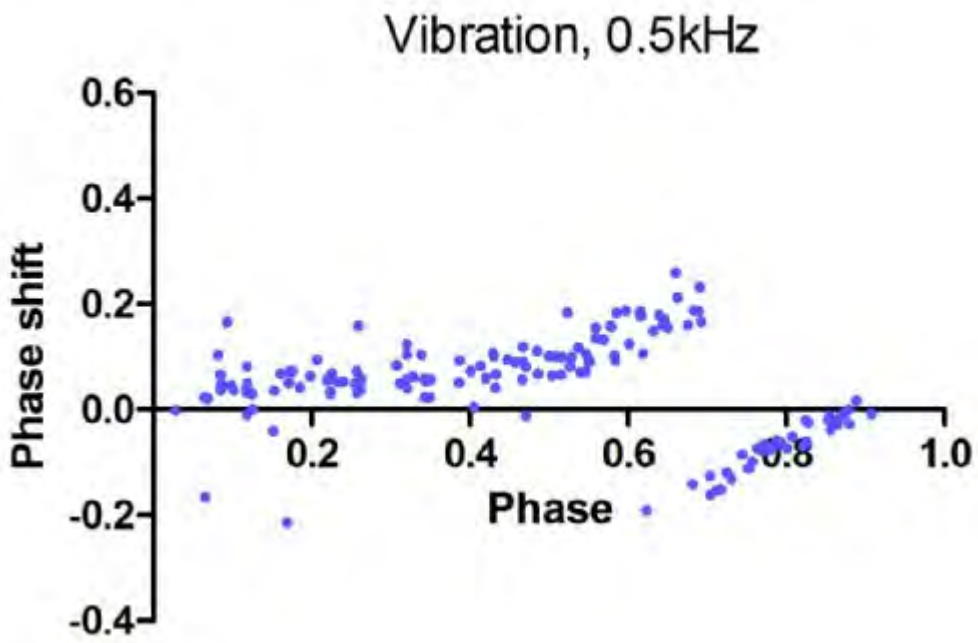
Reinhard Lakes-Harlan[1], Alberto Licona-Backenköhler[1], Ann-Sophie Sturzbecher[1]

[1] Justus-Liebig University Giessen, Integrative Sensory Physiology, Heinrich-Buff Ring 26, 35392 Giessen, Germany

For acoustic communication, the bush cricket *Mecopoda elongata* produces airborne sound chirps in a regular pattern. The chirp timing can be influenced by conspecific sounds, which may result in synchrony (Hartbauer et al. J Comp Physiol A, 2005).
Here we investigate different sound and substrate vibration stimuli in order to evaluate the properties and limits of the song oscillator. The stimuli were applied during different phases of the chirp period and the resulting time-shift of post-stimulus (ps) chirp was evaluated.
Airborne sounds were varied from 10 ms to 1000 ms duration (White Noise, WN), from 50 and 90 dB SPL (WN, 100 ms duration) and for carrier frequency. Substrate borne stimuli had either WN or 500 Hz carrier frequency. Additionally, the response has been tested after the tympanal organs of the experimental animals had been destroyed.
Airborne and substrate borne stimuli in early periods of the chirp phasing typically resulted in a gradual increased ps-chirp period, with a sharp change to shorter ps-chirp periods at a phase of 0.7. Very long stimuli (1000 ms) could also result in the loss of a chirp. For vibrational stimuli, especially in operated animals, the increase in ps-chirp period is weak, but the sharp change at a phase of 0.7 was still present.

The broad range of effective stimuli indicates that the effect is independent from song recognition. The results show a sensory convergence of the hearing and vibrational system for the song oscillator. Furthermore, vibrational influence may partly origin from different legs.
A model of the network for song generation and timing is presented.

Figure: Phase shift of the ps-chirp after presenting a vibration stimulus (100ms duration, 500 Hz) at different phases (3 animals, 50 chirps each).


Vibration, 0.5kHz
Phase shift
0.6
0.4
0.2
0.0
-0.2
-0.4
0.2
0.4
0.6
0.8
1.0
Phase

Co-modulation effect of two antagonistic neuromodulators on rhythmic motor activity

Nils Laudenberg[1], Felix Clotten[1], Carmen Smarandache-Wellmann[1]

[1] University of Cologne, Institute of Zoology, Animal Physiology, Zülpicher Str. 47b, 50674 Cologne, Germany

Locomotor systems have to be well coordinated in order to produce effective behavior. For this reason, they are used to investigate rhythmically active neuronal networks. The swimmeret system of the crayfish, *Pacifastacus leniusculus*, provides the opportunities to study rhythm generation within one central pattern generator (CPG) and intersegmental coordination between neighboring CPG's. The crayfish produces rhythmic swimmeret activity with a network of neurons located at the level of the abdominal nerve chord. The abdominal nerve chord is segmentally organized in a chain of ganglia, with each hemiganglion containing the neurons for the movements of the corresponding swimmeret. In the swimmeret system all neurons are identified which are necessary and sufficient for rhythm generation and coordination. Each swimmeret moves in alternating powerstroke (PS) and returnstroke (RS) movements. This is achieved by the CPG neurons which drive membrane potential oscillations in PS and RS motor neurons (MN). Swimmeret activity occurs in a metachronal wave from posterior to anterior with a fixed phase lag of 25 % between the segmental CPG's, independent of cycle period.
Complex motor activity can be initiated or modified by excitatory and inhibitory 'command neurons'. There are strong indications, that the inhibitory neurons use octopamine (OA) and the excitatory neurons use proctolin (PR) as neuromodulator. We wanted to test if single neuromodulators or a combination of both produces different speeds or strength of activity. Thus, we investigated how neuromodulators shape and influence MN's and CPG neurons activity when used in subsaturated concentrations. For this we performed extracellular recordings of all PS motor-nerves. Additionally, we recorded intracellularly with sharp electrodes from the dendrites of PS MNs and CPG neurons and bath applied different concentrations of OA or PR.
Application of several OA concentrations had different effects on the swimmeret rhythm. High concentrations abolished PS activity and intermediate concentrations evoked an on-off rhythm. Interestingly, subsaturated OA concentrations decreased the cycling period of the rhythm and shortened the phase lag. MN's membrane potential stopped oscillating with high OA concentrations, while in subsaturated OA the duration of the MN's oscillation decreased but the oscillation amplitude did not change. Bath application of several PR concentrations had a dose dependent, activity enhancing effect. The duty cycle and burst duration of each PS was longer during PR application. The duration and amplitude of membrane potential oscillations in MN increased. When both neuromodulators were applied simultaneously we observed an additive effect. The phase lag between each PS burst shortened, but the duty cycle did not change, compared to control conditions. The membrane oscillation amplitude of the MNs increased but the duration of the oscillation shortened. We suggest that OA shortened the phase lag between each PS burst and PR increased the membrane potential oscillation. Regarding the CPG kernel of rhythm generation, we could show that parameters of the CPG neurons are also affected by OA and PR. Moreover, both neuromodulators showed similar effects on the CPG interneurons and the MNs. These findings suggest an important role of OA and PR in shaping the rhythmic motor output so that the crayfish can adapt its behavior to environmental changes.

The distribution of inputs from several brain areas into the teleost Mauthner cell

Kathrin Leupolz[1], Peter Machnik[1], Wolfram Schulze[1], Stefan Schuster[1]

[1]University of Bayreuth, Department of Animal Physiology, Universitätsstrasse 30, 95440 Bayreuth, Germany

A pair of giant reticulospinal neurons forms the center of the fast-start network in the medulla oblongata of most teleost fish. These so-called Mauthner cells receive input from nearly all sensory systems to induce a fast-start response when required. We established preparations that allow stable *in vivo* intracellular recordings from the Mauthner neurons while we delivered sensory stimuli or applied electrical stimuli to sensory nerves and various brain areas. For instance, we investigated the transfer of visual information to the Mauthner neuron. Therefore, we studied latency, amplitude and other aspects of the PSPs (postsynaptic potential) in the Mauthner neuron after either stimulating the eye, the optic nerve or various regions distributed over the optic tectum. We find that each Mauthner cell symmetrically receives visual input from both tectal hemispheres. Interestingly, no region of the optic tectum was distinguished by causing particularly low latency or high amplitude PSPs in the Mauthner neuron. Also, more detailed analyses of the induced PSP waveforms failed to detect any regions that project differently onto the Mauthner cell than the others. Our findings suggest that the Mauthner neuron receives rich visual information from the tectum without much prior filtering. The main part of evidence was obtained in goldfish but the main findings could also be confirmed in an additional species, the archerfish.
Additionally, we examined potential modulating inputs from the cerebellum, the telencephalon and the olfactory nerve, by applying electrical stimuli to various spots on them and recording correlated shifts in membrane potential or changes in sensory-induced PSPs. One of the most interesting findings is a modulating effect of stimuli applied to the telencephalon on the response of the Mauthner cells. We suggest that our techniques will be valuable to dissect the surprisingly rich input structure to the Mauthner neuron.

Electrophysiological characterization of human dopaminergic neurons derived from LUHMES cells

Dominik Loser[1,2,3], Timm Danker[2], Clemens Möller[1], Marcel Leist[4], Udo Kraushaar[3]

[1]Life Sciences Faculty, Albstadt-Sigmaringen University, Anton-Günther-Straße 51, 72488 Sigmaringen, Germany
[2]NMI-TT GmbH, Markwiesenstraße 55, 72770 Reutlingen, Germany
[3]NMI Natural and Medical Sciences Institute at the University of Tuebingen, Markwiesenstraße 55, 72770 Reutlingen, Germany
[4]Doerenkamp-Zbinden Chair for in vitro Toxicology and Biomedicine, University of Konstanz, Universitätsstraße 10, 78464 Konstanz, Germany

The loss of dopaminergic neurons in the substantia nigra plays an important role in the development of the Parkinson's disease. The symptoms of this disease typically occur after around 80% of these neurons degenerated. This cell decay can be caused or promoted by genetic defects or environmental factors including chemical compounds like pesticides. For a proper testing of neurotoxic effects on these neurons, but also for the development of neuroprotective drugs, assays based on animal primary cells lack predictivity since the correlation between animal and human data is weak in some cases. Therefore, models based on human neuronal cells have the potential to overcome the limitations of animal models. One interesting neuronal cell line is the LUHMES (Lund human mesencephalic) line, which consists of immortalized fetal human mesencephalic cells that can be differentiated into fully post-mitotic dopaminergic neurons.
We currently investigate the electrophysiological properties of these neurons using manual and automated patch clamp as well as high-throughput calcium imaging for a functional characterization on both single cell and network level.
We could verify the presence of voltage-gated ion channels, like TTX-sensitive sodium channels and potassium channels, as well as acid-sensing ion channels (ASICs) in these cells using the patch clamp method on a single cell level. Besides these ion channels, the presence of certain neurotransmitter receptors is also an important characteristic of differentiated neurons. Thus, calcium imaging experiments were performed to check for the existence of different receptors in these cells. We found that several key receptor subtypes that are expected to be present in dopaminergic neurons are functionally expressed in our cells, including dopamine, glutamate and acetylcholine receptors. Next, we investigated whether the neurons were capable of forming functional neuronal networks using a high-throughput calcium imaging system. While at rest no network activity was visible, we were able to induce oscillatory network activities by adding different neurotransmitter receptor agonists like Serotonin and Norepinephrine. Furthermore, we were capable of modifying the response by the administration of different known ion channel and neuronal receptor modulators.
The results show that we were able to differentiate the cells derived from LUHMES cells with neuronal electrophysiological characteristics. Addressing these neurons with the calcium imaging system could offer a great opportunity for a high-throughput assessment of the neurotoxic potential of novel drug candidates on a neuronal network in the future.

Recordings in an integrating central neuron provide a quick way for achieving appropriate anaesthetic use in fish

Peter Machnik[1], Elisabeth Schirmer[1], Laura Glück[1], Stefan Schuster[1]

[1]University of Bayreuth, Department of Animal Physiology, Universitätsstraße 30, 95440 Bayreuth, Germany

In many countries, legislation no longer distinguishes between so-called higher and lower vertebrates but requires for all vertebrates appropriate anaesthetisation to moderate stressful interventions. For an anaesthetisation specifically adapted to a planned intervention, it would thereby be essential to select from the range of possible anaesthetic agents that differ in where and how they act. In mammals and birds, anaesthetisation generally meets these requirements. Here, appropriate anaesthetisation does not merely mean the application of an agent to make handling easier or to reduce stress in general, but an anaesthetisation specifically adapted to the intervention to be performed. In ectothermic vertebrates this is regularly not the case. Even with equal legal treatment of all vertebrates, detailed evidence is needed to select the appropriate anaesthesia for a specific intervention. However, the targeted application of anaesthetic agents in ectothermic vertebrates is currently not feasible in most cases due to the serious lack of detailed information how the anaesthetics in use or further possibly potent substances affect the animal's physiology. Comprehensive knowledge on the dose-dependent anaesthetic impact on sensory systems and/or central processing of sensory information in particular would be needed to enable facilities working with fish (both aquaculture and scientific facilities) a similarly efficient anaesthetisation as performed in mammals and birds. Here, we present a straightforward way for obtaining this information rapidly.

We demonstrate that electrophysiological recordings taken in a pair of accessible and individually identifiable command neurons in the brain of fish – the Mauthner neurons – can be used (i) to qualify the impact of a pharmaceutical on various sensory systems, on central processing and motor output, (ii) to narrow down its site of action, and (iii) to quantify the specific dose needed to reach a desired effect. The Mauthner neurons form the centre of an escape response network of fish and some amphibians. Their natural function requires these neurons to integrate information from all sensory systems and to rapidly issue a motor command as required. They can be localised and identified using well-established techniques and are accessible to electrophysiological *in vivo* recording. We were able to employ this system in the approach presented here to determine the effects of four anaesthetics that could be applied more profitably in fish and potentially in some other ectothermic vertebrates (MS-222, benzocaine, Aqui-S, and 2-phenoxyethanol (2-PE)). We show that small numbers of animals ($N \geq 3$) are sufficient for obtaining the needed information and so our method is likely to quickly widen the spectrum of anaesthetics for fish and potentially other 'lower' vertebrates for the required more targeted application in experimentation, treatment and aquaculture.

The findings presented here thereby can be used as a first guide to scientists, veterinarians and aquaculture specialists for a more targeted application of the examined agents. Moreover, the introduced approach could additionally be the basis for quickly exploring further potential agents for a more sufficient future anaesthetic use.

Practical guidelines for anaesthetic use based on our findings in goldfish

Anaesthetic agent	Surgical dose*	Functionality of CNS neurons		Handling in the presence of		Scientific study of	
		affected	vanished	noise	light	hearing	vision
2-PE (ml L^{-1})	0.2	no	no	no	no	yes	< 0.6
MS-222 (mg L^{-1})	60	> 60	n.d.	no	≥ 60	<100	no
Benzocaine (mg L^{-1})	60	> 60	> 100	no	≥ 60	<100	no
Aqui-S (mg L^{-1})	10	no	no	no	no	yes	no

* required dose according to Neiffer and Stamper (2009); n.d. = not determined

Intersegmental CPG coupling in the deafferented walking system of the stick insect

Charalampos Mantziaris[1], Till Bockemühl [1], Ansgar Büschges [1]

[1]Dept. of Animal Physiology, University of Cologne, Biocenter, Zülpicher Str. 47b, 50674 Cologne, Germany

Leg coordination during walking in the stick insect is based on intra- and intersegmental interactions among distributed central pattern generating networks (CPGs) that drive the muscles responsible for movement about each of the main leg joints (Bidaye et al. 2017). Such coupling interactions may be due to sensory signals either generated locally or transmitted from other ganglia through the connectives, which result in intra- and intersegmental CPG coordination (Borgmann et al. 2009). In a previous study, we have shown that CPGs are also weakly coupled in the absence of phasic sensory input (Mantziaris et al. 2017). Contralateral CPGs tended to be coordinated in-phase in the isolated mesothoracic ganglion, whereas in the metathoracic ganglion they were mostly out-of-phase. Moreover, intersegmental influences resulted in increase of coupling strength in both ganglia and in-phase CPG coordination in the metathoracic ganglion. However, intersegmental CPG interactions in the deafferented leg muscle control system have not been analyzed so far, and the underlying neuronal mechanisms still remain elusive.
To address this issue we used the deafferented central nervous system of the stick insect *Carausius morosus*. The activity of CPGs that control the coxa-trochanter joint (CTr) of the animal was assessed by extracellular recordings of the activity of the motor neurons (MNs) that innervate the depressor trochanteris muscle. The muscarinic acetylcholine receptor agonist pilocarpine was applied to induce rhythmic motor activity in leg MN pools (Büschges et al. 1995). Intersegmental CPG interactions were assessed by analyzing synchronization, phase differences, and correlation of ipsilateral depressor activity within pairs of ganglia as well as the whole thoracic nerve cord. To further identify the localization of neuronal pathways mediating coupling between CPGs, we transected the connectives that in vivo mediate the exchange of coordinating information among ganglia.
Our results show that ipsilateral CTr-joint CPGs in the interconnected meso- and metathoracic ganglia are weakly in-phase coupled in the absence of sensory input (mean = 10°, 90% C.I. = [4.2, 16.3], N = 13). Interestingly, unilateral connective transection affected, but did not fully disrupt intra- and intersegmental CPG coordination in the meso- and metathoracic ganglia. In contrast, no consistent intersegmental interactions but rather a tendency for asynchronous activity could be detected among pro- and mesothoracic CPGs (N = 7). Intersegmental CPG coordination was variable throughout the recording of the meso- and metathoracic segments, when all three thoracic ganglia were interconnected (N = 7). Intersegmental input from the prothoracic networks affected the tendency for in-phase coordination observed in the interconnected meso- and metathoracic ganglia. Finally, phase relationships among activity of CTr-joint CPGs in the thoracic ganglia did not resemble those observed in interleg coordination patterns during walking.
In summary, intersegmental coupling between segmental CPGs in the stick insect leg-muscle control system is weak and interactions between the meso- and metathoracic ganglia are the most prominent. Currently, we are looking for populations of intersegmental interneurons in the meso- and metathoracic segments, which may contribute to coordination.

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Intracortical compensatory mechanisms for weakened thalamic input in the mislaminated somatosensory cortex of the reeler mutant.

Anouk JM Meeuwissen[1], Julien Guy[1], Martin Möck[1], Jochen F Staiger[1]

[1]Institute for Neuroanatomy, Barrel Cortical Circuits group, Kreuzbergring 40, 37075 Goettingen, Germany

In the mouse somatosensory cortex, layer IV (LIV) receives most of the thalamocortical synapses and is therefore called the input layer. In the reeler mutant mouse, a loss of expression of the reelin protein causes a mislamination of the cortex, whereby cells normally located in a given layer are spread throughout the cortex instead. The Scnn1acre-tdTomato-Reeler mouse line allows for studying LIV in the mislaminated cortex, as the excitatory LIV-equivalent neurons express tdTomato in a cre-dependent manner. Using this mouse line, tracing studies from our lab have found that even in these conditions of severe mislamination, thalamic axons manage to target their misplaced, LIV-equivalent neurons. In line with these anatomical studies, functional imaging studies from our lab have found that the reeler somatosensory cortex maintains proper sensory activation in response to sensory input. Moreover, electrophysiological recordings showed that the synapses formed by thalamocortical axons on spiny stellate (SpS) neurons, one of the main recipients of thalamic input, are functional. However, the synapses were found to be weaker in reeler as compared to wild-type. This leads to the following question: how does the reeler somatosensory cortex maintain proper functional activation with a weakened thalamic input? A possibility is that compensatory mechanisms arise in the cortical microcircuitry in order to accommodate the weaker thalamic input. Potential mechanisms include an increase in intracortical gain through strengthened excitatory connections between SpS neurons, or a decrease in thalamus-evoked feed-forward inhibition through a weakening of connections between fast spiking (FS), parvalbumin-expressing (PV+) interneurons and SpS neurons. In the present study, we investigate this second hypothesis by measuring the connection strength and probability between FS interneurons and SpS neurons using in vitro paired whole cell voltage clamp recordings. In wild-type animals, in agreement with previous literature, we found reliable connections between cell types. In reeler mice however, we observed a massive reduction in connection probability as well as a marked weakening of unitary inhibitory postsynaptic current responses. When repeating these paired recordings with cesium containing intracellular solution, ensuring blockage of K+ channels and detection of responses more distal from the soma, the connection probability in reeler markedly increased towards levels observed in the wild-type controls. These results suggest that synapses formed by FS interneurons on SpS neurons have a more distal location in reeler compared with wild-type, providing a possible mechanism by which feed-forward inhibition could be weakened in this mutant. Future immunohistochemistry experiments using synaptotagmin-2, a presynaptic protein present in PV+ synapses in the cortex, will be used to investigate this possibility.

Infraslow Oscillations in the Mouse Accessory Olfactory Bulb

Julia Mohrhardt[1,2], Chryssanthi Tsitoura[1,3], Rudolf Degen[1], Minghong Ma[4], Marc Spehr[1]

[1]Department of Chemosensation, Institute of Biology II, RWTH Aachen University, Germany

[2]Department of Psychiatry, Psychotherapy and Psychosomatics, Med. Fac., RWTH Aachen University, Germany

[3]Sainsbury Wellcome Centre for Neural Circuits and Behaviour, UCL, Great Britain

[4]Department of Neuroscience, University of Pennsylvania School of Medicine, United States

The accessory olfactory bulb (AOB) represents the first stage of information processing in the rodent accessory olfactory system. In the AOB, mitral cells receive sensory input from peripheral vomeronasal neurons. This sensory information is (pre-)processed in the AOB and relayed to third- and fourth-order nuclei in the amygdala and hypothalamus.

In both *in vitro* and *in vivo* experiments, we investigate patterns of spontaneous neuronal activity in AOB mitral cells (AMCs). Recently, we demonstrated that a subpopulation of AMCs is intrinsically rhythmogenic and exhibits slow stereotypical oscillatory discharge. Using voltage- and current-clamp whole-cell recordings in acute AOB tissue slices from C57BL/6 mice, we now identify an excitatory circuit within the AOB that entrains oscillatory activity in a second AMC subpopulation. These neurons display periodically increased synaptic input that correlates with their respective rhythmic discharge patterns. Blocking fast glutamatergic synaptic transmission reveals that, in a subgroup of AMCs, entrainment largely depends on an intact glutamatergic network. By contrast, a second subpopulation of entrained AMCs appears insensitive to pharmacological inhibition of glutamatergic input.

Ongoing patch-clamp and optogenetic experiments now aim to identify the exact physiological mechanisms of oscillatory entrainment and synchronization. Together, our long-term goal is to gain a detailed mechanistic understanding of slow synchronous oscillatory discharge in the mouse AOB and thus to dissect the functional role of such rhythmic activity in information processing along the accessory olfactory pathway.

Eslicarbazepine effects on hippocampal sharp wave-ripples in a mouse model of KCNQ2-related encephalopathy

Laura Monni[1], Pawel Fidzinski[1], Matthias Wawra[1], Martin Holtkamp[1]

[1]Charité – Universitätsmedizin Berlin, Experimental Epileptology, Charitéplatz 1, 10117 Berlin, Germany

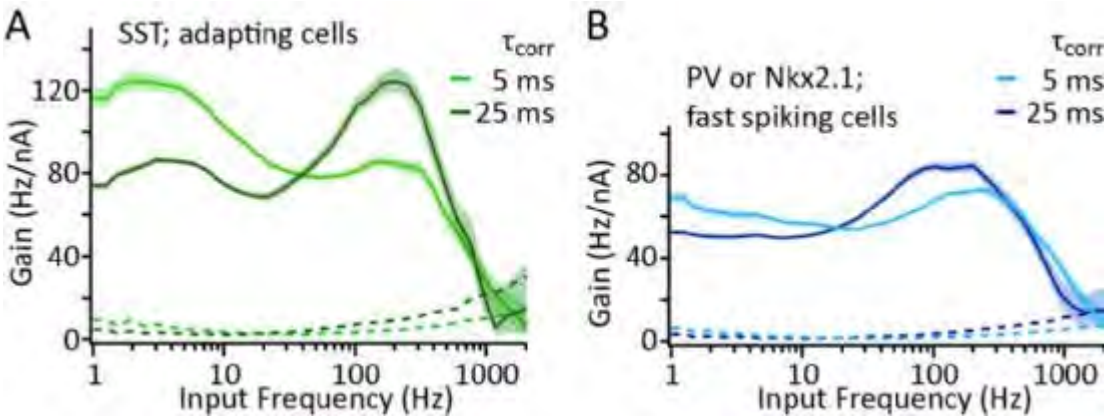
KCNQ2-related encephalopathy is a rare form of childhood epilepsy syndrome caused by a missense mutation of the KCNQ2 gene encoding for a subunit of the KCNQ family of non-inactivating potassium channels. KCNQ2-related encephalopathy is associated with intellectual and cognitive developmental deficits. In the hippocampus, sharp wave-ripple complexes (SPW-R) have been specifically implicated in cognitive functions. SPW-R represent a hippocampal pattern of spontaneous activity with a ripple oscillation component superimposed on slower sharp waves. Here, by employing a mouse model carrying a heterozygous deletion of KCNQ2 gene, we have investigated the effects of the novel antiepileptic drug eslicarbazepine on SPW-R. SPW-R were acquired by local field potential recordings from CA3 and CA1 hippocampal regions in 400 µm-thick mouse brain slices. SPW-R parameters such as incidence, amplitude or ripple power were not different in control and heterozygous KCNQ2 mice. Application of eslicarbazepine at different concentrations (300 and 100 µM) resulted in an increase in amplitude and decrease in incidence of SPW-R. Patch clamp recordings in CA1 pyramidal cells clamped at different potentials revealed that eslicarbazepine altered SPW-R activity role by inhibiting both the excitatory and inhibitory components of SPW-R. We suggest that although KCNQ2 was mutated in the heterozygous mice, its deletion was not sufficient to alter differently SPW-R activity compared to wild-type littermates. Indeed, even if KCNQ2 channels have been shown to have a role in the control of the hippocampal interneuronal excitability, there is still no evidence of a direct implication of these channels in the generation of SPW-R. As consequence, eslicarbazepine showed similar effects in both genotypes. We think that, by blocking largely pyramidal neurons firing through the block of sodium channels by eslicarbazepine, SPW-R initiation became less likely to be triggered. The prolonged inactivation of sodium channels might also reduce the excitation of hippocampal GABAergic interneurons, which contribute to the perisomatic inhibition that has been shown to enforce ripple synchrony.

A cellular basis for cross-frequency coupling?

Andreas Neef[1,2,3,4], Fred Wolf[1,2,3,4], Ricardo Martins Merino[1,2,3,4]

[1]BCCN Göttingen, Biophysics of neural computation, Von-Siebold-Str. 3a, 37075 Göttingen, Germany
[2]Campus Institute for Dynamics of Biological Networks, 37075 Göttingen, Germany
[3]MPI Experimental Medicine, Hermann_rein-Str. 3, 37075 Göttingen, Germany
[4]MPI for Dynamics and Self-Organization, Am Faßberg 17, 37077 Göttingen, Germany

Cortical activity in-vivo is characterized by the occurrence of oscillations at various spatial and temporal scales (Buzsáki and Draguhn 2004). These oscillations arise from synchronized neuronal activity and likely reflect specific aspects of information processing. Gamma oscillations (30-80 Hz), for instance, are thought to dynamically synchronize functionally related ensembles (Singer and Gray 1995, Fries et al. 2001) or allow dynamic routing of information (Fries, 2015), depending on the phase relation between neuronal ensembles (Palmigiano et al., 2017). Phenomenologically, the amplitude of gamma oscillations seems to be modulated in phase with lower frequency oscillations (theta, 4-10 Hz). This so called 'cross-frequency coupling', has been hypothesized to reflect top-down modulation of early signal processing (Canolty et al. 2006, Scheffzuk et al. 2011). Despite the implied relevance of this phenomenon, the biophysical basis of cross-frequency coupling is completely unclear (Aru et al. 2015).
Here, we study cortical neurons under *in-vivo*like conditions, i.e. driven with a continuously fluctuating input, in order to characterize their ability to participate in oscillatory activity. This ability is captured in the frequency-response curve (Higgs and Spain 2009), in other words, in the neurons ability to lock its spikes onto a particular frequency component in the input. In signal-processing terms, we measured the frequency dependent gain of the neurons. The shape of this gain curve show clear differences between neurons of different firing characteristics, e.g. fast-spiking interneurons vs pyramidal neurons. It also depended on the spectral composition of the input. In particular interneurons of the "adapting" type, showed a drastic change in the frequency dependent gain curve, when the input statistic was changed. These predominantly somatostatin positive interneurons, amplify preferentially high frequencies (>50 Hz) if the predominant frequencies in the input are low (correlation time 25 ms). But when the input fluctuates more rapidly (correlation time 5ms), the peak of the gain curves shifts from 200 Hz to just 2 Hz. This demonstrates that adapting interneurons have the ability to selectively amplify high frequency oscillations in their input in particular during episodes, when low frequency components dominate their input. We suggest that this mechanism might underlie cross-frequency coupling in cortical networks.



How versatile behaviors are flexibly supported by local circuits: Dissecting *Drosophila's* wing motor circuit

Nelson Christian Niemeyer[1,2], Jan-Hendrik Schleimer[1,2], Silvan Huerkey[3], Carsten Duch[3], Susanne Schreiber[1,2]

[1] Institute for Theoretical Biology, Humboldt-Universitaet zu Berlin, Philippstr. 13, Haus 4, 10115 Berlin, Germany

[2] Bernstein Center for Computational Neuroscience, Humboldt-Universitaet zu Berlin, Philippstr. 13, Haus 6, 10115 Berlin, Germany

[3] Institute for Biology, Johannes Gutenberg Universitaet Mainz, Colonel-Kleinmann-Weg 2, 55128 Mainz, Germany

Swimming, crawling, flying, brachiating, or running are but some examples of locomotor behaviours based on oscillations (Katz 2016). All of these are controlled by nerve activity in motorcircuits. In some cases, one and the same circuit has to produce several distinct activation patterns to create multiple motor actions. In this way an animal can exercise action selection, by perturbing the circuit to switch between its generated patterns.

In this study, this is exemplified by a circuit of five motorneurons (MN1-5) which is involved in at least three distinct motor behaviours of *Drosophila*: (i) It powers flight, (ii) produces sine song and (iii) produces pulse song. The optimal activity pattern for flight is the so-called "splay-state" (Harcombe and Wyman 1977), i.e., a robust temporal sequence of firing, while the activity driving song has been associated with different activity patterns.

Based on mathematical modeling, we here dissect how the circuit can selectively support both behaviors. To this end, the motorcircuit is described by a gap-junction and inhibition-coupled phase-oscillator network. Drawing insights from coupled phase-oscillator theory (Schleimer and Schreiber 2018), all network states are enumerated given the previously described connectivity as well as three qualitatively different spike generating mechanism that could in principle be produced by these motorneurons (Berger and Crook 2015). Based on the kinetics of indirect flight, optimal activation patterns of the circuit are derived and connections to physiological properties of the involved motorneurons are discussed.

These predictions are corroborated by coupling functions derived from in vivo spike recordings of the wing motor circuit. Methodologically, two algorithms a employed: one based on Fourier decomposition (Kralemann et al. 2008) and a new strategy based on least-squares optimisation. Algorithm performance is evaluated with respect to simulated data and then applied to electrophysiological recordings.

Due to the generic nature of the modelling predictions, the results of this study further our understanding of how single cell properties shape the activity patterns in small networks.

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Reward rate during Upper Alpha Neurofeedback affects learning of Upper Alpha modulation

Mareike Nödler[1], Sjoerd Meijer[1], Mücahit Tasci[1], Lena Geiss[1], Marinus Breteler[1]

[1]Radboud University Nijmegen, Clinical Psychology, PO9104, 65100HE Nijmege

Neurofeedback training (NFT) is supposed to be based on operant conditioning. Building on the observations of prior research, showing that specific feedback (low reward rate) is more effective to learning than sensitive feedback (high reward rate), this study investigated changes in individual upper alpha (IUA) and cognitive functioning after IUA-NFT. Forty- two healthy participants were randomly assigned to either the high (70% of time over threshold (ToT)) or the low (10% ToT) reward rate condition and ran through two base rate measurements (pre/post IUA-NFT) and one single IUA-NFT session consisting of five blocks of three minutes. In preliminary data no significant difference was found comparing the first base rate (BR1) measurement with the second in either of the reward conditions. However, we did observe between-group differences in IUA when comparing session five of the NFT with BR1, i.e. a measure of trainability. Specifically, the high reward rate condition led to an increase in IUA, whereas the low reward rate condition led to a decrease in IUA. No associations were found with cognitive functioning. Contrary to our expectations, a high reward rate seems more effective in learning to enhance IUA than a low reward rate. An extension for future research is the investigation of the effects of other reward rates on IUA modulation.

Searching for neural correlates that control sleep wake-cycles in the circadian clock of the Madeira cockroach

Jenny A. Plath[1], Pablo Rojas[2], Julia Gestrich[1], HongYing Wei[1], Martin E. Garcia[2], Bharath Ananthasubramaniam[3,4], Hanspeter Herzel[4]

[1]Animal Physiology, FB 10, Biology, University of Kassel, Kassel, Germany

[2]Institute of Physics and Center of Interdisciplinary Nanostructure Science and Technology (CINSaT), University of Kassel, Kassel, Germany

[3]Institute of Health, Laboratory of Chronobiology, Charité Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, Berlin, Germany

[4]Institute for Theoretical Biology, Humboldt-Universität zu Berlin, Berlin, Germany

The circadian clock of the Madeira cockroach *Rhyparobia maderae* is the accessory medulla (AME), a small glomerular neuropil located in in the brain´s optic lobes. It is innervated by about 240 neuropeptidergic neurons which are involved in controlling sleep-wake cycles as well as other physiological/behavioral rhythms. One of the crucial neuropeptides for sleep-wake cycle regulation is pigment-dispersing factor (PDF). This neuropeptide is expressed in 12 clock neurons next to the AME (PDFMEs), four of which form a direct connection between the bilaterally symmetric clocks. Since contralaterally projecting circadian clock neurons were shown to control locomotor activity rhythms, these four contralateral PDFMEs were suggested to activate locomotor control areas. Additional work in diurnal *Drosophilae* suggested that an oscillator circuit, the morning oscillator (M-clock) couples to dawn to control activity onset, while an evening oscillator (E-clock) couples to dusk to control sleep onset. The PDF clock cells are also M-clock cells in *Drosophila* that inhibit E-clock cells, hereby delaying their activity. This process depends on PDF. To characterize how activity in the AME of the Madeira cockroach changes daytime-dependently and to identify neuronal M- and E- circuits, we performed long-term loose patch clamp recordings from AMEs *ex vivo*. We found two activity peaks at dusk and dawn reminiscent of M- and E-clock circuits. After application of PDF M-cell activity increased in synchronization and amplitude, while E-cell activity was inhibited delaying the occurrence of the E-peak. Furthermore, we evaluated fast electrical activity changes of clock neurons as well as slow local field potentials in our loose-patch clamp recordings. With new computational analysis methods, we described multiscale events of different duration, frequencies, and amplitudes, as well as their variations over time. We found distinct oscillatory activity in the gamma frequency range, as well as different activity profiles before morning and evening according to the animal's Zeitgeber time. In future experiments we will examine whether morning- and evening peaks in electrical activity of the AME correspond to the activity of ipsilateral PDFME neurons as part of the M-clock and contralateral PDFMEs as part of E-clock. In addition, with a combination of AME-and muscle recordings we will test whether these M- and E-activity peaks control sleep-wake cycles as found in *Drosophila*. [Supported by STE531/18- 2,3 and SPP 2041 STE531/26-1 to MS and by SPP 2041 HE 2168/11-1 to HH]

Changes in hippocampal network oscillations and single cell properties of GAD65 KO mice-a model of reduced GABAergic synthesis

Evangelia Pollali[1,2,3,4], Gürsel Çaliskan[1,4], Thomas Munsch[2,3,4], Volkmar Lessmann[2,3,4], Oliver Stork[1,3,4]

[1] Department of Genetics and Molecular Neurobiology, Institute of Biology, Otto-von-Guericke University, 39120 Magdeburg, Germany

[2] Institute of Physiology, Medical Faculty, Otto-von-Guericke University, 39120 Magdeburg, Germany

[3] ESF graduate school ABINEP

[4] Center for Behavioral Brain Sciences, 39120 Magdeburg, Germany

Mice with targeted disruption of the gene for glutamic acid decarboxylase (GAD65 KO mice) display a postnatal deficit in γ-aminobutyric acid (GABA) synthesis, increased fear and anxiety. In the current study we examine GABA-dependent network activities in the ventral hippocampus (vHP) that may be involved in the emergence of this phenotype.

To this end we performed local field potential recordings from acute horizontal brain slices including the vHP and examined spontaneous sharp-wave ripples (SW-R) and carbachol-induced gamma oscillations from Cornu Ammonis 1 (CA1) and 3 (CA3). We found that the power and the peak frequency of gamma oscillations were significantly increased both in CA3 and CA1 subregions of GAD65 KO mice in comparison to wild-types (WT). In line, spontaneous SW- and Ripple- components of the SW-R activity were altered and SW propagation failure from CA3 to CA1 was observed. Patch clamp recordings, initially from CA1 pyramidal neurons, revealed unaltered intrinsic properties in pyramidal cells but moderate changes in both excitatory and inhibitory post-synaptic currents.

These findings suggest that reduced GABA availability in GAD65 KO mice may trigger long-term alterations in vHP network oscillations, which in turn may underlie the fear- and anxiety- phenotype of GAD65 KO mice. Augmented network oscillations in the vHP might be a risk factor for the development and persistence of fear memories and stress-related disorders.

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Network-specific synchronization of delta oscillations gates sleep regulation in Drosophila

Davide Raccuglia[1], Sheng Huang[2], Anatoli Ender[1], Desiree Laber[1], Agustin Liotta[3], Stephan J Sigrist[2,4], Jörg R P Geiger[1,4], David Owald[1,4]

[1]Institute of Neurophysiology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

[2]Institut for Biology/Genetics, Freie Universität Berlin, Takustrasse 6, 14195 Berlin, Germany

[3]Department of Anesthesiology and Intensive Care and Neuroscience Research Center, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

[4]NeuroCure, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

In mammals brain-wide electrical slow-wave oscillations in the delta band (0.5 – 4 Hz) are characteristic of deep sleep. However, addressing the neuronal origin and the functional significance of compound oscillations remains difficult. Here we discover delta oscillations at the level of a sleep-regulating network in Drosophila that are modulated by sleep need. Using multi-unit optical voltage recordings, we show that single units are synchronized by optogenetically activating input pathways. Synchronization requires NMDA receptor activity and leads to increased compound power, which corresponds to increased sleep drive. Interfering with NMDAR activity abolishes compound delta oscillations and leads to disrupted sleep and facilitated light-induced wakening. We therefore propose that multi-unit synchronization represents an evolutionary conserved optimal strategy for constructing sleep-regulating sensory gates.

Rapid depth perception in hunting archerfish

Caroline Petra Reinel[1], Stefan Schuster[1]

[1]University of Bayreuth, Department of Animal Physiology , Universitätsstrasse 30, 95440 Bayreuth, Germany

Archerfish are well known for their unique hunting technique: They dislodge aerial prey with precisely aimed jets of water fired from their mouths. To then secure their prey against competitors they use their predictive C-starts. These rapid C-start manoeuvers turn the fish to the later point of impact and set the speed so that the fish would arrive just in time. The starts are adjusted on the basis of information on speed, direction, timing and horizontal start position of prey movement that is sampled during less than 100 ms after prey starts falling. Presently it is unclear, if one crucial parameter, the initial height of prey can also be determined during this brief sampling time. Shooters and probably also observing bystanders already know target height - to hit and to shape their jets and could in principle feed this information into their C-start circuitry. We challenged archerfish by launching initially invisible prey objects either from an expected height level and from a location to which the fish were looking or - in interspersed tests - from more lateral positions and from a lower or a larger initial height. Height levels were selected such that the start direction and the linear speed chosen by the starting fish would readily tell us whether the fish had made their C-start decision based on estimates gained prior to prey movement or based on an estimate of initial height gained in the brief interval between onset of prey motion and the C-start. Our findings demonstrate that the fish quickly estimate initial height during the initial falling phase of prey and do not use prior information.

We then examined potential cues that might allow archerfish to so quickly extract distance information. Based on measurements on the fish's binocular aerial vision we analyzed C-starts that either could or could not have used binocular information. This showed that equally precise start-decisions were possible even in absence of binocular cues. We next examined if the fish were using assumptions about the absolute size of their prey or about the distance of their prey from reference structures in the background. However, experiments with unexpected changes from the standard conditions failed to cause any errors. Furthermore, we tested the hypothesis that the fish might infer depth from accommodation or from cues related to image blurring. To test this we created 'fake-flies' whose image could never be focused and whose combined size and degree of blurring should have mislead the fish. However, also the distance of the 'fake-flies' was determined correctly. Our findings strongly suggest that the C-starts are driven on the basis of a remarkably accurate and rapid evaluation of the initial apparent looming of falling prey.

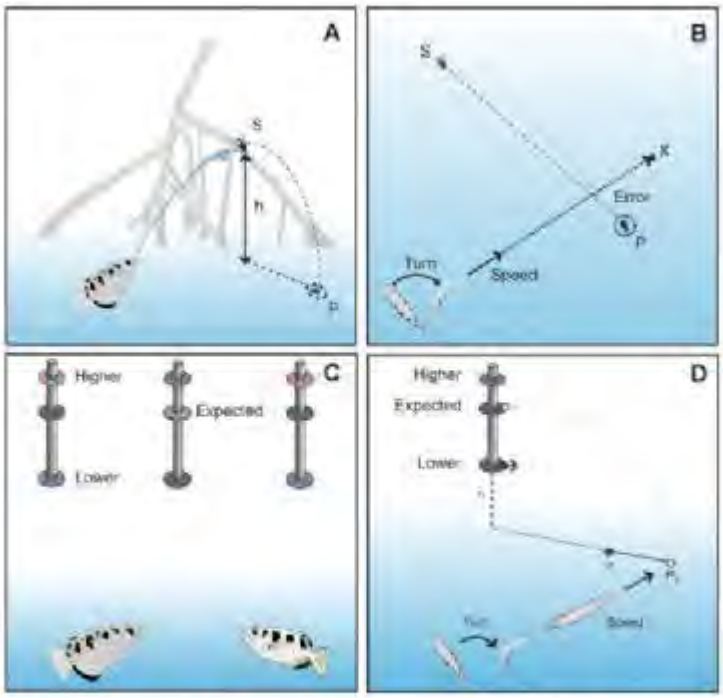

Figure: Do archerfish use prior information to determine the initial height from which prey is falling?
Information on target height h is needed to aim the shots (A) but also to select a matching C-start that quickly turns the fish to the future impact point (P) of falling prey and lends the fish a linear speed matched to impact timing (B). A brief glimpse of less than 100 ms on the prey's initial falling phase is sufficient to select the appropriate C-start. It was long unclear whether the fish would also determine initial height of prey, an essential parameter that determines the location and timing of impact, during the short sampling time of less than 100 ms. (C) To experimentally remove any prior information on height we triggered the fish to always expect starts from a median height level. But in some tests flies were launched from a higher or lower level. These levels were chosen so that analyzing the C-starts would tell us which height - actual or expected - the fish will use to select their C-start. (D) Example to illustrate the idea. Here prey was launched from a lower level than expected. Should the fish drive its C-start based on the expected level, then detectable errors in turn and speed of the C-start would occur.

Multiscale analysis of neuronal activity of the circadian clock of the Madeira cockroach

Pedro Pablo Rojas[1], Julia Gestrich[2], Jenny A. Plath[2], HongYing Wei[2], Martin E. Garcia[1], Bharath Anasthasubramaniam[3], Monika Stengl[2], Hanspeter Herzel[3]

[1]University of Kassel, Theoretical Physics, Heinrich-Plett-Str. 40, 34132 Kassel, Germany
[2]University of Kassel, Animal Physiology, Heinrich-Plett-Str. 40, 34132 Kassel, Germany
[3]Charité-Universitätsmedizin and Humboldt Universität, Institute for Theoretical Biology, Berlin, D-10115, Germany

The accessory medulla (AME), a small neuropil at the ventromedial medulla in the brain's optic lobes, is the circadian clock that controls sleep wake cycles in the Madeira cockroach. Neurons in the AME show rhythms ranging from milliseconds (action potential firing), to ~24h (circadian rhythm), which are endogenous, but also subjected to entrainment and coupling through not fully understood pathways. Neuropeptides such as pigment-dispersing factor (PDF), are suggested to play a key role in coupling neurons in forming the sub-networks that constitute the morning and evening oscillators, which are responsible for anticipation of dusk and dawn, and for synchronization of activity and rest cycles to different photoperiods. Therefore, in order to find evidence of these oscillators and characteristics of the connectivity of the circadian clock, long-term loose patch clamp recordings of the electrical activity of the AME were analyzed. When performed *in-vivo* over days, these recordings contain information that spans 8 orders of magnitude in time. Along with action potential firing at the scale of few milliseconds, it is also possible to observe oscillations and pulsed events in the local field potential from the gamma range up to the order of seconds. Furthermore, consecutive events display even slower and apparently non-random changes in their features, thereby filling the gap up to the circadian timescale. They sometimes resemble some typical dynamic characteristics, such us bifurcations in the underlying dynamical system. A method for analyzing these multiscale oscillations and events is presented here. Through the use of wavelet transform, we are able to detect and extract features from different events. These events are then clustered, in order to separate them into units. This leads to reduced representation, in which each event is characterized by its timing, features and label, which then can be analyzed to find specific patterns of multiscale events. This analysis is also combined with a spectral analysis of the original trace. Some specific examples are presented, in which sequences of events following a pattern of interest are automatically detected, enabling an analysis of the functional connectivity. Finally, the results are connected to the previously published hypotheses of ensembles of neuropeptidergic neurons that control locomotor activity.

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Learning Central Pattern Generator models for the generation of rhythmic activity

Alessandro Salatiello[1], Martin A. Giese[1]

[1]Section on Computational Sensomotorics, Department of Cognitive Neurology, CIN + HIH, University Clinic Tübingen, Germany

A typical objective in large-scale electrophysiology is being able to reconstruct the underlying neural architecture from sparse and noisy measurements. The problem is ill-posed since there are infinite parameter settings of neural models and network architectures that can generate the measured activation patterns. We present a method for the automatic identification of the simplest neural architecture that is able to reproduce a target activation pattern. According to the parsimony principle, such network is the most likely to be implemented in the brain.
As first step, we decided to restrict our analysis on rhythmic activation patterns (such as those observed in the spinal cord and in the lower limbs during locomotion [1]) and on oscillatory neural models. Among the many oscillatory models, we choose the simple two-state Matsuoka oscillator [2]. Such firing neurons are able to generate oscillations through a mechanism of adaptation and mutual inhibition and have already been used to control the locomotion of biped robots, but never to reconstruct electrophysiological signals.
Given a set of target activation patterns y*, our algorithm looks for the simplest (i.e. the smallest) recurrent neural network composed of Matsuoka neurons that is able to reproduce y*. In particular, the algorithm starts off from the smallest architecture (i.e. N=2), and keeps on “adding” neurons until a given stopping condition is met (e.g. reconstruction error lower than a given threshold). In order to fit each proposal network, which constitutes a non-linear dynamical system where both the initial states and the parameters are unknown, we developed an efficient algorithm based on the Unscented Kalman Smoother (UKS) [3], where the state vector was augmented with one additional state for each parameter to be estimated. Since we found that the UKS was highly sensitive to the initial estimates of the (extended) states, we integrated it in a genetic algorithm [4] framework, thus making the optimization algorithm global. Lastly, once the best chromosome is selected, to fine tune the parameters and impose L2 regularization, we perform least squares minimization. This final step further decreases the reconstruction error.
The algorithm was tested on the ten Matsuoka networks described in [2]. For each network, we used as target activation pattern the outputs of only two neurons, corrupted with Gaussian noise. Using the algorithm here described, we were always able to retrieve the correct architecture in each of the ten cases. Future work will involve the use of real electrophysiological data as target signals, and will study the biological plausibility of the identified networks.

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Connectomics of the rat brainstem

Oliver Schmitt[1], Peter Eipert[1], Sebastian Schwanke[1], Julia Beier[1]

[1]Uni Rostock, Anatomy, Gertrudenstr. 9, 18057 Rostock, Germany

A comprehensive mapping of all known connections of the brainstem of the laboratory rat has been build by a systematic evaluation of 1291 tract tracing publications (1972-2018) describing intrinsic connections and 2274 publications which provide observations of extrinsic connectivity. All publications which were selected for extracting manually connectivity data describe neuronal connections in young or adult control rats without lesions and genetic modifications.
The 222 ipsilateral regions of the rat brainstem are linked by 1814 known neuronal connections. 297 of these connections are reciprocal. In average 16,3 connections per region occur in the brainstem connectome. It has a small-world architecture and has been approached best by a degree preserving rewiring model with constant number of reciprocities. When considering the bilateral brain stem connectome 448 regions are interconnected by 5352 connections. 1684 of these links are contralateral and 826 are reciprocal. With regard to network structure, most important regions are the rostral ventral respiratory group, raphe obscurus nucleus, Kölliker fuse nucleus, caudal part of the spinal trigeminal nucleus and locus coeruleus. Interestingly the circular 3 node motiv is slightly more abundant in the empirical connectome than in degree preserving rewiring simulations. This specific local network feature has not been observed in thalamic, cortical, subcortical or spinal connectomes. A further finding regards extrinsic connectivity which is strongest for the locus coeruleus.
In conclusion, we have generated for the first time a brainstem connectome of the laboratory rat and characterized its global structure and regional network features. Now, all known intrinsic and extrinsic brainstem connections can be queried through our online database: http://neuroviisas.med.uni-rostock.de/connectome/index.php.

Neuromodulation of circuit output variability and component variability

Anna C. Schneider[1], Dirk Bucher[1], Farzan Nadim[1]

[1]New Jersey Institute of Technology & Rutgers University Newark, Federated Department of Biological Sciences, Institute for Brain and Neuroscience Research (IBNR), NJIT; 100 Summit Street, NJ 07102 Newark, USA

Behavioral constraints require neural circuits to produce reliable outputs with consistent characteristics. Yet, the underlying neuronal ionic currents and their corresponding channel gene mRNA levels can vary substantially in the same neuron type across animals. This disparity raises the question of how variable components can lead to consistent circuit outputs.

We address this question using the pyloric circuit of the crab stomatogastric nervous system (STNS). Across animals, the pyloric circuit produces a stable triphasic rhythm with remarkably consistent activity phases, but large variability in ionic currents of identified neurons. As in all neural circuits, pyloric activity is influenced by a variety of neuromodulators. When descending inputs are blocked to remove neuromodulator inputs (decentralization), the pyloric rhythm becomes unreliable or stops altogether. Application of any of several neuromodulators activates a stable pyloric pattern specific to that modulator. It is therefore reasonable to assume that neuromodulation is necessary for producing the observed consistent output patterns by reducing component variability.

To examine this hypothesis, we compared the consistency of cycle frequency (coefficient of variation, CV), and activity phases (circular variance) in the decentralized STNS (control) and in the presence of the excitatory neuropeptide proctolin (1 µM). Although proctolin significantly shifted activity phases of some pyloric neurons, variability of the measured parameters was generally reduced. To examine if this reduction resulted from a decrease of variability of the components or cellular properties, we also compared the CVs of ionic current levels, membrane potential resonance, and synaptic currents in control and proctolin. CVs of all factors remained comparable and were not reduced. These results suggested that a neuromodulator, which rescues rhythmic activity, does not necessarily reduce variability of the components.

However, all neural systems are subject to simultaneous actions of multiple neuromodulators, many of which have overlapping and partially convergent actions. For instance, a number of STNS peptide and muscarinic neuromodulators converge to activate a single persistent inward current, IMI, albeit at different levels and by targeting different subsets of pyloric neurons. If the combined actions of multiple neuromodulators are primarily additive, output variability should be reduced by co-modulation through averaging. It is therefore possible that consistent and stable network output is a result of combined action of several neuromodulators on shared target components. We will present data in support of this hypothesis.

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An analysis of cultured hippocampal neuron activity in relation to the circadian rhythm

Sinem Meleknur Sertel[1], Silvio O. Rizzoli[1]

[1]University Medical Center of Göttingen, Neuro and Sensory Physiology, Humboldtallee 23, 37073 Göttingen, Germany

The circadian rhythm is an internal time-keeping mechanism. The consensus view is that every cell integrates a circadian clock input with internal information to generate its own cellular function, which is adapted to the molecular clock rhythm. It has been repeatedly shown that Suprachiasmatic Nucleus and some other cell types can maintain their time dependent behavior in a Petri dish. However, not much is known about hippocampal neurons. We would like to investigate the time dependent firing behavior in disassociated hippocampal cultures in relation to their internal clock, since the electrical activity can have an influence on and be influenced by many pathways. As a starting point, we performed long term calcium imaging on disassociated hippocampal rat neurons at DIV 21 and appreciated the diversity in a network. Our aim is to understand how different spiking behaviors in a network are and how it is connected to the internal clock by interfering with the molecular time keeping mechanism.

Abnormal hippocampal innervation of developing prefrontal cortex in a genetic-environmental mouse model of mental illness

Lingzhen Song[1], Xiaxia Xu[1], Peggy Putthoff[1], Ileana L. Hanganu-Opatz[1]

[1]Developmental Neurophysiology, Institute of Neuroanatomy, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Glutamatergic projections from hippocampus (HP) control the function of prefrontal cortex (PFC) in cognitive processing. Correspondingly, abnormal long-range coupling within prefrontal-hippocampal networks has been identified as a fundamental mechanism underlying cognitive deficits in several mental disorders. Our previous data from mice mimicking the combined genetic-environmental etiology of disorders showed that the functional coupling between these two brain areas is already disturbed during the early development. We hypothesized that the early dysfunction results not only from abnormal activity patterns in PFC and HP, but also from disruption of axonal projections connecting these two areas. Here we tested this hypothesis and explored the anatomy of hippocampal projections to PFC in neonatal dual-hit genetic (abnormal gene Disc1)–environmental (maternal immune activation) (GE) mice. Anterograde tracing of CA1 projections with biotinylated dextran amine (BDA) revealed that at the beginning of second postnatal week they innervate the PFC in both controls and dual-hit GE mice. These projections mainly targeted prefrontal layer 5/6 and synapse both interneurons and pyramidal neurons. However, in line with the reduced activity and functional coupling within prefrontal-hippocampal networks, their density significantly decreased in dual-hit GE mice. Light activation of CA1 axonal terminals in PFC led to network entrainment and firing in investigated mice, yet the patterns of modulation varied between controls and dual-hit GE mice. These results suggest that the decreased glutamatergic projections from hippocampus to prefrontal cortex might act as substrate of developmental hypofrontality in dual-hit GE mice.

Gradients in the cerebellar cortex enable Fourier-like transformation and improve storing capacity

Isabelle Straub[1], Laurens Witter[1,4], Miriam Hoidis[1], Abdelmoneim Eshra[1], Niklas Byczkowicz[1], Sebastian Maaß[1], Igor Delvendahl[1,5], Kevin Drogans[3], Elise Savier[3,6], Ingo Bechmann[2], Jens Eilers[1], Martin Krüger[2], Philippe Isope[3], Stefan Hallermann[1]

[1]Universität Leipzig, Carl-Ludwig Institut für Physiologie, Liebigstraße. 27, 04103 Leipzig, Germany

[2]Institute of Anatomy, Medical Faculty, University of Leipzig, Leipzig, Germany

[3]3Institut des Neurosciences Cellulaires et Intégratives, CNRS, Université de Strasbourg, 5, rue Blaise Pascal, 67084 Strasbourg, France

[4]Department of Integrative Neurophysiology, Center for Neurogenomics and Cognitive Research (CNCR), VU University, De Boelelaan 1085, 1081 HV Amsterdam, The Netherlands

[5]Institute of Molecular Life Sciences University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland

[6]Department of Biology, University of Virginia, Charlottesville, VA, USA

Accurate information processing in our brain relies on precise timing of action potentials in feed-forward neuronal networks. Here, we investigate the mechanisms that support efficient feed-forward information processing in the cerebellar cortex and identify a gradient in the biophysical properties of granule cells (GC), which allows a partial Fourier-like transformation of the mossy fiber (MF) input. GCs closer to the white matter are tuned for higher frequencies, have faster axonal conduction velocity, and preferentially project to the base of the Purkinje cell (PC) dendritic tree to elicit faster postsynaptic potentials. Computational modeling of the cerebellar network demonstrated that these gradients in the biophysical properties improve spike-timing precision of PCs and dramatically reduce the number of GCs required to obtain a specific temporal precision. Our data reveal how Fourier-like transformation and specialized downstream signaling pathways improve temporal precision whilst reducing the number of required neurons in neuronal networks.

Circadian clock connections to specific layers of lamina and medulla in the cockroach *Rhyparobia maderae*

Thordis Arnold[1], Sebastian Korek[1], Azar Massah[1], David Eschstruth[1], Monika Stengl[1]

[1]University of Kassel, Institute of Biology, Department of Animal Physiology, Heinrich-Plett-Str. 40, 34132 Kassel, Germany

The circadian clock of the Madeira cockroach *Rhyparobia maderae* is the accessory medulla (AME) in the brain's optic lobes. It controls sleep-wake cycles and is innervated by many neuropeptidergic neurons such as the pigment-dispersing factor-immunoreactive (PDF-ir) neurons. The 12 PDF neurons next to the AME take part in different circadian clock circuits. Contralaterally projecting PDF-neurons synchronize the bilaterally symmetric clocks and also control locomotor activity rhythms. Ipsilaterally remaining PDF neurons arborize in optic lobe neuropils, possibly modulating photic processing and/or gating photic entrainment of the clock. While previous studies revealed that solely the compound eyes' photoreceptors synchronize the clock with light dark cycles, photic entrainment pathways are not well characterized yet in the cockroach. Thus, we focused on the analysis of connections between compound eye photoreceptors and the circadian clock. With immunocytochemistry and histochemistry on the light microscopic level we examined possible connections between PDF clock cells and photoreceptor terminals in distinct layers of lamina and medulla. Acetylcholinesterase histochemistry distinguished ten main layers of the medulla and three main layers of the lamina. The layers were defined more precisely with antisera against the neurotransmitters histamine, GABA, and serotonin, and against the neuropeptides PDF, corazonin, orcokinin, myoinhibitory peptides, FMRFamides, and allatotropin. We found that light information may be transferred from short photoreceptor terminals in the lamina to the clock via the PDF-, FMRFamide-, 5HT-, and GABA-ir anterior fiber fan that connects the proximal lamina via branches over the face of the medulla (medulla layer 1 = ME1) to the AME. Long photoreceptor termination sites in ME2 overlap with arborizations of neuropeptidergic neurons that connect ME2, ME4 and the AME, such as the single corazonin-ir neuron next to the AME. Most neuropeptidergic neurons examined, as well as the GABA-ir medial layer fiber tract connected ME4 to the AME, apparently forming neuropeptidergic clock inputs as well as outputs. We hypothesize that ME4 integrates ipsi- and contralateral circadian clock inputs and outputs to control circadian rhythms in behavior and physiology. Accordingly, neurobiotin backfills from the contralateral optic stalk combined with multiple-label immunocytochemistry identified contralaterally projecting neuropeptidergic neurons branching in ME4, such as four PDF neurons and many ventromedial neurons. They may serve as potential coupling neurons for the synchronization of both clocks but may also relay photic information form the contralateral compound eye. Future work will examine our hypothesis that ME4 relays photic and non-photic clock inputs and clock outputs via neuropeptide-dependently labeled lines. [Supported by DFG grants STE531/18-2, 18-3 and STE 531/25-1 to MS]

Nucleus incertus is a pontine theta oscillator – electrophysiological *in vivo* studies on urethane anaesthetised rat

Aleksandra Trenk[1], Magdalena Walczak[1], Tomasz Blasiak[1]

[1]Institute of Zoology and Biomedical Research, Jagiellonian University in Krakow, Department of Neurophysiology and Chronobiology, Gronostajowa 9, 30-387 Krakow, Poland

Theta rhythm is a pronounced brain oscillation characterized by the frequency of 3-12 Hz, observable at the level of the local field potential, the pattern of action potential firing and the membrane potential of neurons. The hippocampal network activity underlying the theta oscillations plays an important role in many of the brain-controlled functions, such as navigation in space, memory formation or the generation of different behavioural states. It has been shown that one of the key elements involved in the induction of hippocampal theta rhythmicity, is the nucleus incertus (NI). NI is a bilateral structure formed of GABAergic neurons widely projecting to the forebrain regions. It is located adjacent to the midline of brainstem, right below the fourth ventricle. Theta oscillations in the local field potential of NI were recently described. Nevertheless, the electrophysiological characteristics of the NI neurons and its involvement in the mechanisms of theta rhythm generation are unclear. Therefore, the aim of our research was to characterize NI neurons on the base of their electrophysiological properties in reference to the hippocampal theta oscillations.

Electrophysiological in vivo experiments were performed on 12 Sprague-Dawley rats under deep urethane anaesthesia. This preparation is characterized by spontaneous, cyclical alternations of brain states: activation and slow wave activity (SWA), characterized by the dominance of the hippocampal theta and delta waves respectively. Firing of NI neurons was recorded extracellularly using an array of 32 microelectrodes connected to a multichannel recording system. At the same time, theta rhythm and slow wave activity from the stratum lacunosum-moleculare layer (SLM) of the hippocampal CA1 field were recorded.

We have shown that the rate of firing of the nucleus incertus neurons is brain state dependent. Most recorded NI neurons (90%; 131/145) showed higher firing during activation than during SWA, and reverse correlation was rarely observed (10%; 14/145). Based on the preferences (or lack thereof) to fire in a specific phase of the hippocampal theta rhythm, we identified two groups of NI neurons. The first one are theta phase-locked neurons (46%; 66/145) showing strong preference to fire action potentials at the rising phase of the SLM theta. Interestingly, within this group we have also observed neurons (68%; 45/66) that display rhythmic, theta bursting pattern of firing. The second group of NI neurons fired action potentials independent of the phase of ongoing hippocampal theta oscillations (54%; 79/145).

In conclusion, we have found that NI neurons' electrical activity patterns are more complex than has been previously described. Almost a third of all recorded NI neurons exhibit theta-bursting pattern of firing, suggesting that the nucleus incertus not only modulates theta oscillations, but is itself an oscillator. A fine electrophysiological characterisation of NI neurons can help us better understand the mechanisms underlying the generation and synchronization of theta oscillations.

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Impact of network architecture on stimulus representations in vitro

Szabina Tudja[1,2,3], Samora Okujeni[1,2], Ulrich Egert[1,2]

[1]University of Freiburg, Biomicrotechnology, Department of Microsystems Engineering, 79110 Freiburg, Germany
[2]Bernstein Center Freiburg, University Freiburg, 79104 Freiburg, Germany
[3]Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany

Neuronal connectivity determines activity propagation and perpetuation and thus affects the capability of networks to represent and retain stimulus-related information. In this context, neural encoding requires rapid separability of activity patterns, robustness to variability (e.g. from ongoing background activity), and flexibility for adaptation. The recruitment order of neurons in elicited activity has been demonstrated to comprise these properties both in vivo and in vitro. Yet, how structural properties of self-organizing neuronal networks enable and constrain the emergence of this type of representation remains unclear.

In the current study, we applied electrical stimulation to cortical cell cultures grown on microelectrode arrays (MEA) to model sensory input in the brain, and study representation in a biological neuronal network. We thereby extend and elaborate on previous findings demonstrating encoding capabilities of recruitment order in these networks (Shahaf et al., 2008). To explore the influence of network structure, some cultures were pharmacologically modified towards more homogeneous architectures, or increased clustering through manipulation of PKC activity (Okujeni et al., 2017).

Network responses were represented by the rank order of readout electrodes with respect to the timing of the first spike at each site. We tested the capability of these propagation patterns to represent different stimulation sites by means of machine learning classification (Support Vector Machine). In line with previous findings, recruitment order was found effective for encoding stimulation site and the accuracy of stimulation site identification depended on the number of readout electrodes. Here, we further show that response separability crucially depends on the distance between two stimulation sites, that largely determined the similarity between propagation patterns. Network architecture vastly impacted classification performance, which showed highest for networks with homogeneous structure.

Our observation provide insight into the complex interactions between modularity, richness of activity patterns, and modulation of neuronal responsiveness by spontaneous background activity. Together these findings attribute an important role for network structure in shaping robust activity patterns available for stimulus representation.

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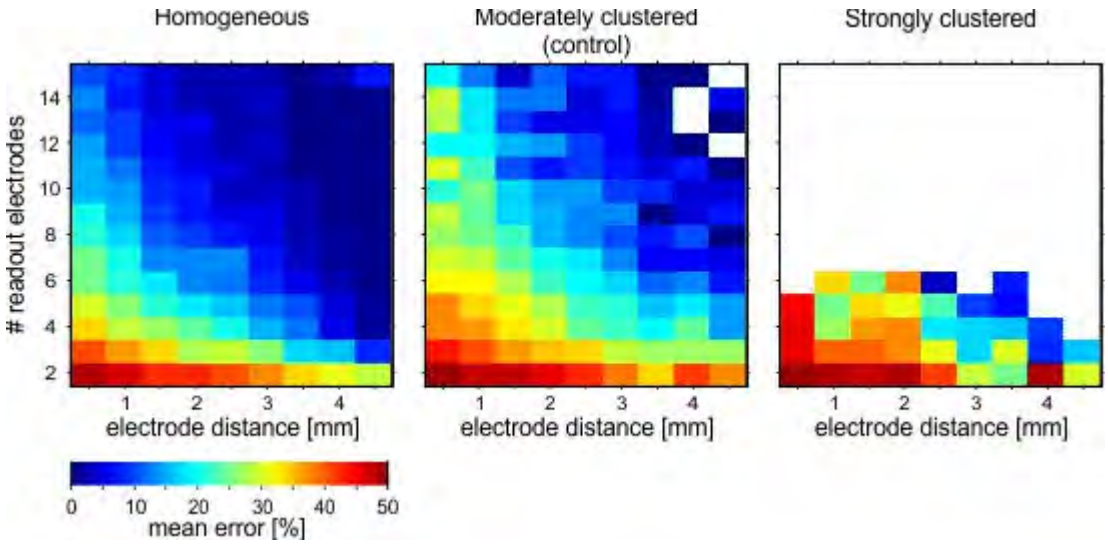
Homogeneous
Moderately clustered
(control)
Strongly clustered
readout electrodes
14
12
10
8
6
4
2
1
2
3
4
electrode distance [mm]
electrode distance [mm]
electrode distance [mm]
0
10
20
30
40
50
mean error [%]

Complex bursts of action potentials in dopaminergic neurons in response to cholinergic agonists administration - – in vivo electrophysiological and pharmacological studies on NR1DATCreERT2 mice

Magdalena Walczak[1], Lukasz Szumiec[2], Jan Rodriguez Parkitna[2], Tomasz Blasiak[1]

[1]Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Cracow, Poland

[2]Department of Molecular Neuropharmacology, Institute of Pharmacology of the Polish Academy of Sciences, Cracow, Poland

Dopamine plays a key role in control of behaviour and motor functions. Amount of released neurotransmitter in the synapse depends on firing pattern of dopaminergic neurons, which is continuum between regular and bursting mode of activity. The latter one results in phasic increase of dopamine release whereas basal level of neurotransmitter is maintained by non-bursting (tonic or irregular) firing of dopaminergic neurons. While functional NMDA receptors are considered to be crucial to evoke dopaminergic neurons' bursts of action potentials, whether other neurotransmitters can also evoke this type of activity remains an opened question. Therefore, the aim of our research was to determine the effect of stimulation of cholinergic receptors on the activity of dopamine neurons lacking functional NMDA receptor.

We have used genetically modified strain of mice (NR1DATCreERT2), which allowed us to induce deletion of NR1 subunit of NMDA receptor selectively on dopaminergic neurons of adult animals. Experiments were performed on urethane anaesthetised animals. We have used multi-barrel, glass micropipettes (five barrels), allowing us to combine single unit, extracellular recordings of midbrain dopaminergic neurons' activity and iontophoretic, local application of drugs (nonspecific agonist of cholinergic receptors – carbachol; muscarinic and nicotinic receptor antagonists – scopolamine and mecamylamine respectively; NMDA and muscarinic receptor agonist-oxotremorine).

Loss of NMDA receptors on dopaminergic neurons decreased their basal firing rate, attenuated bursting and abolished responsivity to NMDA comparing to wild-type animals. After application of non-selective cholinergic agonist carbachol, vast majority of dopaminergic neurons increased their firing rate. Interestingly, some of recorded cells, both in control and NR1DATCreERT2 mice developed slow, oscillatory changes in firing rate, which transformed into robust, complex bursts of action potentials. Neurons tested with oxotremorine application responded with increase of firing rate and similarly to carbachol iontophoresis – some of recorded neurons developed complex bursts.

These results show that agonists of cholinergic receptors can modulate rate as well as pattern of firing of the midbrain dopaminergic neurons. Furthermore, our observations suggest that activation of cholinergic receptors alone, i.e. without the involvement of NMDA receptors, can switch subpopulation of dopaminergic neurons into bursting mode of firing. Our studies suggest that muscarinic receptors can be involved in this phenomenon, since administration of muscarinic receptor agonist was able to evoke complex bursts in dopaminergic neurons.

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E/I ratio is maintained constant in neocortical cultured networks despite variation of the GABAergic neurons proportion

Wenxi Xing[1], Ana D. de Lima[1], Thomas Voigt[1]

[1]Otto-von-Guericke-Universität Magdeburg, Medizinische Fakultät, Leipziger Str. 44, 39120 Magdeburg, Germany

In the mammalian cerebral cortex different cortical areas show a surprisingly constant ratio in the number of excitatory projection neurons (non-GABA) and inhibitory interneurons (GABA). In this study we use cell culture techniques to generate neuronal networks with defined non-GABA/GABA cell ratios to address the question if “atypical” non-GABA/GABA neuron ratios change over time and what influence they have onto the EPSCs and IPSCs balance.

GABA cell depleted neuronal networks were prepared by plating dissociated non-GABAergic precursor cells of the dorsal portion of the cortical anlage from of E16 Wistar rat embryos onto Poly-L-Lysine treated coverslips. These cells were cultivated in serum free culture medium in the presence of a confluent astrocyte feeder layer. After one week (day 7) these networks had a mean neuron density of 1308.86/mm²±42.50 (n=149 cultures) and 99.93%±0.02 of these neurons were identified as non-GABAergic projection neurons in anti-GABA/anti-NeuN/DAPI triple staining.

GABAergic precursor cells were obtained from the medial ganglionic eminence (MGE) of E14 time pregnant homozygote transgenic Wistar rat embryos that express the eGFP derivate Venus in GABA cells (Uematsu et al. Cerebral Cortex 2008). After 7 days in culture 90.61% ± 0. 69 (n = 40 cultures) of all MGE neurons were GABAergic (anti-eGFP/anti-NeuN/DAPI triple staining).

Networks with different GABA/non-GABA content were generated by plating non-GABA cells obtained from the wild type animal at a density of 1500cells/mm². Twenty four hours later 5% (low GABA, T05) and 80% (high GABA, T80) of dissociated MGE precursor cells from transgenic Venus rats were added.

The initial non-GABA/GABA cell ratio set during the plating procedure remained unchanged over the entire cultivation period. These results indicate that on the cellular/network level a broad range of non-GABA/GABA cell ratios can form stable networks and that no adjustment occurs over a 1-month cultivation period.

Giving the result above the question emerges what effect different cell type ratios have onto the excitation/inhibition balance of individual cells. To access the E/I balance, sEPSCs and sIPSCs were recorded in 20-minute long spontaneous voltage clamp recordings in T05 and T80 networks in 155 non-GABA and 169 GABA cells between 6-28DIV. In each cell the E/I balance for amplitude, rise time, decay time and area (e.g. charge transfer) were calculated. The results show that for both transplant types and for both cell types the sIPSPs/(sEPSPs+ sIPSPs) ratio is the same for all four parameters over the entire cultivation period (two-way ANOVA).

These results suggest that the proportion of GABA to non-GABA neurons is not intrinsically regulated on the network level. However, a similar ratio between excitation and inhibition is maintained constant, irrespective of cell numbers, most probably by adjusting number and strength of synapses.

Abnormal CA1 activity causes decreased neonatal prefrontal-hippocampal coupling in a gene-environment model of neuropsychiatric disorders

Xiaxia Xu[1], Lingzhen Song[1], Ileana L Hanganu-Opatz[1]

[1]Developmental Neurophysiology,Institute of Neuroanatomy,Falkenried 94, 20251 Hamburg, Germany

Compromised brain development has been hypothesized to account for mental illness. This concept was underpinned by the function of Disrupted-in-Schizophrenia 1 (DISC1) gene that represents an intracellular hub of developmental processes and has been related to cognitive dysfunction in psychiatric disorders. Mice with whole-brain DISC1 knock-down show impaired prefrontal-hippocampal communication and cognitive abilities throughout development, especially when combined with early environmental stressors, such as maternal immune activation (MIA). While synaptic dysfunction of layer II/III pyramidal neurons in PFC has been recently identified as one source of long-range dysfunction at neonatal age, the specific contribution of abnormal maturation of hippocampus (HP) and prefrontal-hippocampal connectivity to the early deficits is still unknown. Combining in vivo electrophysiology and optogenetics with neuroanatomy in neonatal mice mimicking the dual genetic-environmental (GE) etiology of psychiatric disorders, we identified pyramidal neurons in intermediate/ventral CA1 (i/vCA1) as key elements causing disorganized beta-gamma oscillatory entrainment of local hippocampal circuits. These neurons show abnormal firing as result of sparser dendritic arborization and lower spine density in neonatal GE mice. Moreover, light stimulation of opsin-transfected pyramidal neurons in i/vCA1 induced spiking activity at beta rhythm in prefrontal layer II/III in control mice, but not in GE mice. To further dissect the mechanisms contributing to the abnormal prefrontal-hippocampal coupling, we restricted the DISC1 knock-down to pyramidal neurons in i/vCA1 (GHPE) and analyzed the activity patterns and interactions in PFC and CA1. These data demonstrate the contribution of cellular abnormalities in CA1 and disruption of long-range coupling for the disease-related deficits within developing prefrontal-hippocampal networks.

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Matthias Wilhelm Voigt, Ina Eiffler, Oliver von Bohlen und Halbach

Aggression forges inter-individual behavioural differences in crickets

Julia Sophie Balsam[1], Paul Anthony Stevenson[1]

[1]Leipzig University, Institute for Biology, Department of Animal Physiology, Talstraße 33, 04103 Leipzig, Germany

Recent studies reveal that many invertebrates exhibit consistent inter-individual differences in behaviour, but the proximate cause of such *behavioural syndromes* or *animal personality* is unclear. We are investigating how the social experiences of winning and losing an aggressive encounter influences other aspects of behaviour in adult male crickets (*Gryllus bimaculatus*). Using automated video-tracking (EthoVision), we found that winners of a single fight tend to turn towards a mechanical stimulus directed at one antenna, whereas losers turn away. Surprisingly, this difference was also evident before the fight, although the individuals were socially isolated for 48 h (short term isolated, STI). To test whether this could result from earlier social experience, we repeated the experiment with adult crickets that were isolated as larvae (long term isolated, >14 d, LTI) and therefore had no prior aggressive experience as adults. In this case, we found no difference between winners and losers of a single fight, both tended to turn away from the stimulus. However, 20 min. after a single fight, winners turned towards and losers away from the stimulus, but this difference was no longer evident 24 h later. Contrasting this, winners and losers of 6 consecutive fights at 1 h intervals again showed significant differences in their turning responses that lasted longer than 24 h. We next tested the extent to which social experiences during larval life influences the future adult behaviour profile. To a limited extent, larval crickets interact aggressively with each other, but these interactions had no effect on subsequent behaviour. We next compared cohorts of adult crickets that we raised as larvae either with or without adult males in the colony. Interestingly, larvae raised without adult males were more aggressive and more proactive in their general behaviour as adults compared to those raised as larvae with adult males present (Figure). We conclude that early, pre-adult social experience forges long term, possibly life-long inter-individual changes in behaviour in crickets. Aggressive experience is thus a major determinant of *behavioural syndromes* or *personality*.

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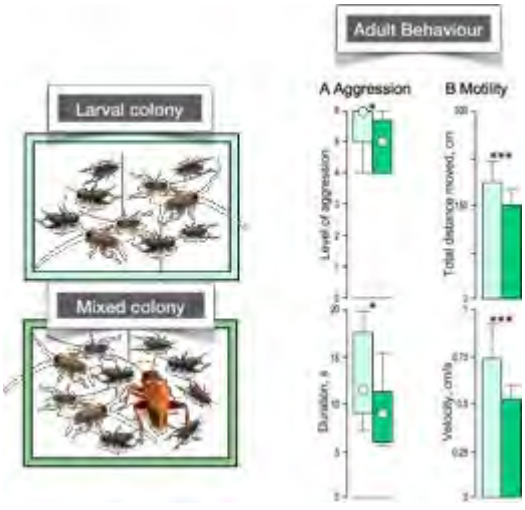

Habituation to appetitive 50-kHz USVs in the playback paradigm in rats

Annuska Berz[1,2,3], Chi-Hsin Chen[1], Markus Wöhr[1,2,3], Rainer K.W. Schwarting[1,2,3]

[1]Philipps-University Marburg, Psychology, Behavioral neuroscience, Gutenbergstraße 18, 35037 Marburg, Germany
[2]RTG 2271 "Breaking Expectations", Philipps-University Marburg, Germany
[3]Center for Mind, Brain and Behavior (CMBB), Philipps-University Marburg, Germany

Rats emit ultrasonic vocalizations (USVs) of different frequencies in order to communicate. The 50-kHz USV calls are emitted during appetitive situations such as rough-and-tumble play or mating. Therefore, these calls indicate a positive emotional state, and in response to their playback, the rats approach the source of the calls, such as when presented from one side of an eight-arm-radial-maze. When repeating the procedure one week later, the rats do not approach the source of the USVs anymore. So far, this so-called 'habituation phenomenon' has not been fully understood. The underlying mechanisms and the brain regions that are specifically involved in the process of receiving and sending 50-kHz USVs are yet to be examined. Studies have verified that the dopaminergic reward system is involved in processing 50-kHz USVs. Previous studies demonstrate an increase in the dopamine levels in the nucleus accumbens during playback of 50-kHz USVs. In addition, learning and memory appears to play a role. Rats injected with scopolamine, a muscarinic acetylcholine antagonist inducing amnesia, approached the sound source during playback of 50-kHz USV one week later, demonstrating a lack of habituation.
In the present study, in order to investigate the reasons underlying the quick habituation to 50-kHz USV playback, the rats are placed on a radial-arm-maze with a speaker on one side of the maze presenting 50-kHz USVs. At the neurobiological level, we are looking at differences of cell activity in several target brain areas by means of immunostaining for cFos, an immediate early gene detecting active cells.
In preliminary behavioral experiments, the habituation effect could be reproduced. The results indicate that alterations in the paradigm can be varied by injecting the dopamine agonist d-amphetamine before 50-kHz playback one week later to induce repeated social approach towards the sound source. The social approach behavior to 50-kHz playback is further investigated by modifying the standard radial-maze playback paradigm, such as by using an elevated arena instead of the maze for changing the context or by pharmacologically manipulating the internal state of the rat.
In addition, previous findings of our lab suggest a difference in 50-kHz USV social approach behavior between certain strains. Therefore, in the present study, we also compared Sprague Dawley and Wistar rats in that aspect, and the results imply differences in response to repeated 50-kHz USV playback in terms of habituation.
Together, these experiments aim to give a better understanding of the behavioral and neurobiological mechanisms underlying communication via USVs, specifically by means of the appetitive 50-kHz USVs.

How humans select and use reliable landmarks for navigation

Norbert Boeddeker[1], Luisa Beckmann[1], Simon Jetzschke[1], Christoph Kayser[1]

[1]Bielefeld University, Cognitive Neuroscience, Universitätsstr. 25, 33615 Bielefeld, Germany

Humans often rely on external references like landmarks for navigation. It is clear that the success of navigating by visual landmarks depends on the landmarks chosen. Natural environments are often cluttered with cues that might serve as landmarks – however not all cues are suitable and allow accurate and precise homing, i.e. navigation back to a previously visited location. The aim of the current study is to provide a better understanding on how humans choose and use landmarks in a visual homing task and to find out whether prior knowledge about object characteristics influences the choice and weighting of landmarks. To this end we manipulated the location of landmarks in homing trials in virtual reality. The virtual world included a large parking lot with various easily distinguishable vehicles and other objects that are normally either mobile (like a food truck and a helicopter) or stationary (e.g. a food booth or a fountain). The task was to learn a specific location on the parking lot, indicated during initial and interspersed training trials, and to return to this target location during subsequent testing trials. Participants were relocated between trials, by placing them on a new position on the parking lot and asked to move to the target location. How do human participants select reliable landmarks and on which criteria do they base their selection? Our results suggest that they rely mainly on objects that are stationary in day-to-day experience. Homing accuracy changes significantly whenever a stationary landmark was relocated. Assuming optimal cue integration, we would expect that precision in any condition with more than one landmark would exceed homing precision in single landmark conditions. However, there was no significant difference between the condition with one stationary landmark and any of the conditions with two landmarks condition. We compared the empirical data with different homing model predictions. These models assume different homing strategies, for example keeping the distance, or the angles between different landmarks constant, or using just one reliable landmark for homing. The comparison indicates that the participants first estimate the reliability of a possible landmark before using it for navigation. In the current study this resulted in a homing performance that relied on objects that normally do not move in daily life.

Traces of negatively valenced objects in a lateral entorhinal cortex - amygdala microcircuit

Vincent Boehm[1], Pinelopi Pliota[1], Klaus Kraitsy[2], Joanna Kaczanowska[1], Wulf Haubensak[1]

[1]Research Institute of Molecular Pathology (IMP), Neuroscience, Campus-Vienna-Biocenter 1, 1030 Vienna, Austria

[2]Preclinical Phenotyping, Vienna Biocenter Core Facilities GmbH (VBCF), Dr. Bohr Gasse 3, 1030, Vienna, Austria

The lateral entorhinal cortex (LEC) is thought to be part of the “external navigation” system, important for goal-directed navigation relative to local landmarks in an environment. It provides local cue information about discrete objects that make up the content of an experience. It is unclear, however, how this information is used within an ethological relevant setting to control the spatial dimension of approach and avoidance behavior (object proximity). To address this question, we performed calcium imaging and optogenetics in freely moving mice during an object-shock conditioning task, in which a 3D object was associated with an aversive shock. Imaging LEC identified several classes of neurons representing different task-related object and valence features. Neuroanatomical tracing studies revealed strong connectivity with the amygdala, a central structure for valence association and behavioral coordination. These connections were actively recruited during spatial object behavior. Optogenetic manipulations revealed that both flexible and stable object-valence representations are multiplexed by different communication channels between LEC and amygdala nuclei. These findings identify the LEC-amygdala projections as an ethological relevant connection, tuning animal behavior towards threatening objects in space.

Influences of aggression on learning in crickets

Kim Julia Borstel[1], Paul Anthony Stevenson[1]

[1]Leipzig University, Institute for Biology, Department of Animal Physiology, Talstraße 33, 04103 Leipzig, Germany

Social experiences such as winning and losing an aggressive interaction with a conspecific influence both future aggression and other non-related behaviours. In insects, our work on adult male crickets (*Gryllus bimaculatus*) has shown that winners tend to be more proactive than losers (Rose et al. Animal Behav. 123:441-450, 2017) and we are now investigating how fighting experience influences their capacity to learn. In our first set of experiments, we applied a simple appetitive olfactory learning paradigm. Mature adult male crickets were isolated for 48 h and deprived of water for ca. 24 h. Weight-matched pairs of crickets were then placed together in an observation arena, shortly after which they interacted aggressively. This generated clear winners and losers; a third cohort of crickets had no fighting experience (naive). Two hours later, and under blinded experimental conditions, individual crickets were presented with an odour (peppermint), followed within a few seconds by a drop of water from a micropipette as reward. This training procedure was repeated 3 times at 5 min intervals. After 60 min, the response to odour alone was tested. Animals were considered as having learnt the odour when they responded with clear searching behaviour, typified primarily by head bobbing and antennal waving in the direction of the presented odour. Non-learners, showed no change in ongoing behaviour. Interestingly, significantly more winners showed learning (71%) compared to losers (31%; CHI-squared test, p = 0,0238), whereas naive crickets had an intermediate learning frequency (50%). We are currently applying additional learning paradigms and preference tests, also with the aim to reveal how neurotransmitters that influence winning and losing in crickets (review: Stevenson and Rillich, Curr. Zool. 62:265-275, 2016) determine experience dependent differences in learning ability. Our main aim is to discover how social and other forms of stress influence learning and memory and the neurotransmitter systems involved.

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Attributing success to oneself versus another: dissociating neural correlates of pride and gratitude

Ke Ding[1], Dian Anggraini[1], Klaus Wunderlich[1]

[1]Luwig-Maximilian University Munich, Department of Psychology, Leopoldstr. 13, 80802 Munich, Germany

Gratitude and pride are both feelings related to accomplishment, whereby the pride attributes success to oneself and gratitude to another. Gratitude and pride are vital to the function of a society, allowing one to create interpersonal relationships and build self-confidence. Despite growing interest in the neural underpinnings of positive emotions and subjective feelings, we know very little about how these emotions are represented in the brain and computationally updated over time by new experience. We developed a novel behavioral task based on the gameshow 'Who Wants to be a Millionaire', which we used together with functional MRI, and computational modeling. We investigated which brain regions are involved in representing gratitude and pride, how the human brain keeps track of these emotions over time and how it updates them when new information is available. We found that gratitude was more associated with neural activities in the bilateral temporoparietal junction (TPJ), which has previously been implicated in theory of mind. In contrast, pride was more associated with neural activities in the caudate nucleus, which is part of the reward system, and hippocampus. Importantly, when we look for neural activity parametrically modulated with the reported magnitude of gratitude feelings we found correlations mainly in the motor cortex (precentral gyrus), reward system (ventral striatum, putamen) and theory of mind network (temporal pole). In contrast, neural activity pertaining to the strength of the feeling of pride was found in the bilateral putamen. Finally, activity in ventromedial prefrontal cortex (vmPFC) was related to an emotional prediction error signal, suggesting that this region might be involved in the process of updating our level of gratitude and pride feelings. Our findings delineate the computational mechanisms and neural circuitry for positive emotions that accompany the attribution of getting reward whether it is due to one's own effort or the help of others.

Developmental peculiarities of perception of speech emotional prosody in schoolchildren with high math abilities.

Elena Dmitrieva[1], Victor Gelman[2]

[1]Sechenov Institute of Evolutionary Physiology and Biochemistry, RAS, Group of Bioacoustics, Torez pr., 44, 194223 St.Petersburg, Russia

[2]North-West State Medical University, St.Petersburg, Russia

A vast body of research considers different aspects of children's cognitive abilities but it is still not fully clear what neuropsychological developmental mechanisms underlie high ability performance in school children. Some authors argue that there is indirect evidence for atypical brain organization in the gifted children and that people with exceptional mathematical abilities have some cerebral asymmetry peculiarities (Winner, 2000, O'Boyle, 2008). It is assumed that children's emotional sphere influences learning process in general, but very little is known about emotional intelligence of mathematically gifted school children. The study analyzes the developmental neuropsychological features of emotion recognition performance in children with high math abilities in comparison with their peers that have common abilities.

Materials and methods: Sample consisted of 69 pupils: control group of children from the public school (C) and mathematically gifted children (M). Two age groups were considered: 11- to 13-year-olds and 14- to 17-year- olds. The children had normal hearing and were right handers. There were approximately equal numbers of girls and boys within each age level. The stimuli used as test items consisted of a sentence spoken in positive (happy) and negative (anger) emotional tones of voice and unemotionally by the professional actor. A computer analysis of the acoustic parameters of these signals was carried out. The stimuli were presented through the headphones at random to the right or left ear, without noise or at ipsilateral white noise background. Simultaneously, white noise of the same intensity as the valid signal was fed to the contralateral ear through the other channel. Listeners had to identify the valence of emotional intonations of test stimuli.

Results. Reaction time (RT) was recorded and accuracy of recognition (AR) was calculated. To examine the data we have used the ANOVA, Mann-Whitney test and correlation analysis. We have found that the factors "age", "sex" and "signal presentation side" influence the accuracy and time of recognition of emotional intonation in both C and M groups. It is shown that the reaction time in children with high math abilities aged 11-13 years is longer than in the control group, but decreases with age and becomes shorter in 14-17 year-olds. At the age of 14-17 only in male mathematicians there is a higher AR as compared to the controls. Data on the AR of emotional intonations indicate the predominant role of the left hemisphere in mathematicians and the right one in the control group, especially in 11-13 year-olds. This data support the assumption that children with exceptional mathematical abilities may have some developmental peculiarities of brain functional organization as compared to controls. The correlation analysis has revealed pronounced and, similar in both groups of studied schoolchildren, interconnections between the recognition indices and the acoustic parameters of the test signals, which ensure the recognition of their emotional prosody at noise background.

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Low frequency oscillatory bursts in the macaque prefrontal cortex predict spontaneous transitions in the content of consciousness

Abhilash Dwarakanath[1], Vishal Kapoor[1], Leonid Fedorov[1], Shervin Safavi[1], Joachim Werner[1], Nicho Hatsopoulos[2], Nikos Logothetis[1], Theofanis Panagiotaropoulos[3]

[1]Max Planck Institute for Biological Cybernetics, Physiology of Cognitive Processes, Max-Planck-Ring 8, 72076 Tübingen, Germany

[2]Department of Organismal Biology and Anatomy, University of Chicago, USA

[3]5Cognitive Neuroimaging Unit, CEA, DSV/I2BM, INSERM, Universite Paris-Sud, Universite Paris-Saclay, Neurospin Center, 91191 Gif/Yvette, France

In multistable perception, the content of consciousness alternates spontaneously between mutually exclusive or mixed interpretations of competing representations. Identifying neural signals predictive of such intrinsically driven perceptual transitions is fundamental in resolving the mechanism and localizing the brain areas giving rise to visual consciousness. Here we employed a no-report paradigm of binocular motion rivalry based on the optokinetic nystagmus reflex read-out of spontaneous perceptual transitions coupled with multielectrode recordings of local field potentials and single neuron discharges in the macaque prefrontal cortex. We show that an increase of oscillatory bursts in the delta-theta (1-9 Hz), and a decrease in the beta (20-40 Hz) bands, along with significant perceptual modulation of single neurons during periods of dominance and perceptual switches, are predictive of spontaneous transitions in the content of visual consciousness. These results suggest that the balance of stochastic prefrontal fluctuations is critical in refreshing conscious perception, casting doubt on a posterior cortical mechanism for visual awareness.

Visualizing BDNF Transcript Usage During Sound-Induced Memory Linked Plasticity

Philipp Eckert[1], Lucas Matt[2], Rama Panford-Walsh[1], Hyun-Soon Geisler[1], Anne E. Bausch[2], Marie Manthey[1], Nicolas I.C. Müller[3], Csaba Harasztosi [4], Karin Rohbock[1], Peter Ruth[2], Eckhard Friauf[3], Thomas Ott[5], Ulrike Zimmermann[1], Lukas Rüttiger[1], Thomas Schimmang[6], Marlies Knipper[1], Wibke Singer[1]

[1]Department of Otolaryngology Head & Neck Surgery, Tübingen Hearing Research Centre, Molecular Physiology of Hearing, University of Tübingen, Tübingen, Germany

[2]Department of Pharmacology, Institute of Pharmacy, Toxicology, and Clinical Pharmacy, University of Tübingen, Tübingen, Germany

[3]Animal Physiology Group, Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany

[4]Section of Physiological Acoustics and Communication, Department of Otolaryngology Head & Neck Surgery, Tübingen Hearing Research Center, University of Tübingen, Tübingen, Germany

[5]Transgenic Facility Tübingen, University of Tübingen, Tübingen, Germany

[6]Instituto de Biología y Genética Molecular, Universidad de Valladolid, Consejo Superior de Investigaciones Científicas (CSIC), Valladolid, Spain

Activity-dependent BDNF (brain-derived neurotrophic factor) expression is hypothesized to be a cue for the context-specificity of memory formation. So far, activity-dependent BDNF cannot be explicitly monitored independently of basal BDNF levels. We used the BLEV (BDNF-live-exon-visualization) reporter mouse to specifically detect activity-dependent usage of *Bdnf* exon-IV and -VI promoters through bi-cistronic co-expression of CFP and YFP, respectively. Enriching acoustic stimuli led to improved peripheral and central auditory brainstem responses, increased Schaffer collateral LTP, and enhanced performance in the Morris water maze. Within the brainstem, neuronal activity was increased and accompanied by a trend for higher expression levels of *Bdnf* exon-IV-CFP and exon-VI-YFP transcripts. In the hippocampus BDNF transcripts were clearly increased parallel to changes in parvalbumin expression and were localized to specific neurons and capillaries. Severe acoustic trauma, in contrast, elevated neither *Bdnf* transcript levels, nor auditory responses, parvalbumin or LTP. Together, this suggests that critical sensory input is essential for recruitment of activity-dependent auditory-specific BDNF expression that may shape network adaptation.

Characterization of C57BL/6J and two transgenic mouse lines in a novel behavioral paradigm for social fear conditioning

Nadine Faesel[1,2], Malgorzata Kolodziejczyk[1], Suemeyra Aksit[3], Michael Koch[2], Markus Fendt[1,4]

[1]Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Germany
[2]Department of Neuropharmacology, Brain Research Institute, University of Bremen, Germany
[3]Integrative Neuroscience Program, Otto-von-Guericke University Magdeburg, Germany
[4]Center for Behavioral Brain Sciences, Otto-von-Guericke University Magdeburg, Germany

Social withdrawal and fear of social situations are symptoms of several neuropsychiatric disorders, e.g. social anxiety disorder. In order to study such symptoms, we established a novel social fear conditioning paradigm in our lab using C57BL/6J mice. In this paradigm, we used a modified version of Crawley's sociability test in which mice have the choice of exploring two wired cups, one containing a stranger mouse and one empty. After a baseline test for sociability, the animals were submitted to a social fear conditioning session, in which each approach to the stranger mouse was punished by a foot shock. After a break of two days, the animals were again tested for sociability, thrice daily on two consecutive days. Our pilot study shows that mice, which have been punished for social approach before, express strong avoidance to a stranger mouse indicating social fear. This social fear extinguishes within the six sociability sessions back to baseline levels of social approach. Our current goal is to investigate the role of two neuropeptides in social fear, namely neuropeptide S and orexin. For that, we are currently testing neuropeptide S receptor knockout mice as well as orexin knockout mice in our novel paradigm.

Enriched environment restores behavioral deficits induced by BDNF haploinsuffiency

Markus Fendt[1,2], Mahmoud Harb[1], Justina Jagusch[1], Thomas Endres[3], Volkmar Lessmann[2,3]

[1]University of Magdeburg, Institute for Pharmacology and Toxicology, Leipziger Strasse 44, 39120 Magdeburg, Germany.
[2]University of Magdeburg, Center for Behavioral Brain Sciences, Leipziger Strasse 44, 39120 Magdeburg, Germany.
[3]University of Magdeburg, Institute of Physiology, Leipziger Strasse 44, 39120 Magdeburg, Germany.

Brain-derived neurotrophic factor (BDNF) is implicated in a number of processes that are crucial for healthy functioning of the brain. Notably, different neuropsychiatric diseases are associated with low BDNF levels in brain and blood. In the present study, we used BDNF haploinsufficient (BDNF +/-) mice to investigate the role of BDNF in different mouse behavioral endophenotypes which are relevant for neuropsychiatric diseases. In detail, we investigated sensorimotor gating (prepulse inhibition of the startle response), emotional learning (fear and safety conditioning), as well as cognitive flexibility (attentional set shifting). Our results showed that BDNF +/- mice had an increased startle magnitude and a deficit in prepulse inhibition. Contextual fear learning was not affected but safety learning was absent. Furthermore, BDNF +/- mice had deficits in all phases of the attentional set shifting task. Notably, in most of these tests, there was a correlation of individual BDNF brain levels with the individual behavioral performance. In a second experiment, we investigated whether a phase of two months enriched environment (including voluntary running) is able to restore these behavioral deficits in BDNF +/- mice. Indeed, BDNF +/- mice exposed to enriched environment did not have exaggerated startle magnitudes and impaired prepulse inhibition scores anymore. Furthermore, safety conditioning could be observed in these mice. Last, first data also suggest less impairments of BDNF +/- mice in the attentional set shifting task after enriched environment. Taken together, the present data strongly support that decreased BDNF levels are associated with several behavioral deficits, and that enriched environment can increase BDNF levels and is thereby able to restore such behavioral deficits.

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Spontaneous alpha oscillations reflect the effort to compensate an individual bias in temporal perception

Laetitia Grabot[1], Christoph Kayser[1]

[1]University of Bielefeld, Department of Biology, Universitätsstr. 25, 33615 Bielefeld, Germany
[1]Cognitive Interaction Technology – Center of Excellence, Bielefeld University, Inspiration 1, 33615, Bielefeld, Germany.

Perceptual decision-making is usually shaped by prior expectations build on recent contextual information, and on persistent internal priors. Time perception is no exception to the rule, as highlighted by temporal recalibration, a phenomenon in which the perception of synchrony is shifted following exposure to a repeated asynchrony. Besides, the perception of simultaneity also seems to build upon an individual and persistent bias: when exposed to a pair of audiovisual stimuli, each individual perceives simultaneity for a specific delay between these two events, and this delay remains stable across time. Previous work suggests that the power of spontaneous alpha activity reflects the effort to overcome this individual bias. Here, we aimed to test this hypothesis by using an audiovisual temporal order judgment task, dissociating in an orthogonal 2*2 design the correctness (correct or incorrect perceived order) and the degree of biasedness (perceived order matching or non-matching an individual's preferred order). We found that a decrease in pre-stimulus alpha power predicts the perception of the non-preferred order compared to the preferred order. We did not find any effect of correctness. These results confirm the hypothesis that spontaneous alpha power indexes the effort to overcome an individual's bias. They are at odds with a classical view proposing that a decrease of spontaneous alpha power improves performance. However, they are consistent with recent studies challenging the classical view, which shows that a decrease of spontaneous alpha power does not improve perceptual sensitivity but increases the overall neural excitability. Our results consolidate this novel perspective and suggest that alpha power determines how much perception can be biased by internal priors.

Differential control of fear and reward behavior in BNST circuits

Wulf Haubensak[1], Nadia Kaouane[1], Sibel Ada[1], Marlene Hausleitner[1]

[1]Research Institute of Molecular Pathology (IMP), Neuroscience, Campus Vienna Biocenter 1, 1030 Vienna, Austria

The ability to discriminate environmental stimuli predicting positive and negative outcomes is critical for survival. The limbic system plays a central role in the encoding of stimulus values. Nevertheless, opposite emotions, like fear and reward-related states, are often processed by a same limbic hotspot, such as the bed nucleus of the stria terminalis (BNST). Therefore we aimed to delineate neuronal circuits of fear and reward-related emotional states within BNST circuits. For this, we developed a combined fear and reward Pavlovian conditioning paradigm during which a sound (Reward-CS) is followed by the delivery of sucrose reward, whereas a different sound (Fear-CS) is followed by a footshock. During the test session, the mice expressed specifically either reward-seeking or freezing responses during Reward-CS and Fear-CS, respectively. We combined this behavioral protocol with pharmacology, calcium imaging and optogenetic manipulations during CSs re-exposure. We first demonstrated that BNST is necessary for both fear and reward expression, as they are both decreased after muscimol pharmacological inactivation. Calcium imaging experiments revealed that BNST neurons are preferentially activated by either Fear-CS or Reward-CS. Using optogenetics, we specifically targeted BNST neurons projecting to one of its two main outputs structures, namely the paraventricular hypothalamus (PVH) or the periaqueductal gray (PAG). Those optogenetic experiments revealed that BNST neurons specifically control reward-seeking or freezing responses depending on their projecting target, i.e. PVH or PAG, respectively. These findings suggest that the BNST acts as a neural hub in integrating positive and negative information to control the expression of fear and reward responses by projecting to different brain targets.

Antidepressant Action of Sugar Treatment is Dependent on Octopaminergic Signalling to the Serotonergic System in Drosophila melanogaster

Tim Hermanns[1], Burkhard Poeck [1], Roland Strauss[1]

[1]Institute of Developemental Biology and Neurobiology, AG Strauß, Hanns-Dieter-Hüsch-Weg 15, D-55099 Mainz, Germany

The motivation to perform a given behaviour is constantly changing in response to external cues, internal states, and is also affected by psychopathological dysfunctions in humans as well as in animals. Imbalance of the motivation system can result in a total loss of motivation to act at all, or to the excessive urge to perform specific behaviours, as manifested in manic-depressive disorders (MDD). A gap climbing-decision assay in which a fly decides whether to climb a just insurmountable gap is an established method to study motivation in the fly [1]. On the other hand we have developed a stress protocol to study the mechanisms which decrease the motivation of the fly for initiating activity: highly repetitive vibrational stress applied over three days significantly reduces the motivation to initiate gap climbing, to walk fast, or to start courting. We established a model for a depression-like state (DLS) because behavioural changes during DLS correlate with reduced serotonin (5-HT) release at the MB α-lobes and can be relieved by feeding 5-HTP (a precursor of 5-HT which passes the blood-brain barrier) or SSRI (selective serotonin reuptake inhibitors). This relief is mediated by 5-HT-1A receptors in the α-lobes of the MB, whereas 5-HT-1B receptors in the γ-lobes control behavioural inactivity.
Surprisingly, also sugar reward can relief DLS. Octopamine is known to be the relevant transmitter translating the sweetness, but not the caloric value, of sugar into the pathway of sugar reward learning in Drosophila melanogaster. To understand the underlying mechanisms of a sugar treatment we investigated the role of octopamine in the motivation system of the fly. Manipulating the octopaminergic system via RNAi against octopamine receptors or inhibition of the production of octopamine affected the antidepressant action of a sugar treatment in stressed flies. Investigating the connection between the octopaminergic and the serotonergic system was the current aim of this study.
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Cortico-limbic interactions in emotional behavior

Dominic Kargl[1], Joanna Kaczanowska[1], Jelena Zinnanti[2], Peter Opriessnig[2], Wulf Haubensak[1]

[1]IMP - Research Institute of Molecular Pathology, Haubensak Group, Campus-Vienna-Biocenter 1, 1030 Vienna, Austria
[2]Vienna Biocenter Core Facilities GmbH (VBCF), Dr. Bohr Gasse 3, Vienna, Austria

Emotions are an inherent component of our mental self, they attribute meaning and significance to our environment. To adequately respond to potential threat, we must assign valence to sensory cues, a fundamental task to survive and a major driving force of emotional behavior. Interestingly, valence-specific activity can be found in diverse brain areas, including cortical and subcortical structures, hence valence information is represented on multiple hierarchical levels. Consequently, a key question in neuroscience is how valence information is distributed throughout diverse brain areas during learning and to what extent this phenomenon is dependent on the interaction between those areas. The central amygdala (CE), the amygdala's major output is known for eliciting fear responses via its projections to the brainstem. Interestingly, the CE also connects to the basal forebrain (BF), a collection of cholinergic nuclei modulating cortical activity, therefore potentially impacting hierarchies far above the CE. Here we sought to characterize the projection of the CE to the BF Nucleus Basalis of Meynert anatomically and its contribution to emotional behavior. Next, we probed for dysregulated functional connectivity brain-wide upon CE lesion with fMRI in the mouse. Subsequent correlation of the behavioral phenotype in a Pavlovian fear learning paradigm with brain-wide functional connectivity allowed us to screen for potential fear learning circuits that can be further addressed functionally.

Expectation and multisensory integration during perceptual decisions

Stephanie, J Kayser[1], Christoph Kayser[1]

[1]Bielefeld University, Cognitive Neuroscience, 100131, 33615 Bielefeld, Germany

To correctly perceive every-day sensory scenarios our brain exploits the redundancies between the information across the senses, but also relies on expectations to facilitate the selection and processing of relevant information. Both prior expectations and multisensory redundancy have been suggested to enhance sensory encoding at relatively early stages, but both can also affect perception by affecting higher-level decision processes. As a result, the similarities and differences in the mechanistic influences of multisensory information and of prior expectations on perception remain unclear. Using a previously established audio-visual motion discrimination paradigm we investigated the functional and neural (using EEG) similarities between multisensory integration and priming within a sensory modality. Human participants discriminated visual random-dot motion kinematograms with time- and trial-by-trial varying motion coherence, while this visual stimulus was either complemented by acoustic motion (of the same direction as the visual motion in 66% of trials), or by a prior visual cue (66% correct). Perceptual performance was significantly higher for congruent acoustic information and a congruent cue, but the accuracy benefit was significantly higher for the multisensory condition. We analysed the EEG data using classification methods to extract EEG activations selective for the encoding of the task-relevant visual motion direction. We then asked which of these EEG components are affected by the acoustic information or the prior cue. This revealed significant multisensory influences at earlier latencies than significant influences of the prior cue, in line with the notion that sensory expectations shape decision processes while multisensory interactions emerge already within sensory-specific brain regions.

Investigation and modelling of monkey and human choice behavior in a transparent coordination game

Sebastian Moeller[1,2,3], Anton M. Unakafov[1,2,3,4], Alexander Gail[1,2,3,5], Stefan Treue[1,2,3,5], Fred Wolf[3,4,5], Igor Kagan[2,3]

[1]Georg-Elias-Müller-Institute of Psychology, University of Goettingen, Gosslerstrasse 14, 37073, Germany

[2]Leibniz ScienceCampus Primate Cognition, Kellnerweg 4, 37077, Goettingen, Germany

[3]German Primate Center – Leibniz Institute for Primate Research, Kellnerweg 4, 37077, Goettingen, Germany

[4]Max Planck Institute for Dynamics and Self-Organization, Am Fassberg 17, 37077, Goettingen, Germany

[5]Bernstein Center for Computational Neuroscience, Am Fassberg 17, 37077, Goettingen, Germany

What facilitates cooperation between individuals? Recent studies in social neuroscience utilized classical game-theoretic concepts of simultaneous and sequential actions. While being convenient for studying strategic decision-making, assumptions of classical game theory may be suboptimal for understanding direct social interactions. This is because real-world agents rarely act strictly simultaneously or sequentially, but rather observe each other and potentially use the others' behavior to adjust their own actions 'on the fly'.

To investigate social decision-making under realistic conditions including mutual action visibility, we created a setup in which two agents sit vis-à-vis separated by a transparent display with two touchscreens; allowing them to observe each other and to interact with the same visual objects by reaching to the shared workspace. We compared humans and rhesus macaques in the coordination game 'Bach or Stravinsky'. Here, each agent has a preferred target associated with a larger reward; one agent's high-value target is the other's low-value target. Selecting the same target adds an equal bonus to the target value for each agent. Thus, joint selection increases reward for both agents, but the agent whose own preferred target is selected earns more. Joint selection of either target is a Nash equilibrium; but selecting only one of these leads to unequal payoffs. Balancing this inequality requires special strategies.

We found that such coordination strategies depend on the ability of agents to observe the others' actions, which in turn depend on reaction times. Action visibility has strategic value: the faster agent foregoes information about the partner's choice for the sake of being able to influence it. To take visibility into account, we developed a novel game theoretical framework of 'transparent games'. Simulations for the transparent Bach or Stravinsky game show that coordination depends on the visibility of partner's actions and reveal 3 main behavior types: *turn-taking* (alternating between the targets), *challenging* (each insisting on its preferable target until one gives in and foregoes its preferences) and temporal *leader-following* (the slower following the faster's choice).

Most human and macaque pairs converged to a Nash equilibrium, coordinating to select the same target. Humans mainly used *turn-taking* to equalize payoffs, while macaques relied on selecting the same side or on *challenging*. Humans also used *leader-following*, but leaders did not always select their own preferred target, resulting in a fair payoffs.

Two monkeys underwent training with a human confederate, who alternated between the two targets in blocks. After several sessions, monkeys adopted largely optimal behavior, coordinating their choices with the confederate. Blocking the view of the human's hand resulted in a coordination loss, implying that monkeys actively observed the human. After this training, when these monkeys played together, they

clearly adhered to *leader-following*. Unlike in humans, the leader mainly insisted on his preference and the 2nd monkey followed. In two sessions where they showed similar average reaction times a *turn-taking* pattern emerged: each monkey led and followed in blocks. Our findings clearly demonstrate the importance of action visibility in emergence and maintenance of coordination and the general usefulness of our approach.

Disconnection of prefrontal cortex and ventral tegmental area alters effort-related responding in rats

Alexandra Münster[1], Wolfgang Hauber[1]

[1]University of Stuttgart, Department of Neurobiology, Pfaffenwaldring 57, 70569 Stuttgart, Germany

Recent evidence suggests that a particular subregion of the prefrontal cortex, the medial orbitofrontal cortex (mOFC), mediates the ability of an organism to work with vigor towards a selected goal. For instance, in rats, pharmacological inhibition of the mOFC increased, while pharmacological stimulation of the mOFC reduced responding under a progressive ratio (PR) schedule of reinforcement [1]. Anatomical studies revealed strong projections from the mOFC to the ventral tegmental area (VTA) [2], a major component of the neural circuitry subserving effort-related motivational function [3]. Thus, it is conceivable that the mOFC-VTA circuitry supports PR responding, however, respective data are missing. Here we analyzed in more detail the role of the mOFC and interactions between mOFC and VTA in rats tested in a PR task.

In Experiment 1, we used an optogenetics approach to globally stimulate mOFC neurons prior to testing rats on the PR task. Results demonstrate that, relative to controls, pre-test optogenetic mOFC stimulation reduced PR responding. This finding is consistent with previous data showing that pharmacological stimulation of the OFC reduced PR responding [1].

In Experiment 2, we used a pharmacological disconnection approach to analyze the role neural pathways linking the mOFC and VTA in effort-related responding. Results indicate that disruption of the communication between the mOFC and VTA altered PR responding. Thus, information transfer between the mOFC and the VTA is necessary for effort-related responding.

In Experiment 3, we used a combined pharmacological/neurotoxin inactivation disconnection approach to analyze the role neural pathways linking the mOFC and VTA dopamine neurons in effort-related responding. Results demonstrate that disruption of the communication between the mOFC and VTA dopamine neurons altered PR responding. These data suggest that the information transfer between mOFC and VTA dopamine neurons is critical for effort-related responding.

Taken together, our data provide further support to the notion that the mOFC plays a key role in effort-related responding. Furthermore, they implicate that the mOFC might control effort-related responding by interacting with VTA dopamine neurons. The idea that the mOFC is a key part of the neural circuitry that governs effort-related responding is in line functional imaging data in humans. Accordingly, the mOFC is one of the regions representing an integrated value of reward type and effort level during effort-based reinforcement [4]. Of note, recent clinical evidence demonstrated in patients with major depressive disorder a decreased functional connectivity of the mOFC that may contribute to anergia, a frequent symptom in depression [5]. In addition, our data may provide further understanding of the neural basis of effort-related dysfunction observed in several psychiatric disorders [3].

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Specificity of pain and fear encoding in neuronal ensembles of the prelimbic mPFC

Manfred Josef Oswald, Sebastian Quiroga[1], Rohini Kuner[1]

[1]Universität Heidelberg, Pharmakologisches Institut, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany

Our ability to experience pain serves an important physiological function to prevent tissue damage. Pain, however, is a complex sensory and emotional experience that can vary widely between individuals and strongly depends on brain state. Imaging studies in humans and rodents have revealed that prefrontal cortical regions such as the anterior cingulate cortex (ACC) and the prelimbic (PrL) and infralimbic (IL) regions of the medial prefrontal cortex (mPFC) are prominent amongst brain areas that are consistently activated during nociceptive stimulation. However, numerous studies also associate the ACC, PrL, and IL with diverse forms of memory, fear, decision-making, attention, and reinforcement learning.

To address how specificity is generated in the mPFC with respect to nociception and pain as opposed to fear we implanted 8 mice with 64-channel probes aimed at the PrL in the right hemisphere. After a recovery period we assessed nocifensive and fear behavior while recording neuronal activity in response to an acute capsaicin pain stimulus to the left hind paw and during a tone-cued fear retrieval session. Of 306 isolated units from 7 paired electrophysiological recording sessions, 9.7 ± 2.1 and 8.6 ± 1.6 % of all units had significantly elevated global firing rates in either the tone-cued fear or capsaicin-induced pain states, respectively. Only a small proportion (4.5 ± 3.1 % of units) shared elevated firing rates in both states. Similar and somewhat larger proportions of units had significantly decreased firing rates in either the pain or fear, compared to the baseline states, again with only a small overlapping proportion. A cluster analysis of spike waveform features and firing rate suggests that 11.8 % of recorded units were putative interneurons, and these also contained similar proportions of units with increased or decreased firing rates in the pain or fear states. This suggests that the unit activity of distinct subpopulations of both principal neurons and putative interneurons is selectively enhanced or suppressed in a state of pain or conditioned fear. In effect this will enhance the dynamic range to encode distinct behavioural states.

To further test for distinct neuronal activity patterns during each of these behavioral states we binarized the unit activity within sliding window bins of 150 - 900 ms and computed principal component scores from the coactivation matrix of all units for each time bin. We then applied a threshold to the PCA scores and computed activity patterns that maximize receiver operating characteristics for freezing and nocifensive behavioural episodes during cued-fear and capsaicin-induced pain states, respectively. Neuronal ensembles were then defined from the resulting single unit coactivation matrix as those with an above chance contribution index derived from the sum of coactivity scores. Pain and fear ensembles clustered differently and Mahalanobis distance measures were significantly distinct. With this approach we obtained estimates for specific ensemble units of 13.9 ± 2.7 % and 14.7 ± 2.0 % of units in a session for either fear or pain states, respectively, and a further 9.1 ± 2.4 % of all units that were part of both ensembles. Our analyses suggest that fear and pain sensations are encoded by specific ensemble units together with a smaller proportion of shared ensemble units contributing to both states.

Neural correlates of mushroom body output neurons measured during flight of a harnessed honey bee on a quad copter

Benjamin Hans Paffhausen[1], Julian Petrasch[2], Tim Landgraf[2], Randolf Menzel[1]

[1]Freie Universität Berlin, Neurobiologie AG Menzel, Königin-Luise-Str. 1-3, 14195 Berlin, Germany
[2]Freie Universität Berlin, Informatik AG Landgraf, Arnimallee 7, 14195 Berlin, Germany

Honey bee navigation is a actively investigated field but the knowledge about the features essential for extracting goal directed long distance navigation remain mostly unknown in such context. To tackle this problem, we recorded neuronal activity during flight in a natural environment. Bees were trained to a feeder 400 m from the hive. Neural activity of high order interneurons (mushroom body extrinsic neurons) were recorded with extracellular electrodes at the alpha lobe. During the recordings, the bees were attached to a quad copter together with the necessary amplifiers and data storing devices. The copter flew along the path the bees would have taken to reach the learned feeder. Additional flight paths were flown at natural speed and height. The spike rates of the recorded neurons were analyzed with respect to the corresponding flight tracks in search for correlations. Preliminary analysis of the data shows a strong, repeatable spike rate change whenever the copter was turning in tight curves. Straight flights however resulted in somewhat repeatable spike rate changes over the same path but with much higher variance. Further analysis as well as more specific flight path will bring light into the role of the mushroom body for navigation.

Dynamics of prefrontal cortical neural ensembles during feeding behaviours in freely behaving mice

Anne Petzold[1], Tatiana Korotkova[1]

[1]Max Planck Research group "Neuronal Circuits and Behavior", Max Planck Institute for Metabolism Research, Cologne, Germany

Feeding is a complex phenomenon encompassing a sequence of different behaviours such as foraging, food detection, approach and evaluation, as well as consumption. While the drive to feed is innate, feeding-related behaviours are shaped by a composition of environmental and internal cues, as well as previous experience and individual preferences (1).

The prefrontal cortex (PFC) has long been implicated in the regulation of feeding behaviours (2, 3), particularly regarding the anticipation and evaluation of the rewarding properties of food, the current state of satiety, and the ensuing motivation to feed (see, e.g., 4, 5). We have recently shown that gamma-rhythmic activation of PFC projections to the lateral septum promotes food-seeking (6). However, it is not known whether the same or different PFC cell ensembles encode various stages of feeding-related behaviours, whether activity of PFC cells depends on the metabolic state of an animal and how it reflects changes in environmental cues.

To address these questions, we investigate the dynamics of neural ensemble activity of the PFC throughout the stages of feeding under different environmental and internal constraints such as varying levels of food availability and satiety. For this purpose, we perform calcium imaging of large populations of PFC excitatory neurons using a miniaturized microscope (7) in the freely moving mouse during spontaneous exploration, foraging, and feeding. Recordings of the same cells across multiple days and behavioural paradigms allow us to identify the cortical neural ensembles encoding various components of such innate behaviours. The concomitant optogenetic perturbation of excitatory ensembles of the PFC allows us to evaluate the functional relevance of PFC neural ensembles in shaping feeding behaviour under varying conditions.

This approach enables us to dissect the contribution of PFC ensemble dynamics to the ongoing adaptation of feeding behaviour in the face of the constantly changing external and internal environment.

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Neuroarchitecture of peptidergic systems and dopaminergic afferents in the mouse central amygdala

Mirjam Richard[1], Angelika Schmitt-Böhrer[2], Philip Tovote[3], Esther Asan[1]

[1]University Wuerzburg, Institute of Anatomy and Cell Biology, Koellikerstraße 6, 97070 Würzburg, Germany

[2]University Hospital Wuerzburg, Center of Mental Health, Department of Psychiatry, Psychosomatics and Psychotherapy, Margarete-Höppel-Platz 1, 97080 Würzburg

[3]University Hospital Wuerzburg, Defense Circuits Lab, Systems Neurobiology, Institute of Clinical Neurobiology, Versbacher Str. 5, 97078 Würzburg

In all mammals, the amygdala is a key component of the brain's emotion system. Its central nucleus (CeA) is composed of several subnuclei with distinct hodological and functional features. Numerous studies in rodents have documented that plasticity of complex inhibitory microcircuits within and between the subnuclei underlies CeA functions in orchestrating adaptive responses to innate and learned emotional stimuli. In both rats and mice, distinct GABAergic neuronal subpopulations differentially (co-)express various neuropeptides throughout the rostrocaudal extent of CeA subnuclei. The CeA is particularly densely innervated by afferents from dopaminergic midbrain neurons. Numerous studies in rats and, more recently, in genetically modified mice using optogenetic techniques, suggested a major role of dopamine on CeA peptide expression, circuit plasticity and function. Morphology of dopaminergic afferents and identified CeA target neurons has been analyzed by light and electron microscopy in the rat, but studies in the mouse are lacking. The aim of the present study is to characterize the neuronal ultrastructure and the (co-)distribution of dopaminergic afferents and their peptidergic targets in the mouse CeA. Electron microscopic findings indicate differences in somatic morphology and interneuronal contacts between neurons of the lateral (CeL) and medial (CeM) subnuclei similar to those found in rats. Immunoreactions for tyrosine hydroxylase (TH) show distinct variations in (ultrastructural) morphology and density of immunolabeled terminal axons between individual CeA subnuclei which resemble those reported for dopaminergic Ce afferents in rats. These characteristics facilitate delineation of subnuclei throughout their rostrocaudal extent, and indicate a subzonation of the posterior CeL, with a particularly dense dopaminergic fiber plexus in the medial CeL. Multiple immunolabelings in combination with in situ hybridization for TH and different peptides (e.g. somatostatin, corticotropin releasing factor, enkephalins, neuropeptide Y) document differential topography and overlap between TH-immunoreactive afferents and identified peptidergic neurons/axon plexus, depending on the specific subnuclear and rostrocaudal localization. Neuroarchitecture of dopaminergic inputs to CeA peptidergic systems likely contributes to CeA subnuclear functions, and will inform future experimental manipulations and interpretation of their outcomes.

Social defeat stress kills insects via a nitric-oxide/serotonin-dependent mechanism

Jan Rillich[1], Paul A. Stevenson[1]

[1]University of Leipzig, Institute for Biology, Talstr. 33, 04103 Leipzig, Germany

In all animals, losers of a conflict against a conspecific exhibit reduced aggressiveness, and this is often coupled with depression-like symptoms, particularly after multiple defeats (chronic social defeat stress). Here we report that chronic social defeat leads to a significant increase in mortality in adult male crickets (Gryllus bimaculatus), via a nitric-oxide/serotonin-dependent mechanism. After a single defeat, crickets show reduced aggression for maximally 3 h, but long-term depression of aggression (>24 h) after 6 consecutive defeats at 1 h intervals. Survival analysis revealed that maximally 11% of crickets die 5 days after experiencing one defeat, and that this is not significantly different to crickets that experienced no defeat (7%, p=0,16). This compares to a significantly higher mortality 5 days after multiple defeat (29%, p<0,0001). We have shown that simple aversive stimuli (AS, wind puffs directed at the cerci) also leads to depressed aggression as shown by losers , but only when paired with the aggression releasing stimulus (RS; Rillich & Stevenson PA. Front. Behav. Neurosci.11/50:1-15, 2017). If this simple stimulation regime is repeated 6 times at 1 h interval, crickets also show long-term depression of aggression, and again significantly higher mortality (21%) compared to crickets that received multiple AS without the RS (9%, p= 0,013). This indicates that increased mortality is due to defeat stress, rather than injury or the physical stress of fighting itself. Since the decision to flee, and subsequent loser depression, is controlled by the joint action of nitric oxide (NO) and serotonin (5HT; Rillich and Stevenson, Front. Behav. Neurosci. in press), we tested if defeat induced mortality is influenced by inhibiting NO synthesis with LNAME, or blocking 5HT2-like receptor with ketanserin, or other 5HT-receptor types with the non-selective antagonist methiothepin. None of the drugs affected mortality 5 days after 1 defeat (10-14%) compared to vehicle (DMSO, 8%). However, whereas we again observed increased mortality 5 days after multiple defeat for crickets that received vehicle (34%), the increase in mortality was prevented by LNAME (13%, p=0,0016) and ketanserin (11%, p<0,0001), but not by methiothepin (28%, p=0,37). We conclude that chronic social defeat stress can kill insects as a consequence of released NO and activation of a 5HT2-like receptor.

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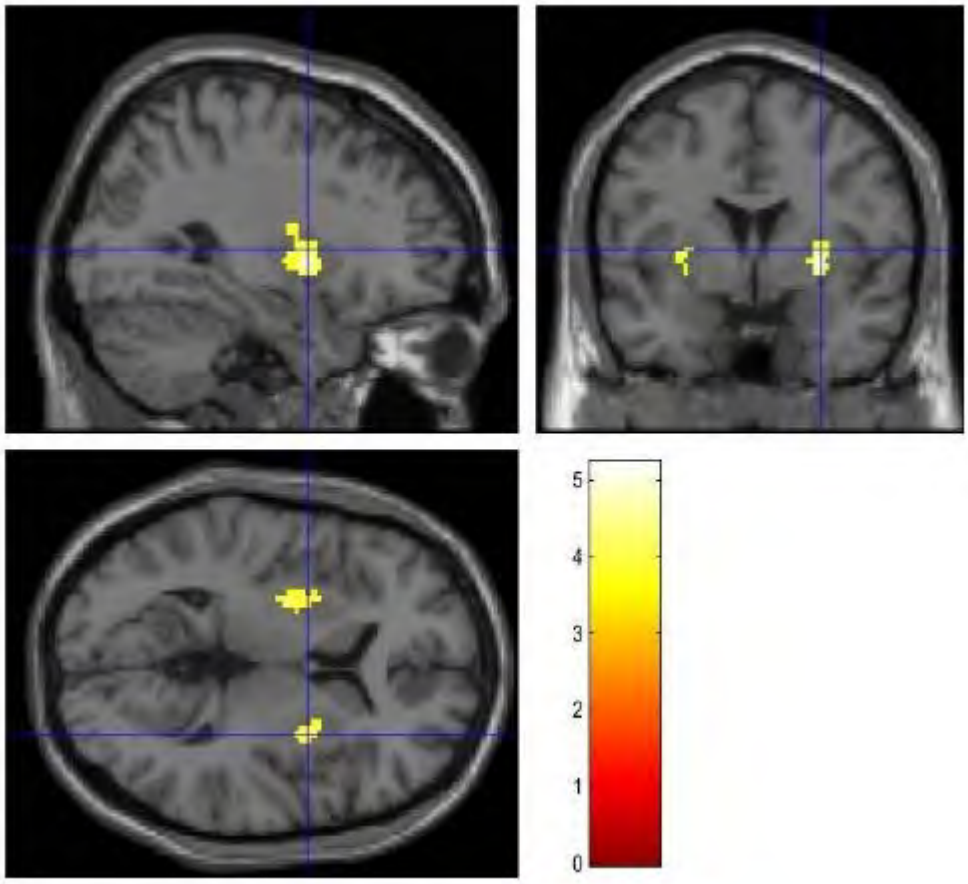
How multiple motives affect the computation of social decisions in the human brain

Anne Christin Saulin[1], Ulrike Horn[2], Martin Lotze[2], Jochen Kaiser[3], Grit Hein[1]

[1]University of Würzburg, Clinic for Psychiatry and Psychosomatic, Würzburg, Germany
[2]University of Greifswald, Functional Imaging Unit, Institute of Diagnostic Radiology and Neuroradiology,Greifswald Germany.
[3]Goethe University, Institute of Medical Psychology, Faculty of Medicine, Frankfurt am Main, Germany.

Often, humans' social decisions are driven by a combination of different social motives, yet little is known at which stage of the decision process different motives interact, and how multi-motive interactions affect neural decision circuitries. To investigate this question, we analyzed prosocial decisions that were driven by either empathy (sharing the emotions of the other) or reciprocity (wish to return a favor), or a combination of empathy and reciprocity.
While undergoing functional magnetic resonance imaging (fMRI), participants performed a social decisions task in which they allocated points in favor of another person (prosocial decision), or in favor of themselves (egoistic decision). Before the social decision task, the empathy and the reciprocity motives were activated separately (single motive condition) or simultaneously (multi-motive condition).
In line with previous findings, results yielded an increased frequency of prosocial decisions for the motive conditions compared to a baseline condition (no motive explicitly activated). Moreover, the combination of empathy and reciprocity resulted in significantly more prosocial decisions than reciprocity alone. These results indicate that the two motives interact, and that empathy enhances reciprocity.
To further investigate the mechanism underlying this enhancement, we conducted hierarchical drift diffusion modelling (HDDM). HDDM characterizes the decision process as a sequential sampling process in which information toward the response options is accumulated over time and a decision is made when a certain information threshold is reached. Based on this model, we assessed whether the single motives and their combination affect A) participants' a priori tendency to make a prosocial decision (z), or B) the speed of information uptake during the decision process (v).
Our results revealed significantly larger z parameters in the multi-motive compared to the reciprocity condition. This indicates that the simultaneous activation of empathy and reciprocity increases participants' a priori tendency to behave prosocially.
To investigate the neural circuitries that underly this increase, we contrasted neural activation during prosocial decisions in the multi-motive condition with activation in the reciprocity condition, and correlated this contrast with the individual increase in the z parameter. The results revealed a bilateral activation of the dorsal striatum (FWE cluster-level corrected ps < 0.018), i.e., a region that is known to be involved in reward-based decision making. The parameter v mirrored a generally increased speed of information uptake for the motive conditions in comparison to the baseline condition.
In sum, we show that prosocial motives and their combination differentially alter the initial tendency to behave in a prosocial manner, and that this shift in prosocial tendencies is tracked by striatal activation.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Mice don't tune in: surprise determines auditory saliency, not selective attention

Karsten Schulze[1], Inga Rauser[1], K. Jannis Hildebrandt [1,2]

[1]
Department of Neuroscience, University of Oldenburg, Oldenburg, Germany
[2]Cluster of Excellence 'Hearing4all', Oldenburg, Germany

Complex acoustics scenes require selective attention in order to extract relevant auditory informationa. Humans are able to selectively attend to one of several streams or certain aspects of sounds. High probability of a cue in one stream or frequency channel will draw attention, such that detection rate for the targets in the attended channel is increased while detection of others is impeded. We asked whether mice show this form of selective attention in three different tasks: (1) detection of single tones in noise (TIN), (2) frequency change detection (FTD) and (3) gap in noise detection using two-stream stimuli.

For all tasks, operant conditioning in a go/no-go paradigm was employed. During TIN detection animals had to indicate the presence of pips of either 10 or 21 kHz. We varied the probability of the target frequency. In the FTD two-stream tasks, we used two intermittent sequences of 10 and 21 kHz tones, and frequency changes in either stream served as targets. Probability of the stream in which targets were presented was varied. For the gap detection task, the two streams of intermediately pulsed narrow-band noise were presented. We used a 10 and a 21 kHz stream with a quarter octave bandwidth. Gap durations varied between 15 and 75 ms. The probability of the gaps in each stream varied per session. If mice listen selectively, performance should depend on the probability of the target: better detection of high-probability targets and decreased performance for infrequent targets.

Surprisingly, performance of the animals was contrary to what would be expected from human psychophysics. In the TIN and in both two-stream tasks, the animals' detection rates were highest for low-probability targets and lowest if the same targets were presented with higher probability. In order to check whether this effect was determined by short term stimulus history or rather by overall target probability, we devised a generalize linear model including overall probability, recent stimulus history and stimulus value as factors. While both stimulus value and overall probability had high predictive power for the behavioral outcome, including recent stimulus history up to five stimuli preceding the target had almost no impact.

Results from the behavioral experiments and the modeling indicate that animals are able track statistics of stimuli over the time course of minutes. However, perceptual saliency is dictated by surprise rather than by expectancy and selective attention.

Lateral prefrontal region 8Av/45 encodes the behavioral relevance of stimulus colors

Philipp Schwedhelm[1,2], Stefan Treue[1,3,4,5]

[1]German Primate Center, Cognitive Neuroscience Laboratory, Kellnerweg 4, 37077 Göttingen, Germany
[2]University of Trento, Center for Mind and Brain Sciences, Via delle Regole 101, 38123 Mattarello (TN), Italy
[3]Bernstein Center for Computational Neuroscience, Am Fassberg 17, 37077 Goettingen , Germany
[4]University of Goettingen, Faculty of Biology and Psychology, Wilhelm-Weber-Str. 2, 37073 Goettingen, Germany
[5]German Primate Center, Leibniz ScienceCampus Primate Cognition, Kellnerweg 4, 37077 Goettingen, Germany

Converging evidence supports the view that the lateral prefrontal cortex (lPFC) of macaques is involved in the generation of an attentional signal (Backen et al., 2018; Bichot et al., 2015; Lennert and Martinez-Trujillo, 2011; Tremblay et al., 2015). This signal is relayed to visual cortex, where it enhances the processing of behaviorally relevant information (Chelazzi et al., 2001; Hayden and Gallant, 2005; Luo and Maunsell, 2015; Martinez-Trujillo and Treue, 2004; McAdams and Maunsell, 2000; Motter, 1994; Schwedhelm et al., 2016), which ultimately leads to behavioral advantages, e.g. higher task accuracy and/or faster reaction times for attended as compared to unattended stimuli (Posner et al., 1980).

To investigate the involvement of primate lateral prefrontal region 8Av/45 in the generation of such a signal, we recorded local field potentials from chronic microelectrode arrays implanted in the lPFC of two rhesus macaques. During data acquisition, the animals performed a feature match-to-sample task and detected unique conjunctions of cued motion and color information in a test stimulus. We then analyzed the data with a machine-learning approach, training classifiers to separate task-relevant variables and decoding this information on a trial-by-trial basis, separately for each monkey.

We found that for both animals, lPFC local potentials recorded from region 8Av/45 were informative about the color of the test stimulus, but not its motion direction. This specialization of 8Av/45 for visual colors, which are locally represented as early as 84ms after stimulus onset points to a putative direct and bottom-up input from visual cortex to lPFC.
Next, our analysis indicated that the animals' upcoming behavior could be robustly predicted from lPFC activity already 152ms after stimulus onset. This signal was mainly related to the detection of matches in the color feature, and was attenuated and delayed for behavioral choices based on motion directions, or unique conjunctions of color and motion. However, in cases in which behavioral responses could not explain differences between trials (i.e. they were the same across all analyzed trials), we still succeeded in decoding whether the color matched the searched-for color, but not whether the motion matched the searched-for motion.

We conclude that the canonical function of macaque region 8Av/45 is the calculation of the behavioral relevance of a given visual color feature, in order to aid the generation of top-down modulatory influences informing goal-directed decision making as well as the deployment of an attentional modulation affecting the responses of neurons in visual cortex. The signal generated by 8Av/45 may thus directly transform into a specific behavioral outcome, or it may be used indirectly, when other visual features are also relevant for a decision, or the generation of a meaningful top-down attentional modulation.
In summary, the information computed at the level of 8Av/45 can be positioned at the intersection between attention and decision, with great relevance for both the planning and execution of reward-based decisions and also the allocation of processing resources onto currently relevant sensory input (Gottlieb, 2018; 2012; Luo and Maunsell, 2018).

Role of cortical areas during pre-stimulus time window in presence of emotional faces and words as distractors: A quantitative EEG study

Tanaya Batabyal[1], Suriya Prakash Muthukrishnan[1], Ratna Sharma[1], Prashant Tayade[1], Simran Kaur[1]

[1]All India Institute of Medical Sciences, New Delhi, Physiology, Ansari Nagar, 110029 New Delhi, India

Pre-stimulus neural activity has been linked to perceptual outcomes during a given task. Intrinsic fluctuations in neural excitability during this period may predict fate of an upcoming stimulus. This seems to be all the more important in case of emotional stimuli, which by virtue of their distractive nature affect behaviour. Further, we wanted to assess, is there any difference in processing of differential distractors i.e words versus face in an emotional interference task, as reflected by their pre-stimulus cortical sources. Thus, in the present study, 17 adults of either gender (25.61 ± 2.78 yr) performed two varieties of emotional interference task (face word and word face), wherein words and faces have acted like distractors respectively. Subjects were instructed to categorise emotion of the face while neglecting the word during face word interference and categorise emotion of the word while neglecting the face during word face interference. Simultaneously, single trial 128 channel EEG was acquired during both the tasks. Further, cortical source activity during pre-stimulus time window of 200ms (to highlight areas associated with premotor response initiation) was compared between the tasks in terms of current source density using sLORETA for 66 gyri. Sixty-three gyri associated with frontal, parietal and temporal areas bilaterally have shown significantly higher activity in face word interference task as compared to word face interference task ($p<0.05/66$). Thus, the current study attempts to explain the plausible implication of higher activity in areas associated with saliency network, dorsal attentional network, ventral attentional network during conflict resolution and monitoring while processing words as distractors and faces as target stimuli. Interestingly rectal gyri which has previously been reported to have a role in emotion processing, showed significantly higher activity in presence of words as distractors during face word interference trials. Thus, the study mapped temporally the probable role of areas associated with neural processing of faces as a complex emotional stream of information and words as potent distractors during an emotional face word interference task.

Fruit flies integrate reward history into foraging decisions

Sophie Elisabeth Seidenbecher[1], Duda Kvitsiani[1]

[1]Aarhus University, DANDRITE, Ole Worms Alle 3, 8000 Aarhus, Denmark

Foraging, or searching for food, is a fundamental behavior exhibited by all moving animals throughout evolution. Every behavior, including foraging, is fundamentally steered by decision-making processes. One particularly interesting type of foraging decision is the decision to return to a spot in the environment that was previously experienced as profitable. We are interested in the basic principles underlying this type of decision. To break down the decision behavior and gain access to these foraging decision principles, we use mathematical models that objectively describe the behavior. These models can give us a set of parameters that can be related to single genes driving this behavior. If all moving animals make foraging decisions according to these principles then we can hypothesize that certain behaviors of foraging and their genetic basis are conserved among different species. We developed an experimental setup to study foraging decisions in male fruit flies using optogenetic rewards and investigated the animal's behavior for different reward probabilities. In particular, we are interested in behavioral changes to varying uncertainty in the environment. Our results so far suggest that, on a population level, the flies base their decisions to return to a rewarded spot more strongly on previous rewards, when the reward probability is low while they become less sensitive to the reward history for higher probabilities. In the long run, our aim is to find computational models for fly foraging decisions and to uncover the key molecular players, by relating single genes and behavior through these models.

A comprehensive anatomical map of the peripheral octopaminergic/tyraminergic system of *Drosophila melanogaster*

Mareike Selcho[1], Dennis Pauls[1], Felix Frantzmann[1], Christine Blechschmidt[1], Basil el Jundi[2]

[1]University of Würzburg, Neurobiology and Genetics, Theodor-Boveri Institute, Biocenter, Am Hubland, 97074 Würzburg, Germany

[2]University of Würzburg, Zoology II, Theodor-Boveri Institute, Biocenter, Am Hubland, 97074 Würzburg, Germany

The modulation of an animal's behavior through external sensory stimuli, previous experience and its internal state is crucial to survive in a constantly changing environment. In most insects, octopamine (OA) and its precursor tyramine (TA) modulate a variety of physiological processes and behaviors by shifting the organism from a relaxed or dormant condition to a responsive, excited and alerted state.
Even though OA/TA neurons of the central brain are described on single cell level in *Drosophila melanogaster*, the periphery was largely omitted from anatomical studies. Given that OA/TA is involved in behaviors like feeding, flying and locomotion, which highly depend on a variety of peripheral organs, it is necessary to study the peripheral connections of these neurons to get a complete picture of the OA/TA circuitry. We here describe the anatomy of this aminergic system in relation to peripheral tissues of the entire fly. OA/TA neurons arborize onto skeletal muscles all over the body and innervate reproductive organs, the heart, the corpora allata, and sensory organs in the antennae, legs, wings and halteres underlining their relevance in modulating complex behaviors.

Behavioral and autonomic defensive responses mediated by periaqueductal gray circuits.

Jérémy Signoret-Genest[1], Nina Scheffler[1], Yavé Lozano[1], Philip Tovote[1]

[1]Defense Circuits Lab, Systems Neurobiology, Institute of Clinical Neurobiology, University Hospital Würzburg, Germany

The mammalian brain evolved to react to threats with stereotypical patterns of activation, defensive emotional states, which give rise to specific behavioural, endocrine and autonomic responses that maximise survival chances. In order to study the precise mechanisms involved, behaviour has classically been used as a proxy for emotions such as fear and anxiety in animal models. In the last few years, our understanding of the neural circuits involved in defensive responses evolved from the implication of particular brain regions to the characterization of cell-type- and input/output-relations of functional cell populations.
However, while behavioural adaptations to threat have been studied in detail, other dimensions of the defense reaction have not received as much attention. Nonetheless, those responses always occur concomitantly and are strongly interrelated; for instance, the autonomic defense response prepares and allows for the expression of appropriate behaviours when animals are under threat.

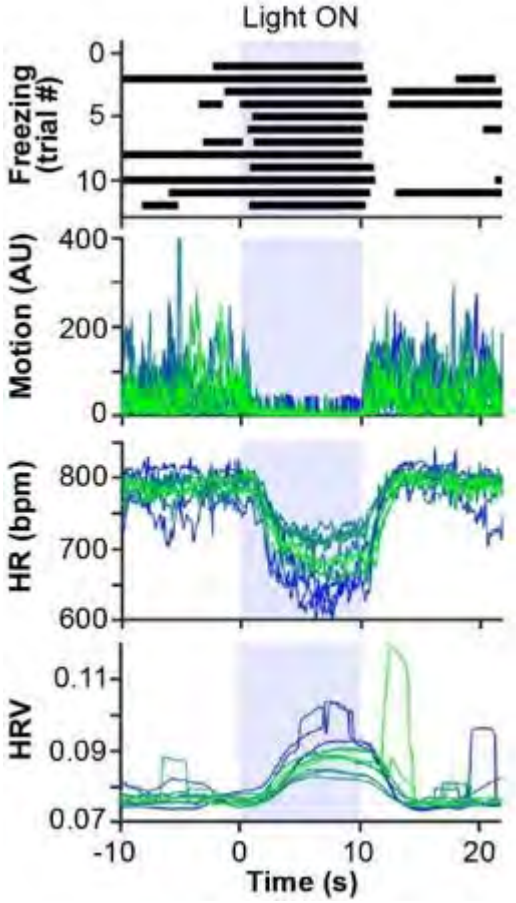
The midbrain periaqueductal gray (PAG) is a major player in mediating multiple aspects of the defense reaction, with the activation of the dorsal PAG (dPAG) eliciting active defensive responses (flight, tachycardia), and the activation of the ventral PAG (vPAG) underlying more passive responses (freezing, bradycardia). More recently, PAG neuronal subtypes and circuit mechanisms mediating threat-induced freezing have been elucidated. Interestingly, specific defensive responses (i.e. behavioural, autonomic, and analgesic) seemed to be parsed into different output pathways from the PAG. Our goal is thus to characterize these functional pathways in terms of their long-range inputs and targets as well as on the level of local microcircuits.

Using in vivo deep brain calcium imaging, we observed switches in neuronal activity depending on the behavioural state of the animal and found that activity of specific PAG neuronal subpopulations (glutamatergic vs. GABAergic) correlates with either active or passive behavioural states in the conditioned flight paradigm. Optical activation of glutamatergic vPAG cells switched active behaviour to freezing and elicited strong bradycardia as well as increased heart rate variability, suggesting activation of the parasympathetic branch of the autonomic system. Furthermore, neuroanatomical tracing studies have revealed glutamatergic projections from to the vPAG to the nucleus of the solitary tract (NTS), providing a route through which the vPAG might exert autonomic control.

Our results confirm PAG cellular subpopulations as neuronal substrates for different behavioural but also cardiac autonomic defensive states. Interference with functionally characterized brain stem circuit elements carrying descending command signals or ascending interoceptive information may allow for selective manipulation of emotion states.

Figure – Optical activation of vPAG glutamatergic cells.
Optogenetic activation (shaded area) of glutamatergic cells in the vPAG elicited freezing (top and second panels), a decrease in heart rate (HR), and an increase in heart rate variability (HRV). Each line represents one trial.

AU: arbitrary units; bpm: beats per minute.



Involvement of rat medial prefrontal cortex in reward and punishment trade-off during perceptual choice

Vanya Valkanova Stoilova[1], Maik Stüttgen[1]

[1]Institute of Pathophysiology & Focus Program Translational Neurosciences, University Medical Center of the Johannes Gutenberg University, Duesbergweg 6, 55128 Mainz, Germany

Adaptive decision making is a complex cognitive process that requires animals to estimate the possible risks, costs and benefits of the available choice alternatives to ultimately select the option exhibiting the highest value. Because natural environments are inherently volatile, the animals must frequently adjust their behavior taking into account both potential rewarding and aversive outcomes of their actions. Therefore, a profound understanding of adaptive decision making requires comprehension of the cognitive and neural mechanisms by which animals integrate information about positive and negative aspects of choice alternatives to select an optimal course of action. However, the computations that the brain is performing to trade off reward and punishment in order to guide behavioral choices have barely been investigated.

To model the uncertainty existing in natural environments in a laboratory setting, we subjected rats to a two-alternative auditory discrimination task employing varying reward and punishment outcome probabilities. Depending on the response of the animal (correct or incorrect), the outcomes could be a drop of water (reward) or a foot shock (punishment). To investigate the trade-off between reward and punishment, we manipulated the probabilities for reward and punishment outcome in a blockwise manner. This allowed us to track the dynamics of decision criterion setting as a function of the integration of reward and punishment contingencies. Recording single-neuron action potentials in rat medial prefrontal cortex (mPFC) during task performance, we found that a subset of PFC neurons' firing rates reflected both previous and upcoming choices. Other neurons signaled response direction, impending punishment, while very few neurons responded to the auditory stimuli. Pharmacological inactivation of mPFC through intracerebral muscimol infusion resulted in reduced discrimination performance and increased response variability, consistent with a model in which the effects or reward and punishment are augmented through mPFC inactivation. The low stimulus discrimination performance of mPFC neurons, along with the tight coupling of neural activity to motor output, suggests that decreased discrimination performance is due to impairment of response selection or execution mechanisms rather than sensory processing.

Single neuron and population dynamics in rodent prefrontal cortex during time reproduction

Kay Thurley[1,2], David Bunk[1], Josephine Henke[1]

[1]LMU Munich, Department Biologie II, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany
[2]Bernstein Center for Compuational Neuroscience Munich, Germany

To identify temporal relationships between events, anticipate actions and guide behavior animals need to estimate durations across several time scales. Timing, however, is often investigated for sub-second intervals and typically tasks are used that only test for single intervals or for the discrimination of two intervals (at least in rodents). The ability to estimate and reproduce intervals chosen from a continuous range of values of several seconds has rarely been investigated. How such durations judgments are processed neurally is not known.
We performed time reproduction experiments with Mongolian gerbils (*Meriones unguiculatus*), while recording neural activity in their medial prefrontal cortex, a brain area that has been in connection to time processing in rodents. The animals were trained to first measure the duration of a visual stimulus lasting between 3 and 7.5 seconds and afterwards reproduce it by running for the same duration in virtual reality.

Similar to previous studies employing other timing tasks, single neurons exhibited diverse firing patterns in our experiments. Some neurons were transiently active, i.e., displayed single or multiple firing peaks, others monotonically decreased or increased their firing rate (ramp-up/-down), yet others combined transient and ramping responses. About half of the neurons showed activity adapted to the stimulus interval. Many neurons were strongly active only either in measurement or reproduction. Cells that responded during both phases typically displayed different activity patterns. Other factors contributing to neural activity were identified fitting generalized linear models and included the animal's running speed or the distance it covered during the reproduction phase.

To characterize the heterogeneous single cell responses, we used dimensionality reduction techniques at the population level to extract common patterns of neural activity and then fed back this information to look at their expression in single cells. With this we were able to quantify the contribution of different response types in the population. In addition, we also examined population activity with these analysis techniques. Using demixed PCA, a specialized version of principal component analysis, we could separate time course-dependent from stimulus-dependent and time/stimulus-interaction components of neuronal activity. We found that population activity was strongly driven by the stimulus during measurement whereas the time course explained most of the population responses during reproduction. Nevertheless, population trajectories shared large similarities between measurement and reproduction.

In sum, we provide a characterization of single neuron and population signals in rodent prefrontal cortex that may contribute to the judgement of duration.

Exogenous attention improves temporal resolution in the auditory systems of humans and mice: perceptual effects and underlying neural mechanisms

Simge Türe[1], Basak Günel[1], Tina Gothner[2], Christiane M. Thiel[1,3], K. Jannis Hildebrandt[2,3]

[1]Department of Psychology, University of Oldenburg, Germany
[2]Department of Neuroscience, University of Oldenburg, Germany
[3]Cluster of Excellence Hearing4all, University of Oldenburg, Germany

A sudden, salient auditory stimulus may capture attention towards a previously unattended auditory object in a multi-source auditory scene. Such a shift of attention can not only serve to allocate cognitive resources to a potentially relevant signal, but also change sensory processing of the capturing sounds. Surprisingly little is known about effect of exogenously triggered attention on auditory processing. Some evidence for changes in sensory processing after involuntary attentional capture comes from the visual domain, where exogenous attention enhances spatial but hampers temporal resolution.

Here, we aimed to characterize both perceptual consequences and underlying neural mechanisms following attentional capture in the auditory domain. We chose to focus on temporal resolution, measured by the ability to detect brief gaps in tones after drawing attention to one out of two auditory streams. To this end, we devised a stimulus consisting of two interleaved sequences of tones, separated by at least an octave and rapid enough to evoke a clear two-stream percept, mimicking two distinct sources. Targets could appear in either of the two streams. A part of the targets was preceded by an attention-capturing cue in either the same stream as the target (valid) or the other (invalid). We used this stimulus set in three different experiments: (1) In a psychophysics study in human subjects we asked the participants to report whether they had perceived a gap in either of the two streams. (2) We tested mice in a corresponding go-no go paradigm. (3) We performed electrophysiological recordings in the primary auditory cortex (ACx) of awake-behaving mice.

Human psychophysics revealed that exogenous attention improves auditory temporal resolution. Invalid cues, drawing attention to the none-target stream, impeded the ability of the subjects to detect gaps, while valid cuing improved gap-detection performance compared to the uncued condition. Behavioral tests of mice on the same set of stimuli confirmed the results from the human study: a benefit of valid versus invalid cued trials in the detection of gaps. Recordings from populations of single-units in ACx provided evidence for a neural correlate of the observed behavior: stronger population responses for validly vs. invalidly cued gaps. Analysis of population dynamics in responses to either to the two tone-sequences showed that the main effect of cueing was a phase-shift of oscillatory activity: advances in the responses to the tone sequence containing the cue and delay in the respective other.

In summary, we found evidence for well-preserved effect of exogenous attention on the encoding and processing of temporal details of an auditory stimulus. The similarity between perceptual effects in humans and mice opens opportunities to further deepen our understanding of the underlying neural mechanisms, including coding in the auditory pathway and the contribution of neuro-modulatory systems.

Applying unsupervised machine learning to study the lateral hypothalamic circuitry underlying motivated behaviour in freely moving mice

Hanna Elin van den Munkhof[1], Tatiana Korotkova[1]

[1]Max Planck Institute for Metabolism Research, Neuronal circuits and behaviour, Gleueler Str 50, 50931, Cologne, Germany

To understand the brain functions, it is crucial to relate neural activity to behaviour. While highly advanced tools exist to characterize neuronal activity in behaving animals, current methods for the analysis of behaviour rely on manual scoring or identification using supervised machine learning (1). Manual scoring is highly subjective, while supervised machine learning is automated but still assesses a limited set of behaviours predefined by a human observer. Since behaviours are regulated by changes in neuronal dynamics occurring at a fast, sub-second, time scale, we are in need of more refined and objective methods to analyse behaviour at high temporal resolution.

Here we apply MoSeq, an unsupervised machine learning algorithm (2,3), to automatically analyse mouse behaviour based on depth images. MoSeq allows for the unbiased identification of behaviour, uncovering novel behaviours with sub-second precision. Wiltschko et al. (2) have shown that mouse behaviour consists of a sequence of reused modules with defined transition probabilities, which can change depending on environment, genetic or neural manipulation.

We use MoSeq to study the lateral hypothalamic circuitry underlying motivated behaviour, with a focus on primary rewards, including feeding. The lateral hypothalamus (LH) comprises multiple cell types, each of which has a unique function in the regulation of innate behaviours. We have recently shown a role of GABA cells in the LH in feeding-related behaviours and arousal (4,5). To assess the functions of other neurochemically defined cell groups in the LH, we now manipulate activity of these cells using chemogenetics (DREADDs), while mice engage in a range of innate behaviours. We aim to gain insight into implication of these LH subpopulations in primary rewards. Ultimately, a better understanding of the neurocircuitry directing motivated behaviour will aid in treatment improvements for various psychiatric disorders, including eating disorders, addiction and depression.

We gratefully acknowledge support by the Max Planck Society and the ERC Consolidator Grant (HypFeedNet, to TK).

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A rat model of reward conditioning using optogenetic VTA stimulation

Vivekanandhan Viswanathan[1], Frank W Ohl[2], Markus Fendt[3], Michael Thomas Lippert[4]

[1]Systems Physiology of Learning, Working Group Neuro-Optics, Leibniz Institute of Neurobiology, Magdeburg, Germany
[2]Systems Physiology of Learning, Leibniz Institute of Neurobiology, Magdeburg, Germany
Institute of Biology, Otto-von-Guericke University, Magdeburg, Germany
Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany
[3]Institute of Pharmacology and Toxicology, Otto-von-Guericke University, Magdeburg, Germany
Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany
[4]Systems Physiology of Learning, Working Group Neuro-Optics, Leibniz Institute of Neurobiology, Magdeburg, Germany
Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany

To survive in an ever changing world, organisms have to adapt to changing stimulus-outcome contingencies. A major driving force for such adaptation is reward. In the mammalian brain, rewards are processed by the mesolimbic dopamine system, which—through the encoding of reward-prediction error—facilitates neuronal plasticity. Here we present a novel paradigm to measure the effects of reward conditioning through its influence on the acoustic startle response. We transduced the VTA of TH::Cre rats with ChR2 and implanted an optical fiber into the VTA, allowing specific stimulation of dopamine neurons. After two weeks of expression time, rats underwent a baseline startle measurement and seven consecutive sessions of optogenetic self-stimulation (US). Animals could only operate the self-stimulation nose poke in the presence of a visual cue (CS), but not in the absence of the cue. Following this operant conditioning, animals underwent a second startle test to assess the influence of visual cue presentation on startle. To compare our results to previous studies, we also include rats that underwent electrical, instead of optogenetic, self-stimulation. In both groups we find a reduction in startle amplitude following the presentation of the paired CS.

Characterization of a mouse model with a central knockout of BDNF

Matthias Wilhelm Voigt[1], Ina Eiffler[1], Oliver von Bohlen und Halbach[1]

[1]University Medicine Greifswald, Institute of Anatomy and Cell Biology, Friedrich-Loeffler-Str. 23c, D-17489 Greifswald, Germany

Neurotrophins, such as brain-derived neurotrophic factor (BDNF), play a role in central functions of the brain like learning and memory (Leal et al. 2017, Monteggia et al. 2004), eating behaviour (Rios et al. 2013, Lebrun et al. 2006) and anxiety-related behaviour (Hashimoto 2007). Loss of BDNF or impairments in the BDNF-signalling are therefore linked with brain dysfunction. However, its role in the postnatal brain has remained difficult to assess, since the BDNF-null mutation is lethal (Rauskolb et al. 2010).

We therefore used C57Bl6/N conditional mutant mice floxed at the BDNF locus and with Cre expression in neurons expressing neurofilament L ($BDNF^{flfl}$, cre^{+}). NFL expression starts shortly after birth, to that these mice loose BDNF expression in pyramidal neurons, projection neurons, Purkinje cells, and motor neurons, but mice do survive. To assess whether the loss of central BDNF affects basic behaviour, we performed several behavioural test with $BDNF^{flfl}$, cre^{+} and their control littermates ($BDNF^{flfl}$, cre^{-}): Open Field, Nest Building, Marble Burying, Dark/Light-Box, Elevated Plus Maze, and Novel Object Recognition (van Gaalen and Steckler 2000). We used female and male mice and performed every test with all mice at the age of three, six, and nine month to cover possible gender and age effect, respectively. Furthermore, we measured food intake and weight of the mice weekly.

We only saw differences between mice lacking BDNF in neurons expressing NFL ($BDNF^{flfl}$, cre^{+}) and their control littermates ($BDNF^{flfl}$, cre^{-}) in some of our analysis (i.e. Marble Burying). Interestingly, changes in behaviour of BDNF knock out mice increased with age. This may be due to the fact, that BDNF expression is highest immediately after birth and decreases with age. We suggest that young mice may cope better with a loss of central BDNF than old mice (i.e. through a higher expression of BDNF by glial cells).

Poster Topic

T25: Learning and Memory

T25-1A Differential functional innervation and corelease from midbrain dopaminergic neurons in amygdala subregions
Ayla Aksoy-Aksel, Anna Seewald, Andrea Gall, Johannes Ungermann, Francesco Ferraguti, Ingrid Ehrlich

T25-2A Behavioral analysis of conditional knockout mice for a presynaptic active zone protein Bassoon in excitatory, inhibitory and dopaminergic nerve terminals
Anil Annamneedi, Gürsel Caliskan, Eike Budinger, Anna Fejtova, Wolfgang Tischmeyer, Oliver Stork, Eckart D. Gundelfinger

T25-3A Learning and memory capacities in classical and operant conditioning tasks underlies individuality in the cockroach *Periplaneta americana*
Cansu Arican, Janice Bulk, Nina Deisig, Martin Paul Nawrot

T25-4A An automated touch screen test battery measuring cognitive decline in mice: minimal experimenter intervention and no food restriction
Dalia Morsi Attalla, Katharina Stumpenhorst, York Winter

T25-5A Concept neurons in the human medial temporal lobe reflect relational processing
Marcel Bausch, Johannes Niediek, Thomas P. Reber, Sina Mackay, Jan Boström, Christian E. Elger, Florian Mormann

T25-6A Fear generalization in a differential mouse fear conditioning paradigm: Role of gender, shock intensity and neuropeptide S (NPS) receptor deficiency.
Jorge R. Bergado-Acosta, Virginia Prameswari, Markus Fendt

T25-7A Augmented ventral hippocampal network oscillations in mouse strains with elevated anxiety and impaired fear extinction
Gürsel Caliskan, Oliver Stork

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Differential functional innervation and corelease from midbrain dopaminergic neurons in amygdala subregions

Ayla Aksoy-Aksel[1,2], Anna Seewald[3], Andrea Gall[1,2], Johannes Ungermann[1,2], Francesco Ferraguti[3], Ingrid Ehrlich[1,2]

[1]Hertie Institute for Clinical Brain Research and Centre for Integrative Neuroscience, University of Tübingen, Tübingen, Germany

[2]Department of Neurobiology, IBBS, University of Stuttgart, Stuttgart, Germany

[3]Medical University of Innsbruck, Department of Pharmacology, Innsbruck, Austria

Dopamine (DA) is a modulatory neurotransmitter that plays a vital role in reward processing, motivation and various types of learning and memory. Dopaminergic innervation to the forebrain structures originates mainly from the ventral tegmental area (VTA) and substantia nigra compacta (SNC) nuclei that are located in the ventral midbrain. In the amygdala, the densest tyrosine hydroxylase positive innervation reaches the intercalated cells (ITC), but innervation is also apparent in the central (CeA) and the basal (BA) nuclei. Manipulation of dopamine signaling in amygdala has been shown to modulate acquisition and expression of fear. However, the cell physiological effects of direct activation of dopaminergic fibers in different amygdala regions is incompletely understood.

To address the properties of dopaminergic fibers from midbrain to amygdala we use an ex-vivo optogenetic approach. Cre-dependent AAV encoding Channelrhodopsin2-YFP was injected into the midbrain in DATCre mice to specifically target dopaminergic cells. As expected, in the amygdala we observed dense projections of YFP containing fibers that were tyrosine hydroxylase positive. To assess the physiological properties of this input we recorded optically evoked postsynaptic currents (PSCs) from dorso-medial (dm) ITC and CeA cells in whole cell patch-clamp mode. We observed short-latency, monosynaptic currents that had reversal potentials and pharmacological signatures consistent with glutamatergic or GABAergic synaptic transmission suggesting co-release from dopaminergic fibers. Interestingly, the majority of co-release in the CeA is glutamatergic whereas in dmITC co-release is mainly GABAergic, and no co-release was detected in basal amygdala. We confirmed the putative synaptic contacts via immunostaining for the presynaptic marker bassoon and in accordance with our co-release data also identified glutamatergic and GABAergic terminals via vGlut and vGAT staining, respectively. Besides co-release, we also explored the effect of phasic stimulation of dopaminergic fibers on spontaneous synaptic activity in dmITC cells and observed reduction of sIPSC amplitude but no effect on sEPSCs. On the other hand, direct application of DA resulted in hyperpolarization in a fraction of dmITCs suggesting differential effect on cellular excitability.

Our results extend the knowledge on the connectivity and function of midbrain dopaminergic input to amygdala subregions and highlight differences that involve fast synaptic transmission.

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Behavioral analysis of conditional knockout mice for a presynaptic active zone protein Bassoon in excitatory, inhibitory and dopaminergic nerve terminals

Anil Annamneedi[1], Gürsel Caliskan[2,4], Eike Budinger[1,4], Anna Fejtova[1,3,4], Wolfgang Tischmeyer[1,4], Oliver Stork[2,4], Eckart D. Gundelfinger[1,4]

[1]Leibniz Institute for Neurobiology, 39118 Magdeburg, Germany
[2]Institute of Biology, Otto-von-Guericke-University, 39120 Magdeburg, Germany
[3]Department of Psychiatry and Psychotherapy, University Hospital, 91054 Erlangen, Germany
[4]Center for Behavioral Brain Sciences (CBBS), 39106 Magdeburg, Germany

Functions of Bassoon, a large scaffolding protein at cytomatrix of the presynaptic active zone, in learning and memory processes are still elusive. Bassoon is present at excitatory, inhibitory, modulatory presynapses and plays an important role in various aspects of presynaptic development, is involved in homeostatic and associative synaptic plasticity, as well as in the regulation of presynaptic autophagy and synapto-nuclear communication. However, using the constitutive Bassoon knockout mice it is difficult to understand its role in different cognitive processes as they display severe seizures, sensory deficits and impaired presynaptic function.

The current study specifically focuses on elucidating the role of Bassoon in learning and memory processes, using conditional knockout approach by crossing the mice having floxed alleles around exon2 of the *Bsn* gene (*Bsn2*$^{lx/lx}$ mice) with different Cre driver lines. Three different conditional *Bsn* mutants were generated: one lacking Bassoon in excitatory forebrain synapses (*Bsn2*$^{lx/lx}$- Emx1 Cre; in short *B2E cKO*), the other lacking the protein in synapses of inhibitory interneurons (*Bsn2*$^{lx/lx}$- Dlx 5/6 Cre; in short *B2I cKO*) and one line with selective deletion of protein in dopaminergic nerve terminals (*Bsn2*$^{lx/lx}$- DAT Cre; in short *B2D cKO*). Specificity of the conditional knockout mice was tested by immunohistochemial analysis. We studied these mutant mice in different learning paradigms.

B2E cKO mice are hyperactive in their home cages and show selective enhancement in background contextual fear memory and improved performance in a pattern separation task. These behavioral changes in *B2E cKO* mice are accompanied by an augmentation of baseline synaptic transmission at medial perforant path (MPP) to dentate gyrus (DG) synapses in the hippocampus and morphological changes including increased dendritic complexity and dendritic length at DG granule cells. A lack of age-dependent reduction in excitability of MPP-DG and altered cellular maturation markers suggest an impaired maturation in the DG of *B2E cKO* mice, arguing for a specific role of Bassoon expression in excitatory synapses during maturation of the MPP-DG network. Our preliminary results on *B2I cKO* mice lacking the protein at inhibitory synapses, display novelty-induced hyperlocomotion and increased anxiety in an open field arena. Further they display social memory and nest building deficits. On the other hand, *B2D cKO* mice display hyperactive behavior only during dark phase of the 24 hr cycle. Further they do not exhibit any behavioral alterations concerning anxiety, novelty recognition, motor coordination or fear memory. Altogether, Bassoon deficiency in different types of synapses has distinct effects on brain performance and causes specific behavioral alterations.

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Learning and memory capacities in classical and operant conditioning tasks underlies individuality in the cockroach *Periplaneta americana*

Cansu Arican[1], Janice Bulk[1], Nina Deisig[1], Martin Paul Nawrot[1]

[1]Institute of Zoology, Computational Systems Neuroscience, Zülpicher Straße 47b, 50674 Köln, Germany

Classical learning experiments in insects are predominantly conducted in flies and bees [1,2]. When an odor is paired with a reward, bees learn to associate both stimuli and respond with the proboscis extension response (PER) at the presence of the odor alone (conditioned stimulus, CS), even before the reward (unconditioned stimulus, US) is presented in consecutive trials. This provides an easy means to test a binary conditioned response behavior [2]. Furthermore, honeybees show inter-individual differences in learning performance[3]. However, in cockroaches individuality in learning is still not investigated, but individuality has been reported in other behavioral traits [4]. The aim of the present study was to emphasize the importance of the American cockroach as model organism for the study of learning and memory by underlining the importance of different learning and memory capacities in different individuals.

We used classical and operant conditioning paradigms in which cockroaches were trained to associate odors or spatial cues (CS) with a reward or punishment (US). In classical conditioning tasks we presented odors stimulus (CS+) paired with reward (sugar solution) or odor stimulus (CS-) paired with punishment (saline solution) to harnessed cockroaches. We tested different classical conditioning protocols by observing the movement of the Maxillum and Labium as conditioned response, which reflects learning success similar to the conditioned PER response in honeybees. This classical conditioning protocol in the harnessed cockroach allows for electrophysiological brain recordings in fix conditions, similar to recordings performed in the honeybee [5]. For operant conditioning, cockroaches were placed in a T-maze where we established different forced choice tasks. For example, when cockroaches entered the dark arm of the maze, light was used as aversive stimulus in a spatial conditioning protocol. The ability to learn the darkness-avoidance was compared between individual cockroaches.

In summary, our results demonstrate that *P. americana* is a good insect model which can be successfully trained in classical and operant conditioning tasks. However, importance should always be rendered to the fact that not all individuals exhibit the same learning and memory abilities and behavioral data should always been dissected on the individual level. Furthermore, established conditioning tasks were used for experiments coupled with neurophysiological recordings.

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An automated touch screen test battery measuring cognitive decline in mice: minimal experimenter intervention and no food restriction

Dalia Morsi Attalla[1], Katharina Stumpenhorst[1], York Winter[1]

[1]Humboldt University, Biology department

The recently developed, non-aversive touch-screen behavioural tasks for animals mimic the human clinical assessment. Nevertheless existing protocols incorporate food deprivation and animal handling, which can induce stress, anxiety and impulsive behaviour. The present study was designed to develop a free-feeding, self-motivated, automated touch screen test battery for group housed mice,in which the majority of food is collected during task performance throughout the day. Hereby, we wanted to achieve less variable and thus more reproducible results.C57BL/6J ID-chippedmice, 10 weeks old, were tested in a 24/7 automated system consisting of a touch screen equipped operant chamber connected to the home cage through a transponder-based sorting system (Fig.1a). Two different cognitive domains were examined in the automated system: working memory was assessed with a spatial location task(TUNL), and, sustained attention and response inhibition was assessed through the five-choice-serial-reaction-time-task. Mice received food pellets from the operant chamber based on their task performance. They were allowed multiple individual chamber visits with short session duration (15 minutes) which maintained motivation. Mice successfully learned the spatial task, that is normally difficult to acquire. Preliminary data show a clear effect on performance when the choice phase is delayed for 6 secondsas compared to only 3seconds(Fig.1b, 1c). In the ID-chip experimental setup mice acquired both tasks, with performance levels comparable to previous protocols, while avoiding animal handling and food deprivation

Figure 1: a) Automated touchscreen experimental setup. b) Number of sessions to reach criterion for each spatial separation level. c) Effect of delay (mixed spatial separation), data presented as mean ± SD. Data from n= 6 mice.

Concept neurons in the human medial temporal lobe reflect relational processing

Marcel Bausch[1], Johannes Niediek[1], Thomas P. Reber[1,3], Sina Mackay[1], Jan Boström[2], Christian E. Elger[1], Florian Mormann[1]

[1]University of Bonn Medical Center, Department of Epileptology, Sigmund-Freud-Straße 25, 53127 Bonn, Germany
[2]Department of Neurosurgery, University of Bonn Medical Center, 53127 Bonn, Germany
[3]Faculty of Psychology, Swiss Distance Learning University, Brig, Switzerland

The human medial temporal lobe is important for relational processing and memory. It contains “concept neurons” that represent semantic rather than perceptual features of presented stimuli. The activity of such neurons has been shown to reflect whether or not their preferred stimulus is kept in working memory. Here we asked whether concept cells could play a role in relational processing.

During 38 experimental sessions, we recorded from 2512 neurons in the amygdala, parahippocampal cortex, entorhinal cortex, and hippocampus of 12 neurosurgical patients performing perceptual or semantic comparisons of visual stimuli. Before the experiment, four images were chosen based on a screening procedure to maximize the likelihood of eliciting selective visual responses. In each trial of the main experiment, subjects viewed one of five possible questions, followed by a sequence of two of the four images that had to be compared. Subjects indicated the sequential position of the stimulus that best answered the question by pressing keys “1” or “2”. Four questions required semantic processing of the stimuli (”Bigger?”, “Last seen in real life?”, “More expensive”/”Older?”, “Which do you like better?”), one question only required perceptual processing (“Brighter image?”). Two control conditions with the same structure but different questions were additionally included in the task.

We detected 61 semantic concept neurons with increased firing during the presentation of one of the images relative to baseline (significant binwise-signed-rank, alpha level of 10^{-5}) and higher firing for both the preferred image and its written name relative to other images and written names, respectively (Hedges’ g greater than 0.3). About half of these concept units responded to the non-preferred stimuli with a delayed but well-defined onset (about 400 ms later) whenever the task required a comparison to the response-eliciting concept. Firing patterns of 22 local pairs of concept neurons resulted in asymmetric population cross correlation peaks on short (<25 ms) as well as longer (200-700 ms) time scales if and only if their preferred concepts had to be compared semantically.

For the first time we could directly monitor the activity of concept cells as a neuronal correlate of relational processing. Task-imposed ordered relations of concepts were expressed in ordered firing patterns of concept neurons. Their sequential activity holds the potential to store concept relations in memory via spike-time dependent or behavioral time scale plasticity and should be the topic of further investigation.

Fear generalization in a differential mouse fear conditioning paradigm: Role of gender, shock intensity and neuropeptide S (NPS) receptor deficiency.

Jorge R. Bergado-Acosta[1,3], Virginia Prameswari[1,2], Markus Fendt[1,3]

1-Institute for Pharmacology and Toxicology, Otto-von-Guericke University, Magdeburg, Germany.

2-Intergrative Neuroscience Program, Otto-von-Guericke University, Magdeburg, Germany.

3-Center for Behavioral Brain Sciences, Otto-von-Guericke University, Magdeburg, Germany.

During Pavlovian fear conditioning, subjects quickly learn to associate an aversive event with a preceding environmental cue. This process is an adaptive learning mechanism, since it allows subjects to predict and to better cope with future aversive or stressful events. Notably, this memory generalizes, i.e. not only the original learned stimulus but also similar stimuli trigger the previously acquired behavioral response. Under certain circumstances, this generalization of fear memories is too extreme ("over-generalization") which then can lead to maladaptive fear responses, i.e. individuals respond with fear to cues that do not predict the aversive event or to cues predicting safety (absence of danger). Such overgeneralization can be observed in anxiety disorders such as phobias, panic disorder, or post-traumatic stress disorder.
In this study, adult male and female mice were conditioned with a differential fear conditioning paradigm using tone stimuli of different frequencies as a CS+ and CS-. Later, their fear memory was tested with an array of ascending or descending frequency stimuli that allowed measuring fear generalization. In different experiments, we tested the effects of (1) the used frequencies, (2) foot shock intensity, (3) gender, (4) deficiency of the neuropeptide S receptor, and (5) previous mild stress. Our results showed that (1) using a CS- of a low frequency and a CS+ of high frequency led to nicely differentiated fear responses which was not the case with the opposite. (2) Conditioning animals with higher foot shock intensities resulted in more generalization of the fear memory, only in male mice. (3) After fear conditioning with moderate foot shock intensities, female mice had a more generalized fear memory than males. Furthermore, (4) mice with NPS receptor deficiency don't showed changes in behavior when compared with their wildtype littermates and (5) preliminary data showed main effects of previous mild stress in both sex, but not gender x stress interaction. Investigating principles of fear conditioning and its generalization in mice may be useful for understanding the pathological basis for a variety of anxiety disorders.

Augmented ventral hippocampal network oscillations in mouse strains with elevated anxiety and impaired fear extinction

Gürsel Caliskan[1], Oliver Stork[1]

[1]Institute of Biology, Department of Genetics & Molecular Neurobiology, Otto-von-Guericke-University, 39120 Magdeburg, Germany

Accumulating evidence suggest that inbred mouse strains differ in several behavioural, molecular and physiological aspects that are relevant to psychiatric conditions related to aberrant memory formation such as post-traumatic stress disorder (PTSD). Specifically, both C57BL/6N mouse (B6N) and 129S2/SvPasCrl (129S) have been shown to have elevated anxiety, impaired fear extinction and generalization of fear. Recent studies from our group suggested that ventral CA3 associative network activity is closely related to (Caliskan et al, 2013, 2015, 2016) the strength of fear memory reconsolidation and extinction which are the key factors for the development of PTSD. Due to an extensive network of axon collaterals within principal calls and also interneurons, CA3 associative network can generate several learning- and memory-related network activities such sharp wave-ripples (SW-R) or gamma oscillations (30-80 Hz). Thus, in this study, we recorded local field potentials from CA3 and CA1 subregions of ventral-to-mid hippocampal slices. We found that the maximum orthodromic PS was significantly reduced in both B6N and 129S in comparison to BJ6 indicating a possible increase in inhibition within the CA3 associative network of B6N and 129S. Furthermore, analysis of SW-R clearly indicated that incidence of SWs were much stronger in CA1 subregion of both B6N and 129S in comparison to B6J. Similarly, gamma oscillations induced by carbachol (5 µM) were much stronger in CA3 subregions of both B6N and 129S evident by significant augmentation of both integrated and peak gamma power without any alterations in gamma peak frequency. Augmented hippocampal oscillations in both B6N and 129S suggests an altered interneuron function as reported before (Caliskan et al., 2016). Together with our previous studies, these data indicate that augmented ventral hippocampal oscillations can be one the contributing factors for increased anxiety and reconsolidation of fear memories predisposing individuals to develop PTSD.

Time to learn: changing the valence of an odor with experience

Florencia Campetella[1], Roman Huber[1], Martin Klappenbach[2], Fernando Locatelli[2], Bill Hansson[1], Markus Knaden[1], Silke Sachse[1]

[1]MPI for Chemical Ecology, Olfactory Coding Group, Hans-Knoell-Str. 8, 07745 Jena, Germany

[2]University of Buenos Aires, Argentina

During its life a fly is constantly being exposed to a wide array of olfactory stimuli, many of them being essential for the fly's survival and reproduction. While some odors seem to be attractive, others result in avoidance behaviors. This attraction or avoidance, also known as hedonic valence of an odorant, is often thought of in the context of a long standing dichotomy: learned or innate. Flies are able to learn to associate olfactory information with a reward or punishment and the circuits involved have been traced back to the neurons of the mushroom bodies. Far less is known about innate behavior and its circuits. We do know that the lateral horn mediates innate attraction and avoidance towards odorants but whether its neurons are able to show plasticity after a learning experience is still not known. In this study we explored this question by assessing if it is possible to change the valence of innately attractive odorants and how this change is reflected in the physiology of neurons and circuits of the lateral horn – the center for innate behavior.

Life history of navigational exploration and social communication in honeybees

Xiuxian Chen[1], Ryuichi Okada[2], Stefan Walter[1], Midori Sakura[3], Yuan Xing[4], Randolf Menzel[1]

[1] Institut für Biologie, Neurobiologie, Freie Universität Berlin, , Königin-Luise-Str. 28/30 , 14195 Berlin, Germany
[2]School of Human Science and Environment, University of Hyogo, Shinzaike-honcho, Himeji, Hyogo 670-0092, Japan
[3]Department of Biology, Graduate School of Science, Kobe University, Rokkodai-cho 1-1, Nada-ku, Kobe, Japan
[4]Technische Universität Berlin, Fakultät Verkehrs- und Maschinensysteme, Berlin, Germany

Early exploratory experience and social interactions influences the brain development and attributes the lasting influences on behaviors throughout the later life history, including decision-making during foraging and social communication. It is still not well understood when and to which extent early experience shapes the behaviors. Honey bees are central place foragers that rely on ephemeral and scattered floral resources. Successful navigation of bees depends on the how familiar they are with the terrain around the hive. Consequently, honeybees explore the environment around the hive in multiple orientation flights, learn about the sun compass and calibrate the visual odometer before they begin their foraging activities and their social communication (waggle dance). It is unknown at which stage of exploration honeybees become foragers, whether they require additional information from social communication and how the specific information acquired during exploration determines their early foraging activities and their social communication. We specifically addressed the question how the knowledge of the landscape in individual bees is bound to their social communication inside the colony and their foraging decisions. The life history from the first day after emergence was monitored in 94 individually marked bees. Their exploratory orientation flights were tracked by harmonic radar, and their indoor activities were monitored by complete video recordings. Special emphasis is given to the relation between out-door flight trajectories and in-door social communication. Young bees followed waggle dances only if they had explored already the environment. About 50 % of the dance followers attended more than one waggle dancing bee involving dancers that advertised different feeding places. The selected dance and the decision for the indicated feeder appears to be connected to the experience collected during the follower ´s individual experience during exploration. The data are used to create a model relating the life history of young foragers to their individual experience during exploration, learning and social communication.

Calcium Imaging of Putative Engram Cells in *Drosophila*

Benjamin Escribano[1], Dominique Siegenthaler[1], Jan Pielage[1]

[1]Technical University Kaiserslautern, Zoology-Neurobiology, Erwin-Schrödinger-Str. 13, 67663 Kaiserslautern, Germany

The association of environmental cues with a reward or punishment is essential for the survival of many animals. Learning, memorising and retrieval of this information in the central nervous system is key to induce specific behavioural adaptations. These processes induce structural and physiological modification of synapses thereby leading to altered activity at the network level. Once network activity is modulated, it can induce adaptive behavioural responses toward the conditioned stimulus.
Here we use the *Drosophila* mushroom body (MB) as a model system for learning and memory. The MB represents the learning and memory center of *Drosophila* and is numerically simple and genetically accessible. Through the implementation of tissue specific drivers and reporter constructs we are now able to specifically express neuronal visualisation or manipulation tools in selective neuronal subtypes of the MB. Here we use a novel tool to specifically label a small number of putative MB engram cells with the calcium-reporter GCaMP6f after aversive olfactory conditioning. By combining multi-photon live imaging with *in vivo* stimulations we aim to gain insights into the circuit mechanisms of memory encoding. Of particular interest are the identification of the memory-relevant MB cell population and potential modulations of odor responses. In combination with silencing and activation experiments we hope to advance our understanding of memory formation and storage.

Circuit Rules of Compulsive Behaviour in *Drosophila*

Johannes Felsenberg[1], Paola Cognigni[1], Sai Parepalli[1], Scott Waddell[1]

[1]University of Oxford, Centre for Neural Circuits and Behaviour, Mansfield Road, OX1 3SR Oxford, United Kingdom

Associative learning uses neural representation of rewards to steer behaviour towards the efficient seeking of desirable outcomes. In the addictive state, reward-seeking can override real-world stimuli and behaviour can become pathological: compulsive and ultimately destructive. We have studied a compulsive appetitive memory circuit in *Drosophila* to identify the features that distinguish a naturalistic appetitive association from situations which lead to destructive memories. This paradigm results in flies so behaviourally driven to their odour cue that they are willing to endure punishment in its pursuit, and neglect food even when starving. Conversely, silencing activity in the relevant neural circuitry results in aversive memories reminiscent of animals in drug withdrawal. By investigating maladaptive behaviours that are not observed in flies trained with a naturalistic appetitive stimulus, we hope to understand how reward representations can be hijacked to generate compulsive behaviour.

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Spatial and image selectivity of hippocampal neurons in virtual reality mazes.

Dustin Fetterhoff[1], Christian Leibold[1]

[1]Ludwig-Maximilians-Universität München, Biologie II, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany

Virtual reality is useful to study spatial navigation because it enables environmental manipulations that would be unfeasible in real world setups. In this study, male Mongolian gerbils (Meriones unguiculatus) were exposed to two different virtual hallways that could be identified based on both turning direction and sequence of images on the walls of straight hallways. Specifically, maze A had two right turns and images of zebra skin, stars and targets, while maze B had two left turns and images of moons, pyramids and leaves. All turns were made at 45 degree angles with plain white walls and images in the next hallways were not visible until turns were completed. Milk reward was automatically given at the end of the maze. One objective of this study was to identify single-unit and population activity of place cells during navigation of a maze with a well-known sequence of images compared to one which had images shuffled between two different image sequences. By shuffling between image sequences, identification of neuronal activity corresponding to spatial location versus image selectivity will be possible.

After learning how to navigate mazes with well-known sequences, a micro-drive with eight individually movable tetrodes was implanted above the right hippocampal CA1 and CA3 regions. Over a period of two weeks, tetrodes were lowered to the hippocampal principal cell layer where single units and local field potential recordings were made before, during and after virtual maze navigation. On testing days, gerbils first ran 20 laps with the well-known images, while the last 20 laps contained the initial image from one maze type but the final two images from the opposite maze type (i.e., mixed maze A contained zebra skin, pyramid and leaf; mixed maze B contained moon, stars and targets.). The turning direction was always consistent with the first image perceived at the start of each lap.

Behavioral results showed that gerbils learned how to navigate virtual mazes. For analysis purposes, mazes were divided into three hallway and two corner segments. Analysis of electrophysiological data collected when images were shuffled between mazes showed that hippocampal principal cells fired specifically to either spatial location, turning direction or to specific images, while some cells only fired during the initial maze but not after shuffling. Single unit remapping analysis showed that more neurons tended to fire based on turning direction, an effect that was more pronounced in the first beginning and middle segments of the maze. In the last segment, image cues more strongly contributed to place cell firing patterns coinciding with the end of the maze and milk reward. Population vector correlations were low between the initially, learned unshuffled mazes. After shuffling images, population vector correlations were strongest between mazes with the same turning direction during the first and middle hallway segments, while the last segments showed an even mixture of correlation strength between image and turning direction. Hippocampus is strongly influenced by spatial cues, but we found an additional response to images linked to rewards at the end of mazes.

In Vivo Recordings Reveal the Encoding of a Conditioned Behavioural Choice in an Identified Neuron

Sabine Feyl[1], Wolfram Schulze[1], Stefan Schuster[1]

[1]University of Bayreuth, Department of Animal Physiology, Universitätsstraße. 30, 95444 Bayreuth, Germany

Fish launch their powerful escape C-starts in response to sudden unexpected events that could signal the imminent attack of a predator. To perform these life-saving manoeuvres, the fish unilaterally contract their trunk muscles, which cause their body to bend into the shape of the letter ‘C’. Much as in the release of a spring, the subsequent straightening, with fins erected, quickly pushes water backwards and accelerates the fish away from the zone of danger. In intact fish, these C-starts are commanded by the pair of Mauthner neurons and their associated networks in the medulla oblongata. The giant Mauthner cells are uniquely identified neurons that integrate incoming sensory information from all sensory systems and then decide on the basis of the current information and in concert with associated networks whether a C-start should be initiated. As soon as one of the two Mauthner cells fires a single spike, the other cell will be inhibited and the fish's body will bend to the side contralateral to the active cell away from the threat.

Although they are often referred to as simple reflexes, C-start manoeuvres can be quite variable. Ideally, they should be unpredictable as long as they move the fish away from the threat. Surprisingly, we were able to train goldfish not to escape but rather to approach a threat. In these experiments we used a commonly used stimulus to elicit C-starts: An exponentially expanding disk, shown on a screen above the tank, mimics the approach of a predator. We trained two fish to show distinctly different escape directionality to the looming stimuli: One group was trained to always escape directly and as far as possible away from the stimulus. The other group, however, could be trained not to escape but rather to rapidly swim towards a position directly under the looming disk. This second group thus quickly produced C-starts to approach the same stimulus that caused escape C-starts in the other group. Even more surprisingly the C-starts of both groups were kinematically fully equivalent.

Given the importance of the Mauthner neurons in initiating the C-starts and in setting the direction of the C-bend, this finding immediately raised an exciting possibility: would the two different training regimes cause distinct and clear differences in how the Mauthner neurons responded to the looming stimulus? If so, then *in vivo* intracellular recordings of PSPs induced by the looming stimulus should show clear and quantifiable differences depending on which prior training – 'escape' or 'approach' – the experimental fish had received before. In these recordings, the looming disk was shown on either side of the trained fish to test, if the training had affected the directionality of the Mauthner cell PSPs. The major finding of this approach is that the PSPs were indeed clearly different, depending on prior training and that the characteristics of the PSPs directly allow us to predict whether the experimental fish belonged to the 'escape' or 'approach' training group. These findings raise exciting opportunities to study how learning translates into changes in the properties of an individual uniquely identified neuron in the vertebrate brain.

Associative olfactory learning in *Drosophila* induces de-correlation of calcium activity in axonal γ-lobe Kenyon cell boutons

André Fiala[1], Florian Bilz[1], Bart Geurten[2]

[1]University of Göttingen, Molecular Neurobiology of Behavior, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

[2]University of Göttingen, Cellular Neurobiology, Julia-Lermontowa-Weg 3, 37077 Göttingen, Germany

Plastic changes in synaptic transmission represent a neuronal substrate underlying learning and memory formation. Since sensory stimuli are typically encoded as sparsely distributed activity across assemblies of many neurons, it is challenging to determine which and how individual synaptic connections change to acquire a stimulus-specific memory. Here we used in vivo calcium imaging in *Drosophila* to monitor learning-induced synaptic plasticity. Fruit flies can learn to avoid an odor stimulus that is temporally paired with a punitive electric shock. We trained fruit flies positioned under a two-photon microscope using this classical aversive olfactory conditioning regime, and monitored odor-evoked calcium activity through a window cut in the head capsule. One odor (CS+) was presented in coincidence with a punitive electric shock. A second odor (CS-) was subsequently presented without punishment. Control animals received the same odorant stimulation, but without the electric shock. The MARCM technique was used to express the calcium sensor GCaMP in single γ-lobe Kenyon cells of the mushroom body, a brain region to which the acquisition of associative olfactory short-term memory could be localized. We measured odor-evoked activity in synaptic boutons along individual axons and across many neurons. Using a subsequent immunohistochemical staining we could assign axonal boutons to specific γ-lobe sub-compartments. A comparison of calcium activity before and after associative learning revealed that associative learning induced bi-directional changes in synaptic bouton activity. Moreover, odor-evoked synaptic bouton activity within and across single Kenyon cells de-correlated as a result of associative learning, and specifically for the CS+. No de-correlation between boutons was observed for the calcium activity evoked by the CS- or the control odor. This reveals a novel principle of how associative memories can be differentially encoded across assemblies of neurons and axonal compartments.

Individual consistency in the learning performance of honeybees

Valerie Finke[1,2], David Baracchi[2], Martin Giurfa[2], Ricarda Scheiner[1], Aurore Avarguès-Weber[2]

[1]University of Würzburg, Behavioral Physiology & Sociobiology, Biocenter, Am Hubland, Würzburg,Germany
[2]Centre de Recherches sur la Cognition Animale, Centre de Biologie Intégrative (CBI), Université de Toulouse, CNRS, UPS, Toulouse, France

Since the first discovery that the individual performance of humans in a variety of cognitive tasks is positively correlated, the study of general intelligence or g factor has been extensively studied in humans. There is now increasing evidence for the existence of a general intelligence in a wide range of vertebrate species and factors underlying those individual differences in cognitive ability. However, the study of correlated performances in cognitive abilities of individuals and consistent individual differences has been mainly ignored in invertebrate species. The ability to learn and discriminate between various stimuli often belonging to different sensory modalities is ecologically highly relevant for honeybees to successfully collect from variable food sources. The honeybee has long been proven as a powerful model organism for the study of various elemental and non-elemental forms of learning and cognition. Thereby a lot of studies reported that honeybees exhibit appreciable variation in their performance in several learning tasks. However, it remained unclear if this variation in learning performance is consistent for individuals across time and if the performance is correlated between different learning tasks. In the present study, it was therefore examined if individual honeybees show consistency in their discrimination performance 1) over time, 2) between discrimination tasks of different complexity and 3) between discrimination tasks involving different sensory modalities. Using differential conditioning we found that the individual test performance was consistent over a time span of three days in free-flying honeybees. Similarly, the individuals' performance in an elemental visual discrimination task and a non-elemental concept discrimination task was positively correlated. Finally, neither a correlation nor a trade-off was found when comparing the performances of individual bees in a visual and olfactory discrimination task. These findings indicate that some individual bees perform consistently better than others in elemental discrimination tasks under free-flying conditions across a reasonable time frame and also between learning tasks which require different cognitive capabilities. Demonstrating correlated cognitive performances of individual honeybees challenge the classical view of invertebrates being merely "reflex machines" which only stereotypically respond to stimuli. Furthermore, such findings stretch the importance of considering individual differences as important factor accounting for variability in the cognitive abilities of invertebrates.

Compass systems during ant learning walks: The earth's magnetic field is the geostable reference system for taking snapshots in *Cataglyphis*

Pauline Nikola Fleischmann[1], Robin Grob[1], Valentin Leander Müller[1], Rüdiger Wehner[2], Wolfgang Rössler[1]

[1]University of Würzburg, Behavioral Physiology and Sociobiology (Zoology II), Am Hubland, 97070 Würzburg, Germany

[2]University of Zurich, Brain Research Institute, Winterthurerstrasse 190, 8057 Zurich, Switzerland

Ant learning walks are short, explorative trips during which so-called novices do not bring back any food items. Instead they circle around the nest entrance and acquire information necessary for successful foraging later on, i.e. they have to learn landmarks and calibrate their compass systems. Learning walks of *Cataglyphis noda*, an ant species inhabiting Greek pine forests, include two different types of turns. (1) Voltes are walked 360° circles without directed stopping phases. The function of voltes is not known yet. (2) Pirouettes are full or partial turns about the ant's vertical axis that are frequently interrupted by stopping phases. The ant's gaze direction during the longest stopping phase is directed towards the nest entrance presumably to take snapshots of the homing direction. Since *C. noda* novices do not use celestial compass cues, like the UV polarization pattern or the position of the sun, as a directional reference system for aligning their gazes back to the nest entrance during learning walks, we tested whether they use the earth's magnetic field for that task. First, we set up an electromagnetic spiral around the natural nest entrance which provided different directional information at any point, and, thus, made the resulting magnetic field useless as a reference system. The gaze directions of novices during the longest stopping phases of learning-walk pirouettes were randomly distributed under this condition. In a second experiment, we set up a Helmholtz coil that offers a precisely controlled magnetic field in the strength of the earth's magnetic field. Ants' gaze directions were randomly distributed when the horizontal component of the geomagnetic field was zeroed. In contrast, after doubling the strength, the gaze directions were still oriented towards the nest entrance. Furthermore, when the horizontal component was rotated, the ants' gaze directions predictably shifted about the same angle. Therefore, the earth's magnetic field is the necessary and sufficient compass cue for *Cataglyphis* novices to align their gaze directions towards the nest entrance during learning-walk pirouettes. As the ants cannot see the nest entrance from their different positions around the nest during learning walks, we conclude that magnetic compass information is integrated into their path integration system. Supported by DFG SFB 1047 (B6) and DFG project Ro1177/7-1, both to WR.

Associative remapping of odor representations by inhibitory network plasticity

Thomas Frank[1], Nila Moenig[1], Chie Satou[1], Shin-ichi Higashijima[2], Rainer Friedrich[1]

[1]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

[2]National Institutes of Natural Sciences, Exploratory Research Center on Life and Living Systems, National Institute for Basic Biology, 5-1 Higashiyama, Myodaijicho, Okazaki, Japan

Intelligent behavior depends on associations between high-dimensional sensory representations and low-dimensional, behaviorally relevant qualities such as valence. Learning of associations involves plasticity of excitatory connectivity but it remains poorly understood how information flow is reorganized in networks and how plastic inhibition contributes to this process. We trained adult zebrafish in an appetitive odor discrimination task and analyzed odor representations in a specific compartment of telencephalic area Dp, the homolog of olfactory cortex. Associative conditioning enhanced the intensity and selectivity of responses to the positively conditioned odor (CS+). Moreover, conditioning systematically remapped odor representations along an axis in coding space that represented valence. Inter-individual variations in odor-to-valence mapping predicted variations in behavioral odor preference. Photoinhibition of interneurons attenuated the representation of the CS+, reversed odor remapping, and reduced inter-individual variations in coding space. These results reveal an individualized odor-to-valence map that is reorganized during learning by plasticity of inhibitory network interactions.

To be in the right place at the right time: *Drosophila* learning in the heat maze

Felix Frantzmann[1], Dennis Pauls[1]

Neurobiology and Genetics, Theodor-Boveri-Institute, Biocenter, University Würzburg, Germany

Time-Place learning is the ability to remember specific events in a spatiotemporal manner. This is essential as many environmental aspects show daily variations such as food sources and mating partners as well as behavioral characteristics of predators. Although the formation and recall of memories are energy-demanding, time-place memories optimize resource localization and survival rate. Importantly, time-place learning is not only an attribute of higher animals since time-place memories as a survival strategy are present in a variety of animals including insects, birds, fish and rodents. Our preliminary experiments so far aimed to establish a visual place learning platform which closely resembles the Morris water maze used for rodents. The Morris water maze like arena is suitable to perform visual learning experiments in insect species including *Drosophila melanogaster*. Next, we will address whether *Drosophila melanogaster* is able to perform time place learning and how time information from the circadian clock is integrated into memory processes. Our preliminary data suggest that place memories are -at least- modulated by the circadian clock.

Neural correlates of decision making in bumble bees in a laboratory environment

Inga Fuchs[1], Benjamin H. Paffhausen[1], Randolf Menzel[1]

[1]Freie Universität Berlin, Institute of Biology, Neurobiology, Königin-Luise-Straße 28/30, 14195 Berlin, Germany

The aim of this study is to analyze the neural correlates of the bumblebee Bombus terrestris while they are navigating through a laboratory based environment making decisions based on the spatial arrangement of local and panorama cues. The wings of the bumblebees are cut making them walking in the test arena that includes access to their colony. The bees are free to choose where to go. They are appetitively trained in the test arena to different tasks, e.g. to choose a colored local cue, a location relative to the panorama or a matching to sample task in which a cue at the access path indicates which direction to choose. The long lasting extracellular recordings are made from mushroom body extrinsic neurons while the animal is still behaving naturally. The mushroom body is known for receiving multimodal sensory information, consolidating and retrieving memory. The intention is to search for neuronal correlates of both basic properties of navigation (path integration, target orientation) as well as high order cognitive performances involving navigation according to the pattern of the panorama and the stimulus at the entrance to the arena.

The synapto-nuclear messenger Jacob alters nucleolar dynamics to facilitate protein synthesis in plasticity

Camilla Fusi[1], Anna Karpova[1], Christina Spilker[1], Daniela C. Dieterich[2], Michael R. Kreutz[1,3]

[1]RG Neuroplasticity, Leibniz Institute for Neurobiology, Magdeburg, Germany
[2]Institute for Pharmacology and Toxicology, Otto-von-Guericke-University, Magdeburg, Germany
[3]Leibniz Group 'Dendritic Organelles and Synaptic Function', Center for Molecular Neurobiology, Hamburg, Germany

Jacob is a protein messenger that encodes and transduces the synaptic and extrasynaptic origin of gluN2B-containing NMDA receptors (NMDAR) to the nucleus and couples NMDAR activity to CREB-dependent gene expression (Karpova *et al*., 2013). Nuclear import of Jacob following activation of extrasynaptic NMDAR leads to long-lasting dephosphorylation of CREB, loss of dendritic arborization and eventually cell death. Vice versa, nuclear import of Jacob following activation of synaptic NMDARs stimulation enhances plasticity related and CREB-dependent gene expression. However, apart from association with CREB still very little is known about the nuclear function of Jacob. Here we report that following nuclear import Jacob also localizes at nucleoli in hippocampal or cortical neurons. Nucleoli are those sub-nuclear compartments where assembly of ribosomal RNA (rRNA) and pre-ribosomal subunits takes place. Nucleolar dysfunction contributes to the pathology of several rare human genetic disorders as well as neurodegenerative disorders (Montanaro *et al*., 2008; Hetman *et al*., 2012). Decreased rRNA synthesis and nucleolar disruption, known as nucleolar stress, are hallmarks of cellular stress associated with aging and neurodegenerative diseases (Pietrzak *et al*., 2011; Lee *et al*., 2014). Interestingly, it has been shown that neuronal stimulation and prolonged neuronal activity, *via* the translocation of the synapto-nuclear messenger AIDA-1d, increase protein synthesis by controlling nucleolar number and thereby regulating processing and maturation of ribosomal RNA (rRNA) (Jordan *et al*., 2007). Here we show that Jacob and AIDA-1d associate *in vivo* and co-localize in dendritic spines and the nucleus. We show that Jacob together with AIDA-1d can link between synaptic activity and control of *de-novo* protein synthesis machinery by regulating nucleolar assembly.

Local amygdala network competition and cooperation in long-term memory

Ki Ann Goosens[1], Yee Fun Lee[2], Seh Hong Lim[1,2], Samiksha Shah[2], Abby Rudolph[2]

[1]Icahn School of Medicine at Mount Sinai, Psychiatry, One Gustave Levy Place, Box 1230, New York, NY 10029
[2]Boston University School of Medicine, 72 East Concord St, Boston, MA, 02118 USA

Long-term fear memories are thought to arise from competitive Hebbian processes across spatially segregated single neurons of the basolateral amygdala (BLA). We sought to examine these processes by using time-lapse microendoscopy to monitor calcium dynamics in BLA neurons across fear conditioning and long-term recall in mice. Consistent with the idea that only a subset of “eligible” neurons are able to encode fear memories, many neurons with tone-shock sensory convergence during fear conditioning were not activated by the tone during fear recall. These neurons became less responsive to the footshock and did not show plasticity during conditioning. Contrary to expectation, neurons activated by the tone during recall (putative memory “winner” neurons) formed small clusters. These neurons became more responsive to the footshock and displayed increased tone-responsivity during fear conditioning. Contrary to Hebbian principles, a majority of tone-responsive neurons during fear recall exhibited no sensory convergence during fear conditioning. Additionally, roughly one-third of tone-responsive neurons during recall completely lacked tone-shock sensory convergence during fear conditioning, suggesting the existence of a previously unrecognized cell class involved in fear expression. These data suggest that competition for the substrate of long-term memory is critically regulated by shock sensitivity during learning, and that long-term memory arises from Hebbian processes that emerge across clustered networks rather than individual neurons.

Characterization of connectivity in synaptic complexes of the mushroom-body calyx in the honeybee *Apis mellifera*

Claudia Groh[1], Annekathrin Lindenberg[1], Christian Stigloher[2], Wolfgang Rössler[1]

[1]University of Würzburg, Department of Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany
[2]University of Würzburg, Imaging Core Facility, Am Hubland, 97074 Würzburg, Germany

Honeybees undergo striking age-related behavioral transitions that offer an excellent experimental model for investigating the neuronal processes underlying the control of behavioral plasticity. In the honeybee, the mushroom bodies (MBs) – sensory integration and association centers in insects – contain large numbers of intrinsic neurons (Kenyon cells, KCs). They receive multimodal sensory input (mostly vision and olfaction) segregated into different subdivisions of the MB calyx. Visual and olfactory projection neurons (PNs) form discrete modular synaptic complexes (microglomeruli, MG) mainly with KC dendritic spines within all calyx subdivisions. In recent years, 3D quantitative imaging of MG in the calyx of Hymenoptera revealed that sensory exposure causes PN bouton pruning, whereas the formation of stable long-term memory leads to volume independent PN bouton increases. Serial-section electron microscopy of young honeybee nurses compared to experienced foragers detected substantial changes at the level of synaptic sites and their connectivity resulting in an increase of synaptic divergence by ~34% in individual MG.

One prerequisite for understanding the mechanisms of this massive synaptic reorganization in the MB calyx is to characterize the PN-KC connectivity and subcellular architecture in the pre- and postsynaptic MG microcircuit. We currently apply 1) serial-section electron tomography (ET) and 2) neurotracing via electroporation (EP) in combination with 3D deep tissue imaging to characterize the architecture and plasticity at the pre- and postsynaptic site of individual MG at high resolution. ET based 3D reconstructions at the MG presynaptic site revealed, for the first time, the detailed ultrastructure of synaptic release sites (active zones, synaptic vesicle pools) within individual PN boutons. Preliminary results at the postsynaptic site based on a combination of labeling only few non-compact (spiny) KCs via EP followed by synapsin-immunolabeling indicates that this class of KCs extends only a single spine to individual PN boutons in young nurses, and that this one-KC-spine per one-PN-bouton relationship is similar in experienced foragers. Given the substantial increase of synaptic divergence within single MG in foragers compared to nurses, this suggests that the pre- and postsynaptic relationship is maintained despite a massive outgrowth of KC dendrites. By further combining the two approaches, we aim to gain more insights into pre- and postsynaptic plasticity of the synaptic connectivity in MG microcircuits. This is crucial for understanding general mechanisms of experience related structural synaptic changes in the MBs promoting behavioral plasticity.

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Neuropeptides in *Cataglyphis* desert ants and their role as potential modulators of behavior

Jens Habenstein[1], Franziska Schmitt[1], Emad Amini[2], Markus Thamm[1], Reinhard Predel[3], Christian Wegener[2], Susanne Neupert[3], Wolfgang Rössler[1]

[1]University of Würzburg, Behavioral Physiology and Sociobiology (Zoology II), Am Hubland, 97074 Würzburg, Germany
[2]University of Würzburg, Department of Neurobiology and Genetics, Am Hubland, 97074 Würzburg, Germany
[3]University of Cologne, Department for Biology, Institute of Zoology, Zülpicher Str. 47b, 50674 Cologne, Germany

Insect societies exhibit a highly sophisticated social organization. Besides the presence of a reproductive and a non-reproductive caste, individual workers within a colony undergo a behavioral maturation with changing tasks along their lifetime. Ants of the genus *Cataglyphis* are favorable experimental models as they show a pronounced age-related polyethism with distinct behavioral-stages. *Cataglyphis* desert ants fulfill tasks such as brood care or nest construction inside the dark nest before they leave their nest to perform visually guided long-distance navigation during foraging trips under bright sunlight. This marked transition from interior workers to foragers provides an excellent possibility to study the intrinsic mechanisms initiating and controlling this remarkable change in behavior. Recent studies started to associate neuropeptides with the modulation of behavioral-stage transitions in social Hymenoptera including *Cataglyphis* ants. Since a profound knowledge about neuropeptides in *Cataglyphis* ants was missing, we biochemically characterized neuropeptides in the brain of *C. noda* by using matrix assisted laser desorption/ionization mass spectrometry (MALDI MS). We further applied MALDI imaging MS (MALDI IMS) and immunohistochemistry, which revealed the localization of 29 neuropeptides in the brain of the ants. To precisely map the localization of neuropeptides, we reconstructed a three-dimensional brain model based on anti-synapsin, anti-serotonin and f-actin stainings. This neuronal brain map differentiates 36 synapsin-rich neuropils and 28 fiber bundles within the *Cataglyphis* brain. For subsequent studies, we focused on corazonin and tachykinin, two potential candidates for modulating the behavioral-stage transitions in *Cataglyphis*. To probe for stage-related differences of neuropeptide expressions between interior workers and foragers, we combined immunostaining and quantitative PCR. Although we did not find any qualitative differences at the level of immunostainings, quantitative PCR revealed that the corazonin titer was significantly higher in foragers compared to interior workers. In ongoing studies, we address how the increase of corazonin levels is regulated, e.g. by first light exposure or an age-related internal program. Furthermore, we will manipulate neuropeptide levels to reveal their specific effects on behavior.

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Imaging Odour Representations and Learning-Induced Plasticity at Mushroom Body Output Neuron Postsynapses

Clare Hancock[1], André Fiala[1]

[1]Georg-August-Universität Göttingen, Molecular Neurobiology of Behaviour, Julia-Lermontowa Weg 3, 37077 Göttingen, Germany

The mushroom body is a key integration centre in the insect brain. Among many others, one process localized to this region is that of learning and memory. By compiling information regarding sensation, internal state, and previous experience, the mushroom body can signal to downstream brain regions to influence the execution of appropriate behavioural outputs.

The olfactory system of *Drosophila melanogaster* provides a well-mapped model system in which to study these processes. By delivering, simultaneously, an odour stimulus and an electric shock, formation of strong associative memories can be induced such that the given odorant would later be avoided. By combining this classic olfactory conditioning paradigm with *in vivo* calcium imaging, we can investigate the neural activity underpinning these behavioural changes.

A coincidence in the arrival of signals conveying olfactory information (represented in the mushroom body by the Kenyon cells) and electric shocks (via a subset of dopaminergic neurons) results in the plastic changes underlying learning-mediated behavioural adaptation. Current models postulate that these changes are represented primarily as modulation of the synapses between Kenyon cells and their principle downstream partners, the Mushroom Body Output Neurons (MBONS). Recent publications have supported this theory, and indicated a role for these output neurons in valence encoding.

This project focusses on these MBONs and their modulation through olfactory associative conditioning. Using the postsynaptically-localized calcium sensor homer-GCaMP, we investigate the changes in Kenyon Cell-MBON signaling induced by odour/shock pairing.

Pavlovian-instrumental transfer is sensitive to outcome devaluation and motivational shifts

Wolfgang Hauber[1], Susanne Sommer[1], Alexandra Münster[1]

[1]University of Stuttgart, Department of Neurobiology, Pfaffenwaldring 57, 50569 Stuttgart, Germany

Stimuli predictive of food can override physiological control and induce food intake in rats regardless of satiety [1]. Thus, it is conceivable that in humans food-predictive stimuli may also override satiety to enhance overeating and contribute to obesity. However, control of food seeking by food-predictive stimuli as a function of the incentive value of food reward as well as motivational states (i.e. hunger, satiety) is poorly understood.

In the present study, we analyzed control of food-directed action by learned food-predictive stimuli using the outcome-specific Pavlovian-instrumental transfer (sPIT) test. In sPIT, rats were first trained to press two levers, each earning a unique food reward (pellets or sucrose), after which they were given Pavlovian training in which two auditory stimuli (tone and white noise) were paired with these same reward. Finally, the effects of the two stimuli on performance of the two instrumental actions were assessed in extinction. In the sPIT test, a Pavlovian stimulus enhances the action with which it shares a specific reward, but not another with which it does not share a specific reward [2]. For example, a sucrose-predictive stimulus increases pressing the lever associated with sucrose reward more than pressing the other lever that earns pellets.

Here, we examined whether a reduction of food reward value by pre-feeding of sucrose or pellets ("outcome devaluation") or a prior shift of motivational state from hunger to satiety alters sPIT. If food-predictive stimuli are indeed able to override satiety and to promote food-directed action, then, sPIT performance should be insensitive outcome devaluation and motivational shifts.

Experiment 1 revealed that performance in the sPIT test was sensitive to outcome devaluation. That is, the stimulus that predicted the devalued food reward was still able to promote instrumental action directed to that reward, however, response vigor was considerably reduced relative to the valued food reward condition.

In Experiment 2, two sPIT tests were performed, one under a restriced feeding regimen with limited access to lab chow and another one under an ad libitum feeding regimen with unlimited access to lab chow. Results show that performance in the sPIT test was sensitive to a shift in motivational state. That is, in a satiated state, stimuli were still able to promote instrumental action with which it share specific reward, however, response vigor was markedly reduced relative to a hungry state.

In Experiment 3, we used a simplified version of the standard task [1] to assess whether stimuli predictive of food can trigger intake of the same food (rather than lever pressing for food). Results show that stimuli can increase food intake in satiated animals, however, the effect size was markedly lower as in hungry animals.

Taken together, our data demonstrate that food-predictive stimuli can promote food-directed instrumental action or food intake, even in a satiated state or if expected food reward has been devalued. However, the ability of stimuli to enhance instrumental action was markedly reduced relative to a hungry state or to the valued food condition. Therefore, our data question the idea that food-predictive stimuli can override physiological control and induce food seeking regardless of satiety [1].

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Signal integration of dopaminergic neurons in *D. melanogaster*

Michael-Marcel Heim[1], Davide Raccuglia[1], David Owald[1]

[1]Institute of Neurophysiology, Charité – Universitätsmedizin Berlin

Evaluation of reward or punishment together with the ability to associate salient features with environmental cues is crucial for goal-directed behavior. In all higher animals, associative learning as well as related tasks, such as motivation and motor control, are enabled by dopaminergic neurons (DANs). DANs of the *Drosophila* Mushroom Body (MB), their learning and memory center, convey signals of salient features. Dopaminergic signaling during associative learning alters the transmission of the MB intrinsic neurons (Kenyon Cells) towards the Mushroom Body Output Neurons (MBONs). As a result, the dynamics of the MBON network in response to neutral stimuli are tuned towards avoidance or approach behavior. Recent discoveries of reciprocal connections between these cell types highlight their role in memory formation and initiation of context dependent behavior. However, little is known about how DANs integrate signals of their synaptic partners. To address this gap in knowledge, we combined Ca^{2+} and voltage imaging approaches paired with neurotransmitter injections in explant *Drosophila* brains. We tested several neurotransmitters and found profoundly different response kinetics between different DAN cell types. These results contribute to a better understanding of DAN computation during memory formation and goal directed behavior.

Functional connectivity analysis of the nucleus reuniens of the thalamus upon remote fear memory attenuation

Hendrik Heiser[1,2], Bianca A. Silva[1], Nana Sato[1], Johannes Gräff[1]

[1]Swiss Federal Institute of Technology (EPFL), Brain Mind Institute, Lausanne, Switzerland
[2]International Max Planck Research School, Georg-August University Göttingen, Germany

Traumatic fear memories are highly persistent over time, but can be updated and attenuated upon recall in a safe environment. While the recall of recent memories is dependent on the hippocampus, remote memory storage is thought to rely more on a distributed cortical network. However, the neuronal circuits underlying remote fear memory attenuation remain largely unknown.

We recently found that the nucleus reuniens of the ventral midline thalamus (NRe), a central hub in the remote memory network, is recruited upon remote fear memory attenuation (Silva et al., 2018). We hypothesize that its connectivity with the medial prefrontal cortex (mPFC), hippocampal area CA1 and the basolateral amygdala (BLA) is critical for remote memory extinction. In line with these findings, the mPFC-NRe circuit seems to be involved in the extinction of recent fear memories (Ramanathan et al., 2018).

Here, we used a combination of retrograde viral tracing and activity-dependent cFos imaging in a remote fear memory attenuation paradigm in the mouse to investigate the functional connectivity of the mPFC-NRe-BLA axis. We found that BLA-projecting neurons in the NRe are clustered in the medial portion of the anterior NRe, and less present in the posterior NRe. Moreover, these BLA-projecting neurons show increased activity during extinction, contrasting a large population of BLA-projecting neurons in the dorsally adjacent rhomboid nucleus. These results may suggest different functional roles corresponding to the physical separation of these projections.

Currently, we are causally investigating the role of the mPFC-NRe-BLA axis in remote fear memory attenuation by chemogenetic functional manipulations. We hypothesize that the mPFC projections to the NRe may modulate the fear memory attenuation induced by the NRe-BLA fibres.

Our findings will improve our understanding of how long-term fearful memories are processed and updated, and specifically how fear responses to a remote traumatic memory can be attenuated. This has important implications for the treatment of traumatic memories in humans, where fear extinction through exposure therapy forms the basis of most treatments for post-traumatic stress disorder (PTSD).

Genetically-encoded differences in cortical dopamine affect phasic dopamine release in nucleus accumbens and modulate the effect of cue salience on associative learning

Anna Huber[1,2], Nebojsa Jovanovic[1], Lydia Oikonomidis[1], Elizabeth M Tunbridge[1], Mark E Walton[2]

[1]Department of Psychiatry, University of Oxford, United Kingdom

[2]Department of Experimental Psychology, University of Oxford, United Kingdom

Using environmental cues to predict rewarding events is essential for adaptive behaviour in humans and animals alike. Dopamine is strongly implicated in this process of reinforcement learning, specifically phasic changes in dopamine release in the ventral striatum, which correlate with a reward prediction error signal necessary for such learning. However, while much is known about striatal dopamine, it is unclear what influence dopamine in other brain regions, particularly the cortex, might play in mediating other factors that influence how such associations are acquired and expressed. To address this issue, we have investigated the behaviour and neurochemistry of a transgenic mouse model which exhibits lower levels of dopamine turnover in the cortex by mimicking a polymorphism found in the human catechol-O-methyltransferase gene (COMT Val158Met).

Using a Pavlovian conditioning paradigm with auditory cues with varying degrees of salience, we found that COMT genotype affects reinforcement learning in a manner that is dependent on cue salience. We then used fast-scan cyclic voltammetry to directly measure phasic dopamine release in nucleus accumbens core (NAcC) as COMT-Met mice and wildtype controls learned Pavlovian associations. Interestingly, we found that COMT genotype influenced phasic dopamine release in NAcC in response to cues, but not rewards.

Taken together, these experiments show that COMT genotype – and by implication, cortical dopamine – impacts striatal dopamine release during learning and controls the selection of a particular behavioural response to a reward-associated, salient cue.

Overall, data suggest cortical-striatal dopamine circuitry may mediate salience effects in associative learning.

Olfactory Learning in Drosophila Larva can be accounted for by Plasticity of the Synapses between Kenyon Cells and Mushroom Body Output Neurons.

Anna-Maria Jürgensen[1], Michael Schleyer[2], Bertram Gerber[2,3,4], Martin Paul Nawrot[1]

[1]University of Cologne, Computational Systems Neuroscience, Zülpicher Str. 47b, 50674 Köln, Germany

[2]Leibniz Institute for Neurobiology (LIN), Department of Genetics, Brenneckestraße 6, 39118 Magdeburg, Germany

[3]Otto von Guericke University of Magdeburg, Center for Behavioral Brain Science(CBBS), Universitätsplatz 2, 39118 Magdeburg, Germany

[4]Otto von Guericke University of Magdeburg,Institute for Biology, Universitätsplatz 2, 39118 Magdeburg, Germany

Efficient coding is particularly relevant in systems consisting of only a small number of neurons. An example is the olfactory system of larval *Drosophila*, which despite its numerical simplicity is able to identify, discriminate, and learn about odors.[8] A transformation from dense coding in the antennal lobe to a sparse code in the mushroom body is one of the mechanisms enabling this efficiency. In this spiking model sparseness is achieved through cellular spike-frequency adaptation in olfactory receptor neurons and Kenyon cells and lateral inhibition onto the Projection neurons at circuit level.[2,4] Taking advantage of the recently released full synaptic connectome of the larval mushroom body we also included realistic network connectivity.[3] Reinforcement-triggered plasticity between the Kenyon cells and the mushroom body output neurons enables learning in *Drosophila*.[5] Thus mushroom body output neurons encode learned valence of stimuli[1,11]. In the larval system, consisting of separate appetitive and aversive pathways,[9,10] a coincidence of odor and reward elicits depression of the synapses between Kenyon cells and output neurons coding avoidance and depression of the output neurons coding approach in the case of punishment.[6,7] Conditioned behavior is reflected in the differences in activation of output neurons coding approach and those coding avoidance. We compare our model results to the results in larval learning experiments.

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Learning of novel semantic relationships by sudden comprehension is associated with a hippocampus-independent network

Jasmin M. Kizilirmak[1], Björn H. Schott[2,3], Hannes Thuerich[2], Kristian Folta-Schoofs[1], Alan Richardson-Klavehn[4]

[1]University of Hildesheim, Institute for Psychology, Hildesheim, Germany
[2]Leibniz Institute for Neurobiology, Magdeburg, Germany
[3]Department of Psychiatry and Psychotherapy, University Medicine Göttingen, Göttingen, Germany
[4]Memory and Consciousness Research Group, Departments of Neurology and Stereotactic Neurosurgery, Otto-von-Guericke-University, Magdeburg, Germany

Sudden comprehension—or insight—during problem-solving can enhance later memory, but the underlying neural processes are largely unknown. We investigated neural correlates of learning from insight using functional magnetic resonance imaging (fMRI) during induced sudden comprehension compared with continued incomprehension. The task employed was a modified German version of the Compound Remotes Associates Task, during which three words are presented for which a fourth solution word needs to be found, so that compound words can be built with each of the other three (e.g., *gown, club, mare*; solution word: *night*). The key feature of this task is that associations between the three problem words are at first remote or latent. Only when the solution is found or presented, does the association between the words become stronger or more direct.

To experimentally induce sudden comprehension or continued incomprehension, either solvable or unsolvable problems, and their solutions or pseudo-solutions respectively, were presented while participants were being scanned (encoding session). They were instructed to search for a solution during the presentation of a triad without its solution. When presented with the correct solution (sudden comprehension) or pseudo-solution (continued incomprehension) shortly afterwards, participants were asked to decide via button press whether the solution was plausible or implausible. Later memory of the problems and their solutions was tested after 24 hours by means of a test in which participants solved old and new sudden comprehension items themselves. To investigate neural correlates of learning from sudden comprehension, fMRI activity in the encoding session for later solved and later unsolved problems was compared.

Irrespective of later memory, we found highly increased activation of the hippocampus bilaterally, the medial prefrontal cortex (mPFC), amygdala, and striatum for sudden comprehension compared with continued incomprehension, despite the novelty and emotional salience of individual words being matched across conditions. Adding to its role in associative novelty, the hippocampus likely responds to a form of conceptual novelty, a novel meaningful relationship between familiar items. Notably, however, mPFC rather than hippocampal fMRI responses were associated with later learning of sudden comprehension solutions. We propose that learning from sudden comprehension may constitute one of the special cases when novel information is directly encoded into semantic memory (mPFC-mediated), similarly to previous accounts of schema- or prior-knowledge-dependent memory. Furthermore, more difficult problems were associated both with activations of the dopaminergic midbrain and greater learning in the memory test, suggesting that comprehending solutions to more difficult problems was intrinsically more rewarding.

Long-term memory improvement by novelty exposure

Jana C. Köhler[1,3], Markus Fendt[2,3], Volkmar Lessmann[1,3], Thomas Endres[1]

[1]Otto-von-Guericke University Magdeburg, Institute of Physiology, Leipziger Str. 44, 39120 Magdeburg, Germany
[2]Otto-von-Guericke University Magdeburg, Institute of Pharmacology and Toxicology, Leipziger Str. 44, 39120 Magdeburg, Germany
[3]Otto-von-Guericke University Magdeburg, Center for Behavioral Brain Sciences (CBBS), Leipziger Str. 44, 39120 Magdeburg, Germany

The Behavioral Tagging hypothesis states that a weak memory trace can be transferred into a long-term memory (LTM) by a novel event, experienced in a critical time window around the weak learning event. It was shown that a preceding or a subsequent exposure to novel but not to familiar stimuli improves LTM in hippocampus- and cortex-dependent memory tasks. To analyze the underlying neuronal mechanisms of the LTM improvement by novelty exposure, we established a spatial object recognition (SOR) paradigm in mice that induces a reliable short-term memory (STM) but no LTM. By exposing mice to novel environments before and after the SOR training session, we could induce a reliable LTM for the object position in this task. The LTM was absent when no or only one novelty session was performed. After having successfully established this paradigm, we now started to analyze the neuronal mechanisms underlying the observed memory improving effects. In particular, we are focusing on the involvement of dopamine (DA) and noradrenaline (NA) signaling. To address the underlying cellular mechanisms of the novelty induced LTM, we are analyzing in a complementary approach whether application of DA or NA before or after a weak long-term potentiation (LTP) inducing stimulus in CA1 of the hippocampus can transfer this weak potentiation into stable LTP (see poster of Klausch et al.).
Overall, these experiments will improve our understanding of the fundamental processes of memory formation and novelty in a behavioral setting. In addition, these results might reveal additional or alternative options for treating pathologies that lead to symptoms of impaired working memory and memory formation such as attention deficit hyperactivity disorder (ADHD).

Plasticity of the start decisions of hunting archerfish

Martin Krause[1], Wolfram Schulze[1], Stefan Schuster[1]

[1]University of Bayreuth, Department of Animal Physiology, Universitätsstrasse 30, 95440 Bayreuth, Germany

Archerfish hunt aerial prey with shots of water and use their so-called predictive C-starts to secure it: Only 100 ms after prey falls ballistically, archerfish elicit a C-start manoeuver that is adjusted in turn angle and speed to where and when prey can later be caught on the water surface. The start decisions are accurately made based on initial speed, height and direction of prey movement and are appropriate for any combination of these variables and for any orientation of the responding fish. We recently discovered that the fish respond to the planar movement of items shown on appropriate screens just as they would for real ballistically falling objects. Based on this we developed a setup that rewards the fish at the time and place that would be appropriate for actually falling natural prey and allows extensive experimental series to dissect the information used by the fish.

Here we explore if the start decisions somehow intrinsically 'assume' ballistic motion patterns: Archerfish select the appropriate C-start for any random combination of initial movement parameters of their prey without requiring any prior information. However, this remarkable ability could require a hardwired assumption that prey falls ballistically. Our setup allowed us to test this. We mimicked trajectories in which a systematic deflection occurred after the fish had already made their C-start decision. As anticipated, the fish initially aimed to the expected ballistic landing point and made large errors which they then had to correct later in their approach path. However, the fish did learn to adjust their start decisions so that they were eventually no longer appropriate for ballistic patterns of falling but for the occurrence of the later deflections. Surprisingly, this did not mean that the fish now no longer could manage ballistically falling objects and had to re-learn how to handle them: When we mimicked two types of objects, one that fell ballistically, the other with a later deflection, and challenged the fish at random with one of the two types, the fish readily made the appropriate decision.

Role of the parietal cortex on the of retrieval of auditory fear memory at ambiguous environment

Sukwon Lee[1], Bitna Joo[1], Ja Wook Koo[1]

[1]Korea Brain Research Institute, Department of Neural Development and Disease, Cheomdan-ro, 41062 Daegu, Korea (South)

Proper evaluation of cue signal (for example which indicate upcoming danger) is critical for animal's survival. To avoid potentially dangerous situation, an animal has to precisely perceive sensory information from its environment and assess probability of a risk. For developing fear response, animal should discriminate the surrounding context with relevant stimuli, and decide to freeze or not. In a pavlovian fear conditioning, pairing of conditional stimulus (CS) with aversive foot-shock (US) increases fear response. However, encountering learned cue at exact same environment is not often in natural situation. Animals would rather adjust their reaction on a same cue in various surroundings based on their prior experience. In many cases, the meaning of cue is changed along with the situation such as context and time. When the context is the same as previously experienced one, animals can easily respond to the cue. But the animals are faced with new or ambiguous context, animals evaluate the cue considering various information. Therefore, the neural circuit for the evaluation of the cue may be different depending on the situation. However, it is not fully understood which brain region is associated with this task.

The posterior parietal cortex (PPC) as a part of association cortex, is known for mediating integration of sensory signals and plays a role in decision making. Previous researches have suggested that the PPC is connected with sensory area, and encode spatial and working memory. For these regions, we hypothesized that the PPC region is critical for the evaluation of cue signal under ambiguous context. To answer the hypothesis, we used fear renewal which enables to examine behavioral effects by changing a context or representing a same context that mice experienced before.

Synaptic $GABA_A$ Receptor Composition in Young Adult-Born Granule Cells Differs from Mature Hippocampal Granule Cells

Meredith E Lodge[1], Jan M Schulz[1], Josef Bischofberger[1]

[1]University of Basel, Department of Biomedicine, Pestalozzistrasse 20, 4056 Basel, Switzerland

γ-Aminobutyric acid (GABA), the main inhibitory transmitter in the adult brain, acts as a trophic factor in young neurons during development and in adult neurogenesis in the dentate gyrus. Due to high cytoplasmic concentrations of chloride ions in new-born granule cells GABA initially acts as an excitatory drive, facilitating neuronal development and integration into the mature network. Here we optogenetically activated somatostatin- (SOM) and parvalbumin-positive (PV) GABAergic interneurons to show the presence of soma-targeting (PV) and dendrite-targeting (SOM) synaptic inputs onto adult-born granule cells from as early as 9 days post mitosis. We have investigated the voltage dependence of synaptic $GABA_A$ receptors in the young granule cells and found that the GABAergic inputs are highly non-linear, showing strong outward rectification. As a consequence, GABAergic peak conductances are about 3-times larger at depolarized potentials as compared to the resting membrane potential (-80 mV). In contrast, this rectification is not present in mature granule cells, suggesting that both PV and SOM interneurons activate synaptic $GABA_A$ receptors in newborn granule cells that differ in composition to those found in mature granule cells. We aim to identify the $GABA_A$ receptors present on young cells that enable this non-linear transmission.

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Sleep improves predictive processing of spatio-temporal sequences

Nicolas D. Lutz[1,2], Ines Wolf[1], Stefanie Hübner[1], Jan Born[1,3], Karsten Rauss[1]

[1]Institute of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Otfried-Müller-Straße 25, 72076 Tübingen, Germany

[2]Graduate Training Centre of Neuroscience / IMPRS for Cognitive & Systems Neuroscience, University of Tübingen, Österbergstraße 3, 72074 Tübingen, Germany

[3]Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Otfried-Müller-Straße 25, 72076 Tübingen, Germany

We live in a world of constant change. To efficiently interact with this world, we need adaptive internal models that can be used to predict upcoming events and detect possible deviations thereof. Internal models are based on similar previous experiences stored in memory. As sleep is known to benefit memory consolidation, we hypothesized that sleep consolidates newly encoded spatio-temporal information and supports their transformation into predictions of future events. Given the variability of stimulus parameters in natural environments, internal models are most useful if they can be applied to different contexts. Recent studies suggest that sleep supports abstraction of encoded information. We thus further hypothesized that sleep supports the transfer of encoded information from one (temporal) context to another. Our results support these hypotheses at the behavioral level. Using high-density EEG, we are currently looking into the neural correlates underlying sleep-dependent prediction processes.

Reward signaling in a recurrent circuit of dopaminergic neurons and Kenyon cells in the *Drosophila* larva

Radostina Lyutova[1], Maximilian Pfeuffer[2], Dennis Segebarth[2,4], Jens Habenstein[2,5], Astrid Rohwedder[3], Felix Frantzmann[2], Mareike Selcho[2], Christian Wegener[2], Andreas S. Thum[3], Dennis Pauls[2]

[1]Julius-Maximilians Universität Würzburg, Dept. of Neurobiology and Genetics, Am Hubland, Biocenter, 97074 Würzburg, Germany

[2]Neurobiology and Genetics, Theodor-Boveri Institute, Biocenter, University of 10 Würzburg, D-97074 Würzburg, Germany

[3] Department of Genetics, University of Leipzig, D-04103 Leipzig, Germany

[4]Institute of Clinical Neurobiology, University Hospital of Würzburg, D-97078 Würzburg, Germany (present address)

[5]Department of Behavioral Physiology and Sociobiology, Theodor-Boveri Institute, Biocenter, University of Würzburg, D-97074 Würzburg, Germany (present address)

Dopaminergic neurons in the brain of the *Drosophila* larva play a key role in mediating reward information to the mushroom bodies during appetitive olfactory learning and memory. Using optogenetic activation of Kenyon cells we provide evidence that a functional recurrent signaling loop exist between Kenyon cells and dopaminergic neurons of the pPAM cluster. An optogenetic activation of Kenyon cells paired with an odor is sufficient to induce appetitive memory, while a simultaneous impairment of the dopaminergic pPAM neurons abolishes memory expression. Thus, dopaminergic pPAM neurons mediate reward information to the Kenyon cells, but in turn receive feedback from Kenyon cells. We further show that the activation of recurrent signaling routes within mushroom body circuitry increases the persistence of an odor-sugar memory. Our results reveal that sustained activity in the underlying circuitry is a conserved mechanism in insects and vertebrates to consolidate memories.

Memory enhancement by ferulic acid ester across species

Birgit Michels[1], Hanna Zwaka[1,2], Ruth Bartels[2], Oleh Lushchak[3], Katrin Franke[4], Thomas Endres[5], Thilo Kähne[6], Volkmar Leßmann[5,7], Alexander Dityatev[7,8,9], Ludger Wessjohann[4], Bertram Gerber[1,7,10]

[1]Leibniz Institute for Neurobiology, Genetics of learning and memory, Magdeburg, Germany
[2]Free University Berlin, Institute of Neurobiology, Berlin, Germany
[3]Precarpathian National University, Department of Biochemistry, Ivano-Frankivsk, Ukraine
[4]Leibniz Institute of Plant Biochemistry, Department of Bioorganic Chemistry, Halle/ (Saale), Germany
[5]Otto von Guericke University, Medical Faculty, Institute of Physiology, Magdeburg, Germany
[6]Otto von Guericke University, Institute of Experimental Internal Medicine, Magdeburg, Germany
[7]Center for Behavioral Brain Sciences (CBBS), Otto von Guericke University, Magdeburg, Germany
[8]German Center for Neurodegenerative Diseases (DZNE), Molecular Neuroplasticity Group, Magdeburg, Germany
[9]Otto von Guericke University, Medical Faculty, Magdeburg, Germany
[10]Otto von Guericke University, Institute of Biology, Magdeburg, Germany.

In humans, *Rhodiola* is used in traditional medicine for its attention- and memory-enhancing remedy. However, the effective compound(s) to mediate these effects remain obscure. Therefore, we exploited the potential of the plant *Rhodiola rosea* and identified the constituent ferulic acid eicosyl ester (FAE-20) as a memory enhancer. We show that food supplementation with dried root material from *Rhodiola rosea* dose-dependently improves odor-taste reward associative memory scores in larval *Drosophila* and prevents the age-related decline of such appetitive memory in adult flies. Task-relevant sensory-motor faculties remain unaltered. From a parallel approach (Michels B, unpublished) we found a massive rewarding effect of acute *Rhodiola* stimulation) with the effective, *Rhodiola rosea*-derived compounds Beta-sitosterol glucoside, FAE-20 and ferulic acid. We show that both *Rhodiola rosea*-derived FAE-20 and synthetic FAE-20 are effective as a memory enhancer in larval *Drosophila*. Synthetic FAE-20 partially compensates age-related memory decline in adult flies, as well as genetically-induced early-onset loss of memory function in young flies. Furthermore, it increases excitability in mouse hippocampal CA1 neurons, leads to more stable context-shock aversive associative memory in young adult (3-month-old) mice, and increases memory scores in old (> 2-year-old) mice.

Brain Electroencephalographic modules segregation as biomarker of learning

Francesca Miraglia[1], Fabrizio Vecchio[1], Paola Maria Rossini[2]

[1]IRCCS SAN RAFFAELE PISANA ROMA , BRAIN CONNECTIVITY LABORATORY , VIA VAL CANNUTA 247 , 00166 ROME , Italy

[2]FONDAZIONE POLICLINICO UNIVERSITARIO A.GEMELLI IRCCS, ROME, Italy

Aim of the present study was to understand whether by modeling
brain function in terms of network structure makes it is possible to find markers of prediction of motor learning performances in a Sensory Motor Learning task.By applying graph theory indexes of brain segregation -such as Modularity and Transitivity- to functional connectivity data derived from EEG rhythms,we further studied pre- (baseline) vs post-task brain network architecture in order to understand whether motor learning induces changes in functional brain connectivity.Results showed that, after the training session with measurable learning, Transitivity increased in alpha1 EEG frequency band and Modularity increased in theta and decreased in gamma bands, suggesting that brain segregation is modulated by the cognitive task.Furthermore, it was observed that theta Modularity in baseline negatively correlated with the performance improvement; namely, lower this connectivity index in baseline pre-task period, higher the improvement of performance with training.The present results showed that the brain segregationis modulated by the cognitive task and that it is possible to predict performances by the study of pretask EEG rhythms connectivity parameters.

Performance-dependent regulation of the extracellular matrix in auditory cortex and hippocampus during learning and long-term memory formation

Hartmut Niekisch[1], Julia Steinhardt[1,2], Julia Berghäuser[1,3], Jana Kasper[1], Erika Kaschinski[1], Sara Bertazzoni[1], Judith Weber[1], Jeet Singh[4,5], Jessica Mitlöhner[4], Renato Frischknecht[4,6], Max F.K. Happel[1]

[1]Leibniz Institute for Neurobiology, Department Systems Physiology of Learning, D-39118 Magdeburg, Germany
[2]University of Lubeck, Department of Neurology, D-23562 Lubeck, Germany
[3]Technical University Dresden, Department of Psychology, Institute of Clinical Psychology and Psychotherapy, D-01187 Dresden, Germany
[4]Leibniz Institute for Neurobiology, Department Neurobiology and Molecular Biology, D-39118 Magdeburg, Germany
[5]University of Bergen, Department of Biomedicine, The Retinal Microcircuits Laboratory, 5009, Bergen, Norway
[6]Friedrich Alexander University Erlangen-Nuremberg, Department of Biology, Animal Physiology, D-91058, Erlangen, Germany

Higher organisms have to establish new memories continuously via learning and provide long-term storage of remote memories in order to adapt in an ever changing environment. In this context, the extracellular matrix (ECM) in the vertebrate brain has been suggested to limit synaptic plasticity and behavioral flexibility. Therefore, learning-related downregulation of the ECM may hence promote synaptic plasticity required for learning. However, the impact of a dynamic ECM regulation on both, learning-related plasticity and life-long memory storage is still elusive.
Recently we have revealed that the ECM in auditory cortex (ACx) of Mongolian gerbils is in control of the behavioral flexibility underlying cognitively demanding reversal learning tasks (Happel et al., 2014, PNAS). In the present study, we measured the abundance of brevican and tenascin-R in two learning-relevant brain regions, the ACx and hippocampus (CA) of mice (C57BL/6NCrl) during learning and long-term retrieval in an Go/NoGo-discrimination task of frequency modulated (FM) tones (4-8kHz vs. 8-4kHz). Using post-training semi-quantitative Western blot analysis, we found a general downregulation of considered total ECM proteins in early training, which are gradually recovered during training and remote recall. Specifically, successful retrieval correlated with a region-specific transient upregulation of brevican levels in auditory cortex, suggesting a performance-dependent recovery. This biphasic regulation of brevican may assist transient sensory cortical plasticity to facilitate initial learning and subsequently promote the long-term consolidation of cortex-dependent memory.

Behavioural characteristics of aversive colour learning in honeybees

Morgane Nouvian[1], C. Giovanni Galizia[1]

[1]University of Konstanz, AG Galizia, Universitätstrasse 10, 78457 Konstanz, Germany

In classical conditioning, a previously neutral stimulus (conditioned stimulus CS, e.g. a colour or an odour) is paired with a valence (unconditioned stimulus US, e.g. a reward or a punishment). As a result, the CS alone elicits a behaviour, e.g. aversion or attraction: the UR (unconditioned response) previously elicited by the US only, now is a CR (conditioned response), elicited by the CS. While the role of the US has been studied extensively (e.g. how strong a reinforcer needs to be), the landscape of CS stimuli is less understood. We use the honeybee, Apis mellifera, to study how different colours can be learned. Colour learning has been extensively studied in appetitive paradigms, in which a particular colour was rewarded with sugar. Here, we investigated the aversive variant: we trained bees to associate colours with an aversive US, mild electric shocks. We examined how pairing shocks with human blue (λ=465nm), human green (λ=520nm) or UV (λ=375nm) lights affected the subsequent behaviour of bees towards these same colours, using an automated Y-maze. In these Y-mazes, naïve bees display a strong phototactic behaviour. We found that after training with blue or green lights, honeybees avoided the arm of the Y-maze containing this light. The avoidance strategies adopted by the bees, however, were different for these two colours: bees trained to blue avoided this colour mostly by staying in the dark arm, and indeed phototaxis to blue light was also reduced. On the other hand, bees trained to green avoided this colour by choosing an alternative colour when available, and their phototactic behaviour was not affected. Finally, training with UV was unreliable, sometimes eliciting avoidance but often not. Thus, aversive learning of colours does not seem to be a unitary phenomenon, but rather depends on the wavelength being used. In particular, different wavelengths appear to become associated with different URs. While this may be linked to the intrinsic biological value of these different colours (e.g. related to flower petals, green vegetal background, or sky UV), it remains unclear how the neural circuitry responsible for these distinct associations differs.

Learning relative value in Drosophila

Emmanuel Perisse[1,2], Pedro F. Jacob[2], Luis D. Suarez[2], Scott Waddell[2]

[1]Institute for Functional Genomics, Neuroscience, 141 rue de la Cardonille, 34094 Cedex 5 Montpellier, France
[2]Centre for Neural Circuits and Behaviour, University of Oxford, Tinsley building, Mansfield road, Oxford, OX1 3SR, UK

Animals use memories of past experience to make appropriate decisions. We previously showed that flies can memorize information that correlates to the intensity of electric shock to make appropriate value-based decisions using specific mushroom body (MB) intrinsic circuits. Surprisingly, we found that during learning rewarding dopaminergic (DA) neurons are required during learning to assign relative value (better or worse than) signals to odours associated with different intensities of electric-shock punishment. Using in vivo neuronal silencing during learning, we found specific subsets of rewarding and punishment DA neurons targeting the MB that are specifically required for relative aversive value coding. MB output neurons (MBONs) with dendrites within the MB zones targeted by the necessary DA neurons are also necessary for relative aversive value coding, suggesting a possible functional role for recurrent connectivity. In vivo calcium imaging during learning revealed that learning induces shock intensity-dependent persistent depression of the conditioned-odour drive to the relevant MBONs. We therefore propose that memories of relative aversive value are written within the DA-MB-MBON network and compared via recurrent MBON-DA neuron recurrent circuits.

A depletion of dietary phytoestrogen in the adult C57BL/6 mice affects contextual fear and hippocampal plasticity

Syed Ahsan Raza[1], Gürsel Çaliskan[1], Oliver Stork[1,2]

[1]Department of Genetics & Molecular Neurobiology, Institute of Biology, Otto-von-Guericke-University Magdeburg, Germany

[2]Center for Behavioral Brain Sciences, Magdeburg, Germany

Phytoestrogens are naturally occurring non-steroidal compounds that possess estrogenic effects and are primarily found in nuts, oilseeds and soy products. During recent years in neuroscience, their use as dietary estrogen supplements and as modulators of endogenous estrogen functions including cognition and emotion, has received increasing attention. The neural circuits that involve estrogen and androgen receptors may be functionally modulated via the direct effects of phytoestrogens. In the present study, we investigated the effects of decreased phytoestrogen intake (~6 weeks) in adult C57Bl/6 male mice in behaviour and slice physiology. This mice with low dietary estrogen intake showed a decreased contextual fear memory at a remote time point (~2 weeks) accompanied with decreased anxiety levels in an open field test. In slice physiology, we observed a profound decrease in long-term potentiation at the Schaffer collateral-CA1 pathway in ventral hippocampus, however no effect on plasticity was evident in dorsal hippocampus slices. Next, we tested whether the LTP deficit can be rescued by providing an estrogen analogue produced by the gut flora. Indeed, acute slices perfusion with equol -an isoflavone metabolized from daidzein- was able to rescue the observed LTP deficit. Furthermore, analysis of network oscillations in the ventral hippocampus revealed that carbachol-induced (5 µM) cholinergic gamma oscillations had increased gamma peak frequency without any alterations in the gamma power. Together, our data imply that nutritional phytoestrogens may have profound effects on the plasticity in the ventral hippocampus circuit and ventral hippocampus-dependent fear memory.
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Characterization of an optogenetically activated dopaminergic reward signal

Michael Schleyer[1], Alice Weiglein[1], Juliane Thöner[1], Anne Voigt[1], Timo Saumweber[1], Bertram Gerber[1]

[1]Leibniz Institute for Neurobiology, Genetics of Learning and Memory, Brenneckestr. 6, 39118 Magdeburg, Germany

A hungry animal may use its previous experience with food-associated stimuli to guide its search for food. Once a food source is found, however, it is adaptive to stop searching and rather exploit the food source. Using the larvae of Drosophila melanogaster as study case, we study the role of a single, identified dopamine neuron in these processes.

In their search for food, Drosophila larvae prefer an odor previously paired with food reward relatively more than an odor that previously was presented unpaired with reward. We show that the larvae track down a reward-associated odor only if there is something to gain, i.e. only if the odor predicts more food than currently present. Moreover, after training with odor and sugar reward larvae specifically search for sugar but not amino acids, and vice versa. That means, larvae establish memories that are specific for sugar vs. amino acid rewards - which allows them to organize their search for food according to their current needs.

Using a combinational approach of behavior experiments and optogenetic activation, we currently characterize single dopaminergic central brain neurons (DANs) for their role in establishing and gating associative memories. Here we present one DAN as study case:

(1) its activation is sufficient as internal reward signal, even with only one training trial;

(2) the valence of the memory established by this DAN is dependent on the relative timing between odor presentation and DAN activation;

(3) this DAN carries a sugar rather than an amino acid reward signal, and therefore training with this DAN makes larvae specifically search for sugar;

(4) the microbehavioral ‘footprint’ of the memory induced by this DAN matches that of a sugar-induced memory;

(5) the retrieval of a memory established by this DAN is acutely suppressed by the activation of the same DAN.

In summary, this single DAN carries a sugar-specific internal reward signal that can establish memories of opposite valence depending on the relative timing with the odor, and gates the behavioral expression of the established memory. Our findings challenge the notion that dopaminergic neurons always carry a common-currency value signal, and reveal an elegant mechanism to prevent further search once the sought-for item is found.

Neuronal processing of multimodal reward associations in the honeybee

Fabian Schmalz[1], Wolfgang Rössler[1], Martin Strube-Bloss[1]

[1]Universität Würzburg, Department of Behavioral Physiology & Sociobiology , Biozentrum, Am Hubland, 97074 Würzburg, Germany[1]

During their daily foraging flights, honeybees find adequate food resources by processing stimuli of different modalities (e.g. visual cues like colorful petals or olfactory cues like flower bouquets). The hereby naturally occurring stimulus-reward association of a conditioned stimulus (CS) with an unconditioned stimulus (US) has been well studied in numerous behavioral essays, separately for each modality. So far, there is a lack of data covering the integration of multimodal stimuli. Our project addresses this need by a combination of behavioral and electrophysiological assays that give insights in the neuronal integration of multimodal stimuli. We perform classic conditioning experiments comprising positive (PP) and negative (NP) patterning while recording extracellularly from mushroom body (MB) output neurons (MBON) which encode stimulus-reward associations. MBONs receive input from Kenyon cells (KC), which are the principal neurons forming the MB. Different layers of KCs receive input from different modalities and this modality separation is conserved in subpopulations of MBONs. However, about 50% of the MBON population integrates olfactory and visual information from across KC-layers. Our established approach therefore allows us to study neural principals of multimodal integration and its modulation due to learning.

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Enhanced feedforward inhibition in the hippocampus of a Down Syndrome mouse model

Jan Michael Schulz[1], Josef Bischofberger[1]

[1]University of Basel, Department of Biomedicine, Institute of Physiology, Pestalozzistr. 20, 4056 Basel, Switzerland

Increased inhibition is believed to cause cognitive deficits in Down syndrome. We have analyzed $GABA_AR$-mediated inhibitory postsynaptic currents (IPSCs) in CA1 pyramidal cells in hippocampal brain slices from Ts65Dn and wt mice. We observed that miniature IPSCs were normal in slices from Ts65Dn mice, indicating unaltered numbers of GABAergic synapses. Next, we stimulated Schaffer Collaterals to evoke excitatory PSCs (EPSCs) and IPSCs. The IPSC-to-EPSC ratio was 1.4 ± 0.2 (n=7) in Ts65Dn mice, significantly larger than 0.6 ± 0.1 (n=9) in wt littermates ($P<0.01$). The latencies of IPSC onset (~5.5 ms) were about twice as long as the latencies of the EPSCs (~3 ms), indicating that IPSCs were mediated by di-synaptic feedforward inhibition. To identify the involved interneuron population, we used targeted cell-attached recordings from identified interneurons. Specifically the recruitment of parvalbumin (PV)-positive required much lower stimulation intensities in Ts65Dn mice, Together, these results suggest that increased recruitment of PV+ interneurons may be the main cause for elevated inhibition in DS.

A deep learning strategy for automatic segmentation of fluorescent labels in brain sections

Dennis Segebarth[1,6], Matthias Griebel[2,6], Alexander Dürr[2], Cora Rüdt von Collenberg[1], Corinna Martin[1], Lucas B. Comeras[3], Dominik Fiedler[4], Anupam Sah[5], Nikolai Stein[2], Rohini Gupta[1], Manju Sasi[1], Ramon O. Tasan[3], Maren Lange[4], Nicolas Singewald[5], Hans-Christian Pape[4], Michael Sendtner[1], Christoph Flath[2,7], Robert Blum[1,7]

[1]University Hospital Würzburg, Institute of Clinical Neurobiology, 97078 Würzburg, Germany

[2]University of Würzburg, Department of Business Management and Economics, 97070 Würzburg, Germany

[3]Medical University of Innsbruck, Department of Pharmacology, 6020 Innsbruck, Austria

[4]Westfälische Wilhelms-University, Institute of Physiology I, 48149 Münster, Germany

[5]University of Innsbruck, Department of Pharmacology and Toxicology, Institute of Pharmacy and Center for Molecular Biosciences Innsbruck, Innsbruck, Austria

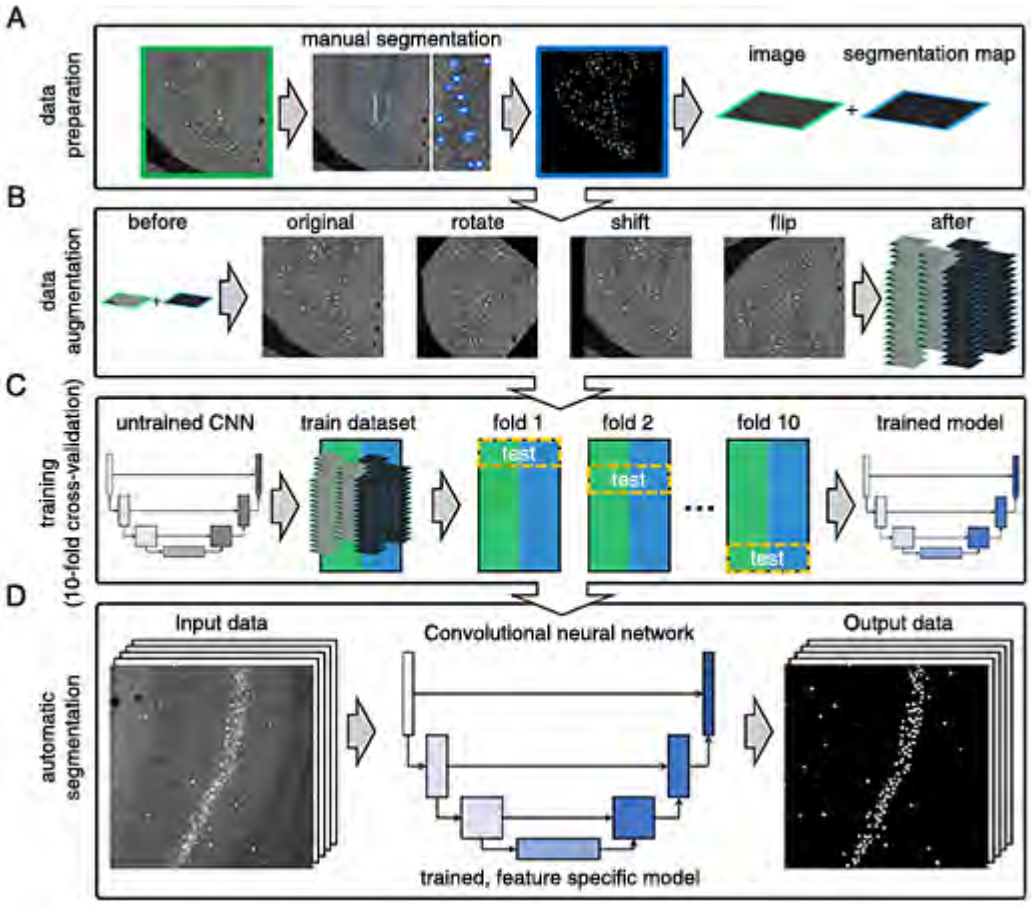
[6]These authors contributed equally

[7]Senior author

The visualization of cells by fluorescent labels, for instance by immunohistochemistry, is a standard technique in neuroscience that allows the identification and localization of specific cell types in brain tissue. Furthermore, this technique is commonly used to quantify experimental or disease-related changes in the number of neurons or the abundance of macromolecules. While the imaging of fluorescence by microscopy is straightforward, the subsequent data analysis is rather demanding. Currently available computational approaches often fail to capture the whole complexity of the underlying image, as they rely on a high signal-to-noise ratio to correctly separate regions of interest (ROIs) from background noise. Therefore, manual analysis by human experts blinded to the experimental conditions is still one of the most commonly used ways to analyze microscopy images. Manual counting or segmentation of fluorescent labels in large imaging datasets, however, is extremely time-consuming and based on heuristic criteria, thus limiting both objectivity and reproducibility.

In recent years, machine learning strategies have shown their remarkable capacities in image recognition tasks. A major advantage of these strategies is that they do not require a ROI pre-definition, but that they can learn the recognition of the desired fluorescent features directly from human experts in a training dataset.

In this study, we addressed the need for an automatized, yet flexible strategy that captures the whole complexity of immunofluorescent imaging data rather than focusing only on the most intense ROIs. We developed a machine learning strategy that learns from multiple human experts to automatically segment labeled neurons. The extensive use of data augmentation methods further allowed us to decrease the required training dataset to biologically feasible sizes. We show that our approach reaches expert-like performance in the segmentation of cFOS-positive nuclei and the somata of Parvalbumin-positive interneurons in brain sections of mice after behavioral training. Furthermore, we demonstrate that our approach is flexible enough to be generalized on independent labeling data from different laboratories. The framework we designed is intended to be used also by non-AI researches to create label-specific models for individual or general demands.


A
data preparation
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training (10-fold cross-validation)
untrained CNN
train dataset
fold 1
fold 2
fold 10
trained model
test
test
test
D
automatic segmentation
Input data
Convolutional neural network
Output data
trained, feature specific model

Novel tool to manipulate putative Drosophila engram cells

Dominique Siegenthaler[1], Benjamin Escribano[1], Vanessa Bräuler[1], Jan Pielage[1]

[1]TU Kaiserslautern, AG Pielage, Erwin-Schrödinger-Strasse 13, 67663 Kaiserslautern, Germany

The understanding of behavior on the molecular and circuit level critically depends on selective genetic control of neuronal populations involved in that behavior. In this study we establish a new tool to gain genetic access to a sparse and dynamic population of neurons in the Drosophila learning and memory center. We find selective reporter activation after protein-synthesis dependent long-term memory (LTM) formation. Using the modular design of the tool to express different effector genes we measured responses of these potential engram cells during natural recall and assessed their function in aversive olfactory LTM. Neuronal silencing and activation experiments show that conditioning-dependent labeled cells are required for LTM expression and are sufficient for LTM recall. Together, our results show that the new tool enables genetic access to putative engram cells to gain novel insights into molecular and circuit mechanisms underlying memory formation.

Protein expression and phosphorylation during consolidation of relief learning in rats

Elaheh Soleimanpour[1], Jorge R. Bergado Acosta[1,2], Peter Landgraf[1], Dana Mayer[1], Evelyn Dankert[1], Daniela C. Dieterich[1,2], Markus Fendt[1,2]

[1]Institute for Pharmacology and Toxicology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany.
[2]Center for Behavioral Brain Sciences, Otto-von-Guericke University Magdeburg, Magdeburg, Germany.

Humans and animals can learn to associate environmental cues with the presence, cessation, and absence of aversive events. These different forms of event learning – called fear, relief and safety learning, respectively – are highly adaptive since thereby humans and animals can better cope with future aversive events. During the last years, we investigated the neuropharmacological basis of relief learning in rats. Among other things, we demonstrated that NMDA and dopamine receptors within the nucleus accumbens are crucial for the acquisition of conditioned relief. The aim of the present study was to investigate the molecular pathways underlying relief learning, with a focus on the expression level and phosphorylation ratio of selected proteins in different regions of the rat brain. To this purpose, two groups of rats were submitted either to relief conditioning or to a sham conditioning procedure. A third group served as naive controls. 45 minutes or 6 hours after the conditioning sessions, the rats were sacrificed and the brains were dissected into 5 different regions, namely the prefrontal cortex, nucleus accumbens, dorsal striatum, dorsal hippocampus, and amygdala. Then, the expression of the proteins CREB, ERK1/2, CaMKII, 14-3-3, neuroligin, MAPKII, and SEK1MKK2, as well as the phosphorylation of CREB and ERK1/2 were measured in these brain samples. Western blot analyses revealed significantly different protein expression levels between the relief-conditioned and the sham-conditioned groups in all analyzed brain areas. Most changes were observed in the dorsal hippocampus and the amygdala. For example, after 45 minutes, relief conditioning lead to increased levels of ERK1/2, pCREB, 14-3-3, and SEK1MKK2, but decreased levels of CREB in the dorsal hippocampus. Furthermore, CREB and ERK1/2 levels were increased but SEK1MKK2 levels decreased in the amygdala. This data indicates that the ERK/CREB signaling pathway is involved in the consolidation of relief learning. Currently, we investigate whether and how these changes in ERK/CREB signaling are accompanied by changes in microRNA expression.

Appetitive and aversive learning of amino acids in larval *Drosophila*

Naoko Toshima[1,2], Michael Schleyer[1], Bertram Gerber[1,3,4]

[1]Leibniz Institute for Neurobiology (LIN), Genetics of Learning and Memory, Brenneckestrasse 6, 39118 Magdeburg, Germany
[2]JSPS Overseas Research Fellow
[3]Otto von Guericke University Magdeburg, Germany
[4]Center for Behavioral Brain Science (CBBS), Magdeburg, Germany

Although amino acids are important nutrients for *Drosophila melanogaster*, how flies detect amino acids and how the behavioural response to amino acids are regulated are largely unknown. Previously Toshima & Tanimura (2012) found that adult *Drosophila* enhance the feeding preference to amino acids when they were deprived of amino acids. Contrary to the adult flies, which can survive without obtaining amino acids, larvae continuously require to ingest protein source for growth. Larval brain consists of relatively small number of neurons, that is ten times fewer than adult brain. Nevertheless, larvae are intelligent enough to exhibit associative learning of odours and taste stimuli. Given that associative learning is related to feeding motivation, it is intriguing to ask whether larvae show reward learning to amino acids. Schleyer et al. (2015) showed that sugar and amino acid induce independent appetitive memories. That is, although fructose and aspartic acid induce similar intensity of appetitive memory, fructose memory is not abolished in the presence of aspartic acid. Similarly, aspartic acid memory is abolished in the presence of aspartic acid, but not in the presence of fructose. We then performed learning experiments for 20 individual amino acids, and found that larvae learn all individual amino acids as reward (Kudow et al. 2017). To see further detail of amino acid learning, here we used an amino acid-mixture as the reinforcer. We also tested genetically modified flies to investigate which neurons contribute to amino acid learning.

Later than expected: Theta-Alpha-Gamma coupling and phase-amplitude shift in memory-based temporal expectation

Vincent van de Ven[1], Oana Iosif[2], Fren Smulders[1], Peter de Weerd[1]

[1]Maastricht University, Cognitive Neuroscience, PO Box 616, 6200 MD Maastricht, Netherlands
[2]University of Aberdeen, Scotland

Background: Theta (4-8 Hz), Alpha (8 - 12 Hz) and (high) Gamma (70 - 90 Hz) oscillations may play different but interacting roles in short-term memory formation, retention and retrieval. Previous studies investigated changes in oscillatory power or phase-amplitude coupling in some combination of two of these bands as a function of memory operations or performance. We investigated θ-α-γ cross-frequency interactions in an associative memory task with a time component, in order to use variations in learned temporal expectancies to unveil changes in cross-frequency coupling in the absence of sensory stimulation.
Methods: Participants learned cue-target pairs that were uniquely associated to a short (1s) or long (2s) cue-to-target delay. Participants were not aware of the delay variations between pairs. During memory testing, cue-target pairs could be shown with the implicitly learned or a novel delay. Oscillatory brain activity was measured using scalp electroencephalography.
Results: During memory testing, participants responded slower when cue-target pairs were tested with a different delay than what was learned (and thus 'expected'). When a target was shown later than expected from learning, parietal θ-power decreased when the target was (implicitly) expected to appear but remained absent, compared to when a target was shown at the expected (long) delay. In addition, and temporally coinciding, we found evidence for a shift in occipital α-phase, resulting in high-γ amplitude to shift phase-locking from α-trough to α-peak when the target was expected but remained absent. Further, α-γ phase shift correlated with 1) magnitude of θ-power decrease and 2) elongated response times for targets presented later than expected.
Computational simulation: A simple computational model using Kuramoto coupled oscillators in which θ-α phase coupling strength (K) was allowed to vary over time, suggested a possible role of θ-α phase coupling changing with temporal expectancy, with γ amplitude locked to θ-trough but changing locking to α-phase, explaining all main empirical oscillator effects.
Conclusion: Our findings are novel and exciting, though preliminary. We suggest that these findings and insights of θ-α-γ cross-frequency interactions underlie memory-based allocation of attention to process temporally expected sensory events.

Learning processes and brain connectivity in a cognitive-motor task in neurodegeneration: evidence from EEG network analysis

Fabrizio Vecchio[1], Francesca Miraglia[1], Davide Quaranta[2], Giordano Lacidogna[2], Camillo Marra[2], Paolo Maria Rossini[2]

[1]IRCCS San Raffaele Pisana, Brain Connectivity Laboratory, Via di Valcannuta, 247, 00166 Rome, Italy

[2]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

EEG rhythms are linked to any kind of learning and cognitive performance including motor tasks. The brain is a complex network consisting of spatially distributed networks dedicated to different functions including cognitive domains where dynamic interactions of several brain areas play a pivotal role. Brain connectome could be a useful approach not only to mechanisms underlying brain cognitive functions, but also to those supporting different mental states. This goal was approached via a learning task providing the possibility to predict performance and learning along physiological and pathological brain aging. Eighty-six subjects (22 healthy, 47 amnesic Mild Cognitive Impairment, 17 Alzheimer Disease) were recruited reflecting the whole spectrum of normal and abnormal brain connectivity scenarios. EEG recordings were performed at rest, with closed eyes, both before and after the task (Sensory Motor Learning –SmoL- task consisting of a visual rotation paradigm). Brain network properties were described by Small World index (SW), representing a combination of segregation and integration properties. Correlation analyses showed that alpha 2 SW in pre-task significantly predict learning ($r=-0.2592$, $p<0.0342$): lower alpha 2 SW (higher possibility to increase during task and better the learning of this task), higher the learning as measured by the number of reached targets. These results suggest that, by means of an innovative analysis applied to a low-cost and widely available techniques (Small World applied to EEG), the functional connectome approach as well as conventional biomarkers would be effective methods for monitoring learning progress during training both in normal and abnormal conditions.

Distracting a *Drosophila* — How do Fruit Flies "Remember" to Visually Orient?

Anjana Venkataramanan[1]

[1]Johannes Gutenberg University Mainz, Institute of Developmental Biology and Neurobiology, Col.-Kleinmann-Weg 2 - SB II, 55099 Mainz, Germany

Visual working memory in *Drosophila melanogaster* allows flies to continue the approach of a landmark that became temporarily out of sight. This memory has been located in the ring neuron system R3 of the ellipsoid body in the central complex. In particular, NO/cGMP signaling is required to establish an engram for the vanished landmark in R3 neurons [1]. Here, we ask whether the canonical cAMP/PKA signaling pathway is additionally required for this type of short-term memory.
Our experimental setup involves the Detour Paradigm [1] which includes a cylindrical LED arena beyond a water-filled moat. Visual landmarks in the form of dark vertical bars are displayed on opposing sides of the arena acting as illusions of escape routes to the fly, prompting them to walk back and forth. A distraction in the form of a new black bar is introduced at the 90 degree angle when the fly reaches the middle of the arena and is subsequently removed 1 second after the fly notices it. Turning towards the original landmark at the 45 degree angle as soon as the distraction is removed serves as a positive choice while turning in the opposite direction indicates a loss of memory for that particular turn. Thus, a cumulative percentage is arrived for each fly after them performing ten trials in the arena. Multiple Comparison Statistical Tests were done between groups of mutant flies, their respective controls and WT-CS in order to reveal a significant difference between them.
Rescue experiments of heterozygous *Pka-C1* mutants revealed the requirement of PKA in R3 neurons. Moreover, this lack of PKA function can be rescued by overexpression of Synapsin, a target of PKA phosphorylation [2,3]. Synapsin phosphorylation by PKA is believed to foster the transfer of synaptic vesicles from the reserve pool to the recycling pool. Analysis of the *synapsin* mutant (*syn97*) revealed a requirement of just one of the two canonical PKA phosphorylation sites. Interestingly, this PKA-site is edited out in most of the syn mRNAs. Consequently, knocking-down the RNA editing enzyme Adenosine deaminase (*Adar*) rescued the heterozygous *Pka-C1* mutant phenotype.
In addition, genetic interaction studies revealed that the AKAP (A-Kinase Anchoring Protein) Rugose (RG) is a negative regulator of PKA function in R3 neurons. This is in sharp contrast to the positive interaction of PKA and RG in Kenyon cells of the mushroom body in olfactory-memory formation [4]. Overexpression and knock-down experiments in the R3 ring neurons suggest that RG sequesters PKA to the Golgi network, thus preventing its action at the pre-synapse [5].

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The formation of aversive olfactory memories in *Drosophila* larvae is regulated through insulin signalling in the mushroom bodies.

Annekathrin Widmann[1], Nazli Güllü[2], Melanie Eschment[2], Kathrin Böpple[2]

[1]Georg-August-University of Goettingen, Molecular Neurobiology of Behavior, Julia-Lermontowa-Weg 3, 37077 37077 Göttingen, Germany

[2]University of Konstanz, Department of Biology, Universitätsstraße 10 78464 Konstanz, Germany

Establishing a memory is a highly complex and dynamic process. It consists of different phases, which dependent on various neuronal and molecular mechanisms. In *Drosophila* it was shown that memory formation after aversive Pavlovian conditioning consolidates from a labile short-term component to more stable and longer lasting form within in hours. This process requires the timely controlled action of different neuronal circuits, neurotransmitters, neuromodulators and molecules that were initially identified by classical forward genetic approaches. Beside this gradual transition another memory component, the anesthesia-resistant memory (ARM), which is resistant to cold shock treatment and independent to the requirement of a *de-novo* protein synthesis, exists in parallel. In *Drosophila* adults both consolidated memory phases ARM and LTM compete with each other. A LTM gating mechanism prevents the adult *Drosophila* from forming an energetic costly aversive LTM in favour to a less costly, but less stable ARM under critical nutritional circumstances. However, the biochemical underpinnings of this gating mechanism are poorly understood. Despite the fact that memory formation was only sporadic analyzed at its larval stage it seems to follow a similar logic. After classical odour-high salt conditioning larvae forms different memory components: a labile larval short-term memory (lSTM) and a larval anesthesia-resistant memory (lARM), which are separated on a molecular level. Given the non-redundancy and numerical simplicity of the larval nervous system, the occurrence of only two memory components offers a unique prospect for studying the biochemical and neuronal principles upon the cellular and molecular basis of switching between different memory phases in dependency of the physiological state. By defining the physiological state of *Drosophila* larvae by feeding different sugars prior to the training regime held some promising findings. The internal physiological state acts as a binary switch between the formation of lARM and lSTM. Administration of high concentration of sugar prevents *Drosophila* larvae from forming lARM after aversive olfactory conditioning. Additionally, it seems that existence of lSTM is prolonged. One promising candidate is insulin signaling (InS), which is an important sensor of the nutritional status of an animal and it is highly conserved in the animal kingdom. However, it its role in memory formation remains largely unknown. Here, downregulation of the insulin receptor (InR) and the insulin receptor substrate (Chico) in the Mushroom body Kenyon cells (MBKCs) of larvae leads to inhibition of forming an larval STM and favours the formation of lARM. This binary switch between different memory phases in the dependency of the physiological state of larvae may held the key of understanding the underlying molecular mechanism and biochemical pathways of the coexistence of different memory phases and evolution of two different consolidated memory phases in *Drosophila*.

Auditory fear conditioning in serotonin transporter knockout rats: Differential effects on overt behavior and ultrasonic vocalizations

Maria Willadsen[1,2,3], Tina Meller[1], Maja Paaschburg[1], Rainer Schwarting[1,2,3], Judith R. Homberg[4], Markus Wöhr[1,2,3]

[1]Philipps-University of Marburg, Behavioral Neuroscience, Gutenbergstr. 18, 35032 Marburg, Germany
[2]RTG 2271 „Breaking Expectations“, Philipps-University of Marburg, Germany
[3]Center for Mind, Brain and Behavior (CMBB), Philipps-University of Marburg, Germany
[4]Department of Cognitive Neuroscience, Radboud University, Nijmegen, Netherlands

Serotonin (5-hydroxytryptamine, 5-HT) is an important modulatory neurotransmitter. While the 5-HT system is complex and includes several 5-HT receptors, a key component of the system is the 5-HT transporter (5-HTT also known as SERT), since it controls the amount of 5-HT available in the synaptic cleft. Interestingly, the 5-HT system has been repeatedly implicated in cognitive flexibility, with alterations in its components resulting in exaggerated persistence of expectations. For instance, genetic alterations in the SLC6A4 gene (e.g. 5-HTTLPR) have been associated with impairments in learning, such as extinction. The important role of this gene variation was confirmed by means of genetic rodent models, yet little is known about underlying behavioral and neurobiological mechanisms.

To assess impairments in extinction learning due to altered 5-HT transmission, we applied an auditory fear conditioning paradigm in homozygous and heterozygous 5-HTT knockout rats and compared them to wildtype littermate controls. In our established auditory fear conditioning paradigm, rats learn to associate the presentation of a tone with a mild electric shock and the conditioned emotional response is measured by means of freezing behavior and aversive 22-kHz ultrasonic vocalizations (USV). Rats emit 22-kHz USV in aversive situations, such as predator exposure, aggressive encounters, and fear conditioning. It is widely believed that they reflect a negative affective state and serve an important communicative function as alarm calls.

Our present results show that freezing behavior did not differ between genotypes and while a prominent freezing response was evident during acquisition, this response was of similar strength in all three genotype conditions. Likewise, during extinction, freezing behavior was high and slowly decreased towards the end of the extinction phase, with a similar response pattern present in all three genotype conditions. Interestingly, however, the emission of aversive 22-kHz USV was strongly dependent on genotype and very low 22-kHz USV emission rates were detected in homozygous 5-HTT knockout rats.

Together, our present findings suggest that 5-HTT deletion has only a minor impact on freezing behavior, while prominent effects on the emission of aversive 22-kHz USV during fear conditioning were detected. This indicates that the neurobiological mechanisms involved in the regulation of freezing behavior and aversive 22-kHz USV emission are, at least partly, independent.

Amygdala intercalated neurons form an interconnected and functionally heterogeneous network

Martin Zeller[1,2], Olena Bukalo[3], Kenta Hagihara[4], Ayla Aksoy-Aksel[1,2], Andrea Gall[1,2], Andrew Holmes[3], Andreas Lüthi[4], Ingrid Ehrlich[1,2]

[1]Hertie Institute for Clinical Brain Research and Centre for Integrative Neuroscience, University of Tübingen, Tübingen, Germany

[2]Department of Neurobiology, IBBS, University of Stuttgart, Stuttgart, Germany

[3]Laboratory of Behavioral and Genomic Neuroscience, NIAAA, NIH, Bethesda, MD, USA

[4]Friedrich Miescher Institute, Basel, Switzerland

The intercalated cells (ITCs) constitute densely packed clusters of inhibitory neurons in the amygdala, a brain structure involved in emotional behaviour. In particular, the amygdala is critical for associative fear learning. It is the site of long-term storage for associative fear memories, and neural activity in the central amygdala (CeA) mediates fear responses such as freezing. In a process called fear extinction, learned fear responses can be suppressed in a context-specific manner, which requires activity in the medial prefrontal cortex (mPFC).

Within the circuitry of learned fear, the ITCs have been conceptualized in the past mainly as an inhibitory relay of the mPFC and basolateral amygdala (BLA) to CeA, making them the top-down “off-switch” for the CeA during the extinction of learned fear. Recent studies additionally suggest close reciprocal interactions between ITC clusters and functional heterogeneity between clusters, whereby the dorsomedial cluster (dmITC) is activated during fear and the ventromedial cluster (vmITC) during extinction. To explore how the wiring of the ITC circuit could support such functional heterogeneity, we expressed channelrhodopsin-2 in single ITC clusters using a Cre-dependent viral strategy, and measured optogenetically evoked postsynaptic currents in acute brain slices of mice. By combining whole-cell voltage clamp recordings with pharmacology, we provide evidence that the dmITCs and vmITCs mutually inhibit each other via $GABA_A$ receptors, which could support opposing activity patterns during different behavioural states. This circuit architecture also provides a mechanistic explanation for ITC activity patterns observed with *in vivo* calcium imaging, and for the differential effects of pharmacogenetic interventions in dmITCs and vmITCs on fear expression. To further dissect the functional basis of heterogeneity among ITCs, we have begun to investigate projection specificity of single clusters.

Our data raise the intriguing possibility that ITCs do not only serve as an inhibitory relay for top-down control of amygdala output, but also form a functionally heterogeneous and interconnected network that is likely involved in multiple emotional processes.

Representation of stimulus-, task-, and choice-related information in rodent auditory cortex revealed by chronic current-source density recordings

Marina M. Zempeltzi[1], Martin Kisse[1], Shivam Maurya[1], Sümeyra Aksit[1], Lars T. Boenke[1], Michael G.K. Brunk[1], Frank W. Ohl[1,2,3], Matthias Deliano[1], Max F.K. Happel[1,2]

[1]Leibniz Institute for Neurobiology, System Physiology of Learning, WG CortXplorer, Brenneckestraße 6, 39118 Magdeburg,Germany

[2]Otto-von-Guericke University,Magdeburg, Germany

[3]Center for Behavioral Brain Sciences (CBBS),Magdeburg, Germany

The fundamental role of the primary auditory cortex (A1) in auditory learning and memory is well-documented. However, primary sensory cortex has been considered classically to process, extract and represent mainly sensory stimulus features, while the learning- and choice-related meaning of a stimulus might be more associated with higher-order brain areas. In this study, we therefore chronically recorded current source density (CSD) distributions from A1 of Mongolian gerbils (Meriones unguiculatus), while animals performed multiple reversals of the choice-outcome contingency in a Go/NoGo shuttle-box frequency discrimination task to investigate cortical circuit mechanisms underlying flexible auditory guided behaviors and decision making. We could demonstrate that not only sensory, but also task- and choice-related information is represented in the neuronal population code distributed across cortical layers. A detailed behavioral analysis based on performance levels and receiver operator curve (ROC) characteristics revealed different behavioral strategies and allowed us to correlate them with distinct cortical activation patterns. We found distinct differences of spatiotemporal columnar circuit activity between classes of choice and contingency in a layer-specific manner. Strongest recruitment of particularly infragranular layers corresponded to trials in which animals showed a conditioned response independent of the contingency, while supragranular activity was highest during correct hit trials. During correct rejections we generally found the lowest columnar activity suggesting an active inhibition of cortical processing. We further applied multivariate statistics and classified spatiotemporal activity patterns representing stimulus- or task-related features by linear support vector machine learning. While stimulus information was most prominently represented during tone-presentation, representation of task-related information about the meaning of the stimulus exceeded the physical tone for up to 500ms. Using a non-parametric Bayesian Friedman ranking test, we could show that particularly infragranular layers contribute most to task-dependent (top-down) representations in auditory cortex.
The current study further expands our understanding of cortical circuit processing modes in sensory cortex that codes task-relevant information in order to guide sensory-based learning, decision making and behavioral adaptation during strategy change.

A cellular source of second-order reinforcement in *Drosophila*

Christian König[1], Afshin Khalili[1], Thomas Niewalda[1], Shiqiang Gao[2], Bertram Gerber[1,3,4]

[1]Leibniz Institute of Neurobiology, Department Genetics of Learning and Memory, Brenneckestrasse 6, 39118 Magdeburg, Germany

[2]University of Würzburg, Julius-von-Sachs-Institute, Julius-von-Sachs Platz 2, 97082 Würzburg, Germany

[3]Otto von Guericke University Magdeburg, Institute for Biology, Universitätsplatz 2, 39106 Magdeburg, Germany

[4]Center for Behavioral Brain Sciences (CBBS), Universitätsplatz 2, 39106 Magdeburg, Germany

In insects odors are coded by the combinatorial activation of ascending pathways including their third-order representation in mushroom body Kenyon cells. Kenyon cells also receive intersecting input from ascending and mostly dopaminergic reinforcement pathways. Indeed, in *Drosophila*, presenting an odor together with activation of the dopaminergic mushroom body input neuron PPL1-01 (PPL1-γ1pedc or MB-MP1) leads to a weakening of the synapse between Kenyon cells and the approach-promoting mushroom body output neuron MBON-11 (MBON-γ1pedc>α/β or MB-MVP2). As a result of such weakened approach tendencies, flies avoid the shock-predicting odor in a subsequent test. Thus, increased activity in PPL1-01 stands for *punishment*, whereas reduced activity in MBON-11 stands for *predicted punishment*. Given that punishment-predictors can typically themselves serve as second-order punishments, we tested whether presenting an odor together with silencing MBON-11 would lead to learned odor avoidance, and found this to be the case. In turn, activation of MBON-11 together with odor presentation led to learned odor approach. Thus, the levels of activity in MBON-11 are a source of second-order reinforcement.

Poster Topic

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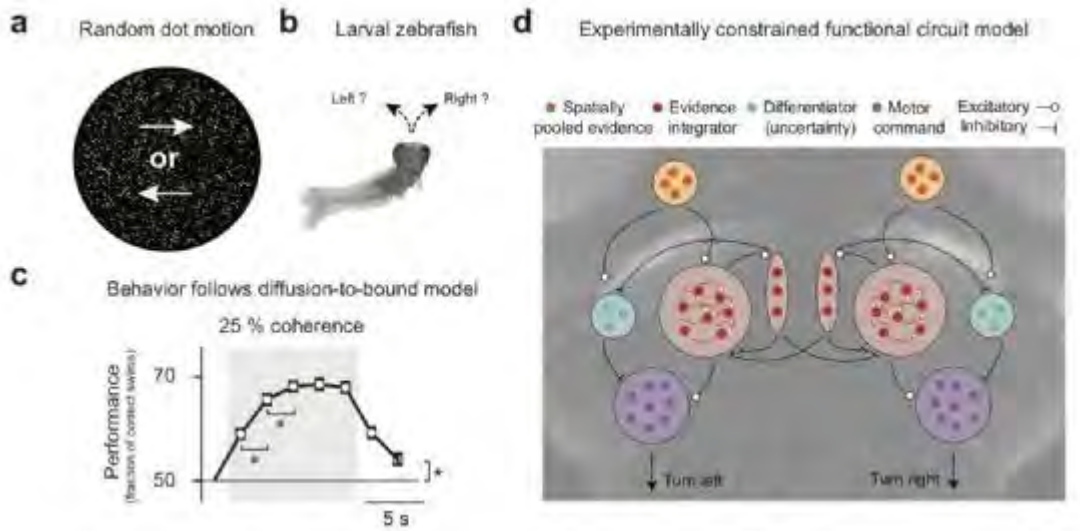
Neuronal mechanisms of evidence accumulation and perceptual decision making in the larval zebrafish

Armin Bahl[1], Florian Engert[1]

[1]Harvard University, Department of Molecular and Cellular Biology, 16 Divinity Avenue, 02138 Cambridge, MA, USA

Sensory evidence accumulation is a crucial part of any perceptual decision making process. Even though behavioral performance in psychophysical experiments can be well explained by abstract mathematical models of integration and thresholding, it remains elusive how such mechanisms are implemented on the level of neuronal networks. Comprehensive understanding of these underlying processes requires explorations of brain-wide circuit dynamics during individual trials. This is difficult to achieve in mammals where analysis is usually restricted to local circuits, allowing observations of only a very small fraction of the overall networks at any given time. Here we approach this problem by adapting a classical assay based on noisy random dot motion kinematograms, usually used in primate studies, to larval zebrafish. We characterized the delay and accuracy of individual swimming decisions and found that larvae can reliably integrate and remember such motion stimuli over many seconds and that their behavior follows precisely the classical diffusion-to-bound model. We then performed unbiased two-photon functional imaging experiments of the whole brain, identifying key circuit elements involved in the integration process. In particular, we found several neuronal clusters in the anterior hindbrain. One cluster represented the integrated sensory evidence, reminiscent of the diffusive variable in the model, while a second cluster represented sensory uncertainty. We propose that these two units together implement, in a biophysically plausible manner, the thresholding operation such that a third cluster, a motor command unit, is only activated when integrated evidence exceeds uncertainty. Analyzing these structures on the level of individual cells and trials allowed us to build a realistic neural network model, which not only quantitatively reproduced our experimental imaging data, but also the behavior of freely swimming fish.



Activity-induced changes in ion concentrations switch cellular and network dynamics

Mahraz Behbood[1], Susana Andrea Contreras[1], Jan-Hendrik Schleimer[1], Susanne Schreiber[1]

[1]Institute for Theoretical Biology, Humboldt-Universität zu Berlin and Bernstein Center for Computational Neuroscience Berlin, Philippstr. 13, 10115 Berlin, Germany

While electrical signaling relies on the exchange of ions across membranes, we usually assume that their concentrations are largely unchanged. Extensive neural activity, however, can in physiological and pathophysiological conditions result in the accumulation of ions in the extracellular space (Zandt et al. 2015, Gulledge 2013). Here, we demonstrate that such concentration changes can induce qualitative transitions in single-cell dynamics that bear relevance not only for information processing of the cell itself but also strongly influence network behavior. Taking a mathematical modeling approach, we here use conductance-based neurons to explore the effect of changing ionic concentrations on neuronal dynamics.

Specifically, we find that an activity-induced accumulation of extracellular potassium ions can induce a codimension-two bifurcation, leading to a switch in the spike-generating mechanism with consequences for a neuron's encoding properties and spiking statistics. We show that while Na^+-K^+-ATPases contribute to reversing this trend, their action takes time and the fast spiking dynamics of the neuron are transiently pushed into a different regime. Specifically, we combine a conductance-based neuronal point model with previously described dynamics of the Na^+-K^+-ATPase and allow intracellular and extracellular ionic concentrations to vary. At first glance, the net current produced by the pump reduces a cell's excitability comparable to the adaptation mediated by slow potassium channels. In addition, the transiently unbalanced changes in ionic concentrations also result in a less predictable, yet computationally relevant transition in neuronal dynamics. Particularly, for neurons with an initial spike generation via a saddle-node-on-an-invariant-cycle bifurcation, ion accumulation shifts spike generation into a homoclinic regime (Hübel, Schöll, and Dahlem 2014) and eventually can even transition into a region of excitation block, i.e. a mode of depression in neural activity.

In this study, we apply a bifurcation analysis to the system that combines the slow extracellular concentration changes with fast spiking activity. These results form the basis for a spatial network analysis which includes neuronal coupling via synapses and a shared extracellular space. The dynamical network states observed with potassium accumulation can lead to increased synchronization as well as suppression of neural activity. We expect these mechanisms to bear relevance for phenomena like spreading depression and epilepsy.

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Modelling Actin Dynamics in Dendritic Spines

Mayte Bonilla-Quintana[1], Christian Tetzlaff[1,2], Michael Fauth[1], Florentin Wörgötter [1,2]

[1]Georg-August-Universität Göttingen, III. Physikalisches Institut - Biophysik, Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience, Göttingen, Germany

Excitatory synapses in the cortex are key elements for information processing and storage in the brain. The postsynaptic part of these synapses dwells in dendritic spines that are small protrusions from neural dendrites. Interestingly, these spines experience shape and size fluctuations that are correlated with changes of the strength on excitatory synaptic connections, hence linking morphological and functional properties of the synapse. However, spine volume also varies spontaneously. In order to investigate the nature of such shape fluctuations, we propose a mathematical model.
The main structural component of dendritic spines is a network of actin filaments; therefore, our model is based on actin dynamics. Actin filaments continuously undergo a treadmilling process in which actin monomers are polymerised at the barbed end of the filament (localised close to the cell membrane), while the filament is depolymerised or severed at the pointed end. Polymerisation generates an expansive force which can push the cell membrane forward; and thus, increase the size of spines. Furthermore, filaments can branch and form new barbed ends; also polymerisation at the barded end and depolymerisation at the pointed end can be inhibited by capping proteins. These events vary the number of barbed ends and are modelled by a stochastic process.
The proposed model resembles dendritic spine membrane deformations resulting from the balance between the expansive force generated by polymerisation of barbed ends and membrane tension (described by the Helfrich free energy). Importantly, the model allows simulations with asymmetric spine shapes with few foci of actin treadmilling and long periods of time (up to days). The distribution and nucleation of such foci of actin activity and its effects on the spine shape fluctuations are investigated.

Activity patterns in a mathematical model of a gap-junction coupled network of heterogeneous neurons.

Hans Albert Braun[1], Aubin Tchaptchet[1]

[1]University of Marburg, Institute of Physiology, Deutschhausstr. 1, 35037 Marburg, Germany

The nervous system is composed of neurons of enormous heterogeneity. It is well known among experimentalist that no neuron, not even from the same brain area and of the same type, reacts in exactly the same way as any other one. The possible impact of such neuronal heterogeneity on neuronal network synchronization has been examined in a mathematical model of a network of nearest neighbour gap-junction coupled neurons during increasing coupling strength.

Heterogeneity has been introduced by Huber-Braun model neurons [1] with randomization of the temperature as a scaling factor which can generate an enormous diversity of impulse pattern, including burst discharges, chaotic activity and two different types of tonic firing – all of them also experimentally observed in the peripheral as well as central nervous system.

When the network includes all these type of neurons, randomly selected, a particular phenomenon can be observed. At a certain coupling strength the network goes into a completely silent state although all neurons have originally been spontaneously firing driven by subthreshold oscillations. When parts of the neurons with specific patterns are taken out, especially the tonic firing neurons from the lower and upper extremes of temperature range, spontaneous firing can be reinstalled with further increasing coupling strength. Reinstalled firing develops from slowly increasing subthreshold oscillations and is always of the tonic firing type and already fairly well synchronized [2].

Examination of voltage traces and interspike-intervals of individual neurons suggests 1) that all neurons, irrespective of their original pattern, go through a well-known bifurcation scenario as observed on current injection eventually leading to subthreshold oscillations without spikes and 2) that synchronization continues in the silent state at the level of subthreshold oscillations. These data demonstrate that neuronal synchronization is far away from being only a matter of synaptic coupling. The dynamics of the individual neurons play a major role.

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Self-organized reactivations maintain and strengthen memories despite synaptic turnover.

Michael Fauth[1], Mark van Rossum[2]

[1]Universität Göttingen, Drittes Physikalisches Institut, Friedrich Hund Platz 1, 37077 Göttingen, Germany
[2]University of Nottingham, Schools of Math and Psycholgy, University Park, NG7 2RD Nottingham, United Kingdoms

Long-term memories are believed to be stored by the synapses in the cortex. However, recent experiments report a continuous creation and removal of these synapses, such that only a small fraction of them is persistent over a few months. This poses the question how long-term memories are retained by such a variable substrate.
We study the formation and retention of Hebbian cell assemblies as model for associative memory. Such cell assemblies can reactivate in rest phases, when the network receives no external input. In combination with Hebbian plasticity, this suffices to maintain the assemblies although the synapses that originally encoded the memory are gradually removed and replaced. Interestingly, a few minutes of rest every day can even strengthen their connectivity without any further external reactivation. This self-organized strengthening, which is reminiscent of offline-memory gains observed during sleep, makes the assemblies not only more robust against prolonged intervals without resting but also leads to improved associative properties.
We show that the strengthening emerges from the convergence to an attractive stationary state at high connectivity levels which also underlies the long-term retention of the assemblies. Hence, the stabilisation against the continuous synapse creation and removal, and the improvements in memory performance observed during sleep or resting may be two effects of the same dynamics.

Non-random connectivity of networks subject to homeostatic structural plasticity

Júlia V Gallinaro[1], Stefan Rotter[1]

[1]University of Freiburg, Bernstein Center Freiburg, Hansastrasse 9a, 79104 Freiburg, Germany

Cortical networks have connectivity features that deviate from that of simple random networks. These include a skewed distribution of synaptic weights [1], indegrees and outdegrees of neurons [2], as well as an over-representation of bidirectional connections [1,3]. It is not yet known, however, how these deviations emerge and how they relate to function. In this study, we have explored how non-random connectivity and neuronal activity are related by simulating recurrent networks of leaky integrate and fire neurons with structural plasticity. Using a rule based on firing rate homeostasis [4,5], we grow networks in which neurons are made to fire at individually specified rates, drawn from experimentally reported distributions [6]. The obtained networks have an interesting structure that includes non-random features similar to those of cortical networks. Through network analysis, we show how the firing rate distribution relates to structural properties of the network, such as its indegree or the abundance of bidirectional connections. Our analysis indicates that some of these features emerge always in combination with, and possibly as a consequence of, other features. We also found that self-consistency analysis of recurrent networks is generally a powerful tool for understanding the intricate relationship between network structure and activity dynamics.

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Short-term ITD (interaural time difference) estimation of natural sound stimuli via effective models of binaural brainstem nuclei

Sebastian Groß[1,2,3], Christian Leibold[1,2]

[1]LMU Munich, Computational Neuroscience, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany
[2]Bernstein Center for Computational Neuroscience, Munich, Germany
[3]Graduate School of Systemic Neurosciences, Munich, Germany

The most important cue to localize low-frequency sounds (<1,500 Hz) in the azimuthal plane is the interaural time difference (ITD). Extraction of ITDs in the mammalian brainstem is performed by neurons of the medial superior olive (MSO) and the low-frequency limb of the lateral superior olive (lLSO). However, a single neuron responds differently for varying stimulus frequencies and it is thus unclear how ITDs are read out on a neuronal population level when considering non-trivial (non-sinusoidal) sound stimuli which have a broad spectrum.

To determine a possible encoder of ITDs for non-trivial stimuli, we have developed fast and effective models of the gerbil MSO and lLSO to simulate large populations of neurons, identified by the three parameters which fully characterize each individual neuron: the best frequency (BF), the characteristic delay (CD) and the characteristic phase (CP). The distribution of these parameters is taken from experiment.

In our model, the hemispheric difference model (2-channel model) fails to decode large ITDs (>300 µs) for high best frequencies (>1,000 Hz). We find that a possible decoding strategy can be formulated in the two-dimensional space spanned by the firing rates of MSO vs. lLSO in each hemisphere. We decode ITDs from this two-dimensional space via short-term estimates, where the length of the estimation window is optimized to approximate the ground truth ITD. This method of ITD estimation can be applied to analyze complex auditory scenes such as multiple concurring sound sources at different locations (cocktail party effect) and sound sources moving along the azimuthal plane (ITD variation).

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Reproducible neural network simulations: model validation on the level of network activity data

Robin Gutzen[1], Michael von Papen[1], Guido Trensch[2], Pietro Quaglio[1], Sonja Grün[1,3], Michael Denker[1]

[1]Institute of Neuroscience and Medicine (INM-6) and Institute for Advanced Simulation (IAS-6) and JARA Institute Brain Structure-Function Relationships (INM-10), Jülich Research Center, Jülich, Germany

[2]Simulation Lab Neuroscience, Jülich Supercomputing Centre (JSC), Institute for Advanced Simulation, JARA, Jülich Research Center, Jülich, Germany

[3]Theoretical Systems Neurobiology, RWTH Aachen University, Aachen, Germany

Modern neuroscience relies on simulations of neural network models to bridge the gap between the experimentally observed activity dynamics in the brain and the theory of neural networks. The quantitative assessment of both experimental and simulated data is the basis for a rigorous validation practice and an indispensable part of any simulation workflow. Moreover, the variety of simulation tools and frameworks, and levels of model description also requires the validation of model implementations against each other to evaluate their equivalence. Despite rapid advances in the formalized description of models [1], data [2], and analysis workflows [3,4], there is no accepted consensus regarding the terminology and practical implementation of validation workflows. This situation prevents the generic, unbiased, and rigorous comparison between published models and data sets, which is a key element of building biologically realistic models in computational neuroscience.

Here, we relate terminology from the literature on validation to our domain and argue for the establishment of standardized statistical test metrics that enable formalized quantitative validation workflows. Furthermore, we emphasize the importance of validation testing on the level of network activity. Despite the importance of validating the elementary components of a simulation, such as single cell dynamics for networks of spiking neurons [5], constructing networks from validated building blocks does not entail the validity of the simulation on the network scale. Therefore, we here describe and discuss a set of complementary tests based on features of network dynamics, ranging from mono- and bivariate statistics such as firing rates and correlation coefficients to more model specific features such as statistics of spatiotemporal spiking patterns. Furthermore, we also present the formal implementation of these tests in the Python module NetworkUnit (RRID:SCR_016543) and the Elephant (RRID:SCR_003833) analysis framework.

We illustrate a corresponding example workflow that practically demonstrates the iterative comparison, or *substantiation*, of a spiking neural network model simulation against its reproduction on the SpiNNaker [6] neuromorphic system, validating the ability of the SpiNNaker system to accurately simulate network models [7,8,9]. Using that framework we also showcase the application to the validation of a network model on experimental data [10].

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Mapping cell types in the reptilian brain with single-cell transcriptomics

David Hain[1], Tatiana Gallego-Flores[1], Maria Antonietta Tosches[1], Gilles Laurent[1]

[1]Max-Planck-Institute for Brain Research, Max-von-Laue Str. 4, 60438 Frankfurt am Main, Germany

Reptiles and mammals shared a common ancestor ~320 million years ago. Understanding similarities and differences between reptilian and mammalian brains allows one to draw inferences on the brain organisation of the stem amniote ancestor.

All brains consist of multiple types of cells which perform different functions. Recent transcriptomic approaches have allowed the large-scale, unbiased classification of cell types in the brain of different species (Zeisel *et al.* 2018, Saunders *et al.* 2018, Davie *et al.* 2018, Raj *et al.* 2018). Recent work in our lab (Tosches *et al.* 2018) classified cell types in the cerebral cortex of reptiles through single-cell RNA sequencing and mapped them by in situ hybridisation of marker genes.
Expanding on this work, we are classifying and mapping cell types in all parts of the reptilian brain of *Pogona vitticeps*. This allows us to identify subpallial structures (e.g. striatum, CeA), thalamic and hypothalamic nuclei, as well as several other diencephalic nuclei.

Comparing gene expression and anatomical location of cell types between species allows us to infer homologous regions between mammals and reptiles. This will shed light on the evolution of the amniote brain, as well as brains in general.

Neural model for the visual recognition of agency and social interaction

Mohammad Hovaidi-Ardestani[1,2], Nitin Saini[2], Martin Giese[1]

[1]Department of Cognitive Neurology Hertie, Institute for Clinical Brain Research Centre for Integrative Neuroscience, University Clinic Tübingen, Germany

[2]IMPRS for Cognitive and Systems Neuroscience, University of Tübingen, Germany

INTRODUCTION: Humans derive spontaneously judgements about agency and social interactions from strongly impoverished stimuli, as impressively demonstrated by the seminal work by Heider & Simmel (1944). The neural circuits that derive such judgements from image sequences are entirely unknown. It has been hypothesized that this visual function is based on high-level cognitive processes, such as probabilistic reasoning. Taking an alternative approach, we show that such functions can be accomplished by relatively simple computations that can be implemented by physiologically plausible neural circuits, exploiting an appropriate hierarchical (deep) neural model of the visual pathway.
METHODS: Using a deep neural network for the construction of dictionaries of low and mid-level feature detectors, we built a hierarchical neural model that reproduces elementary psychophysical results on animacy and social perception from abstract stimuli. The lower hierarchy levels of the model consist of position-specific neural feature detectors that re selective for oriented contours and intermediately complex shape features. The next-higher level is formed by position-variant neurons that are selective for shapes and their orientation in the image plain. The output of these neurons is processed by a form and a motion pathway that computes the body axis, the relative positions, speeds and accelerations of moving agents, exploiting established neural circuits (gain fields, motion energy detectors). The top level of the model combines these extracted features using simple feed-forward neural circuits and elementary classifiers.
RESULTS: Based on input video sequences, the model successfully reproduces results of Tremoulet and Feldman (2000) on the dependence of perceived animacy on motion parameters and the body axis. The animacy percept is stronger for agents that abruptly change their direction and speed, and if the movement is aligned with the body axis. In addition, the model classifies correctly six categories of social interactions that have been frequently tested in the psychophysical literature (following, fighting, chasing, playing, guarding, and flirting) (e.g. Scholl & McCarthy, 2012; McAleer et al., 2008). In addition, we show that the model can be extended for the processing of simple interactions in real-world movies.
CONCLUSION: Using simple physiologically plausible neural circuits, the model accounts simultaneously for a variety of effects related to animacy and social interaction perception. Even in its simple form the model proves that animacy and social interaction judgements partly might be derived by very elementary operations in hierarchical neural vision systems, without a need of sophisticated or accurate probabilistic inference. The model makes specific predictions about neurons involved in the visual processing of abstract social stimuli.

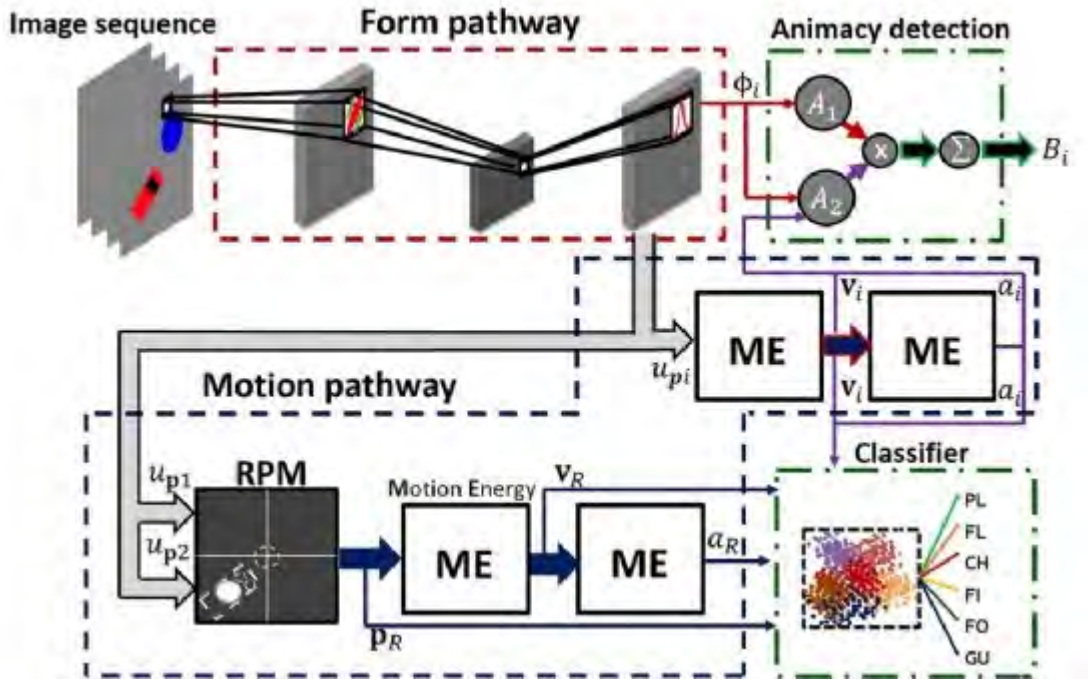
Image sequence
Form pathway
Animacy detection
ϕ_i
A_1
A_2
x
Σ
B_i
Motion pathway
u_{pi}
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Classifier
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BrainTrawler: A Web-based Framework for Iterative Exploration of Big Brain Network Data

Joanna Kaczanowska[1], Florian Ganglberger[2], Wulf Haubensak[1], Katja Bühler[2]

[1]Research Institute of Molecular Pathology IMP, Vienna, Austria

[2]VRVis Research Center, Biomedical Visualization, Vienna, Austria

Recent advances in neuro-imaging allowed big brain-initiatives and consortia to create vast resources of brain data that can be mined by researchers for their own projects. Exploring the relationship between genes, brain circuitry, and behavior is one of key elements of neuroscience research. This requires fusion of spatial connectivity data at varying scales, such as whole brain correlated gene expression, structural and functional connectivity. With ever-increasing resolution, those exceed the past state-of-the art in several orders of magnitude in size and complexity. Current analytical workflows in neuroscience involve time-consuming manual aggregation of the data and only sparsely incorporate spatial context to operate continuously on multiple scales. Incorporating techniques for handling big connectivity data is therefore a necessity.

We present a novel web-based framework to explore heterogeneous neurobiological connectivity data of different types, modalities and scale for interactive visual analytics workflows. It enables domain experts to combine data from large-scale brain initiatives with user-generated data, by utilizing the hierarchical and spatial organization of the data. Connectivity data at different resolutions, such as mesoscale structural connectivity and region-wise functional connectivity can be queried on different levels on a common hierarchical reference space. On-demand queries on volumetric gene expression and connectivity data enable an interactive dissection of networks, with billions of edges, in real-time, and based on their spatial context. Relating data to the hierarchical organization of common anatomical atlases allows experts to compare multimodal networks on different scales. Additionally, 3D visualizations have been optimized to accommodate domain experts' needs for publishable network figures.

We demonstrate the application of our approach by analyzing fear-related functional neuroanatomy in mice. Further, we show its versatility by comparing multimodal brain networks linked to autism. Importantly, we achieve cross-species congruence in retrieving human psychiatric traits networks, which facilitates selection of neural substrates to be further studied in mouse models.

For the future, we are aiming to extend this prototype to create a holistic framework for interactive exploration of neurobiological data. This should not only allow to access the data, but also include the import, preprocessing as well as computing network statistics in the web.

Stabilization of Hebbian cell assemblies by synaptic consolidation

Jannik Luboeinski[1,2], Christian Tetzlaff[1,2]

[1]III. Institute of Physics - Biophysics, University of Göttingen, Dept. of Computational Neuroscience, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

[2]Bernstein Center for Computational Neuroscience, University of Göttingen, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

It still remains a largely unresolved issue how memory consolidation is implemented in the brain. For the microscopic level, the candidate mechanism is a biomolecular process called synaptic consolidation, which stabilizes the state of synapses that have undergone long-term potentiation or depression. Synaptic consolidation transfers synapses from an early-phase state, lasting several hours, to a late-phase state, lasting at least several days [1, 2, 3]. While there are theoretical investigations of synaptic consolidation at single synapses [4, 5, 6], investigations at the network level, particularly with regard to Hebbian cell assemblies, are missing. Hebbian cell assemblies are groups of neurons with strong connections within the group, making the neurons tend to fire together. They are assumed to serve as memory representations in the brain [7]. We examine how synaptic consolidation can stabilize Hebbian cell assemblies in a theoretical network model, and thus, how synaptic consolidation can account for memory consolidation.

Our synapse model is based on a calcium-dependent model that accounts for spike-timing dependent as well as activity-dependent plasticity [8]. To describe late-phase dynamics, we use synaptic tagging and capture, which is a concept widely used to describe synaptic consolidation [1, 2]. In synaptic tagging and capture, proteins can be captured by synapses that are tagged, which leads to the stabilization of synaptic changes. In general, we consider that a synapse is tagged and that protein synthesis is initialized, if the synapses of a neuron have received a sufficient level of early-phase synaptic changes [6]. Different to previous studies, we consider multiple protein pools because it was shown that late-phase potentiation and depression feed on specific pools [3]. Employing the synapse model, we connect spiking neurons to a network and apply learning and recall stimuli with varying parameters. We then use Mutual Information to measure how much the uncertainty in synaptic weights and neuronal activities is reduced by learning and recall. For a wide range of parameters, we find long-term reduction in uncertainty, which corresponds to the formation and stabilization of Hebbian cell assemblies.

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Sparse Coding Predicts Optic Flow Specificities of Zebrafish Pretectal Neurons

Hanspeter A. Mallot[1], Gerrit Ecke[1], Fabian Mikulasch[1,2], Sebastian Bruijns[1], Thede Witschel[1], Aristides B. Arrenberg[1]

[1]University of Tübingen, Institute for Neurobiology, Auf der Morgenstelle 28, 72076 Tübingen, Germany
[2]Chalmers University, Gothenburg, Sweden

Zebrafish pretectal neurons exhibit specificities for large-field optic flow patterns associated with rotatory or translatory body motion. We investigate the hypothesis that these specificities reflect the input statistics of natural optic flow. Realistic motion sequences were generated using computer graphics simulating self-motion in an underwater scene. Local retinal motion was estimated with a motion detector and encoded in four populations of directionally tuned retinal ganglion cells, represented as two signed input variables. This activity was then used as input into one of two learning networks: a sparse coding network (competitive learning) and backpropagation network (supervised learning). Both simulations develop specificities for optic flow which are comparable to those found in a neurophysiological study (Kubo, F. et al., 2014, Neuron 81:1344-59), and relative frequencies of the various neuronal responses are best modeled by the sparse coding approach. We conclude that the optic flow neurons in the zebrafish pretectum do reflect the optic flow statistics. The predicted vectorial receptive fields show typical optic flow fields but also “Gabor" and dipole-shaped patterns that likely reflect difference-fields needed for reconstruction by linear superposition. For a full version of this paper, see https://arxiv.org/abs/1805.01277.

A functional network model of the neocortex can reproduce spiking dynamics in monkey motor cortex during delayed reach movements

Martin Paul Nawrot[1], Thomas Rost[1], Alexa Riehle[2], Sacha J van Albada[3], Vahid Rostami[1]

[1]University of Cologne, Computational Systems Neuroscience, Zülpicherstr 47B, 50674 Köln, Germany
[2]Institut de Neurosciences de la Timone (INT), UMR 7289, CNRS-Aix Marseille Université, Marseille, France
[3]Institute of Neuroscience and Medicine (INM-6) and Institute for Advanced Simulation (IAS-6) and JARA Institute Brain Structure-Function Relationships (INM-10), Forschungszentrum Jülich, Germany

The performance of a behavioral task in a human or monkey requires precise processing of sensory cues, rapid decision-making, and the accurate control of behavior. At the level of cortical processing this involves the highly dynamic and successive activation of neuronal populations across different cortical areas. During each repetition of the task, i.e. in the single trial, the cortical networks are in a different initial activity state. This results in a considerable trial-to-trial variability of single neuron responses. This variability dynamically and consistently reduces during task performance, both in sensory cortices [1] and motor cortices [2,3].

Here we study spiking neural network models of the neocortex with cluster topologies that enable multistability and attractor dynamics, a feature that can subserve functional roles such as optimization, decision-making, or action selection. Multi-stable attractors can be realized in cortical networks through clusters of strongly interconnected excitatory neurons [4-6]. However, we show that this network topology results in the loss of the excitation-inhibition balance and is not robust against overall modulation of network activity. Moreover, it leads to widely separated firing rate states of single neurons [7] inconsistent with experimental observations.

To overcome these problems we propose the incorporation of two biologically plausible mechanisms. At the network level, recent anatomical and physiological studies point to increased local inhibitory connectivities and possible inhibitory clustering through connection strengths [8-11]. We therefore suggest a combination of excitatory and inhibitory clustering [7] to restore local excitation/inhibition balance. At the cellular level, we could previously show that spike frequency adaptation (SFA) strongly affects cortical variability dynamics [12] and a recent study with large-scale recordings [13] argued for the importance of SFA to explain cortical activity dynamics.

We find that inhibitory clustering is necessary to achieve realistic spiking activity in terms of a biologically realistic firing rate, spiking regularity, and trial-to-trial spike count variability. Including SFA at the single cell level adds a second transitory component to task-related variability dynamics that is also observed in vivo. Together, both mechanisms make these networks highly robust against parameter fluctuations due to homeostasis or neuromodulation.

With the appropriate stimulation of network clusters we could qualitatively and quantitatively reproduce in vivo firing rate and variability dynamics for different task conditions as observed in multiple single unit recordings form motor cortex of the behaving monkeys [2].

Acknowledgments

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Data driven exploration of mouse behavior in the Go/No-Go task.

Lukasz Piszczek[1], Manuel Pasieka[2,3], Andreea Constantinescu[1,3], Wulf Haubensak[1]

[1]IMP - Research Institute of Molecular Pathology, Campus-Vienna-Biocenter 1, 1030 Vienna, Austria
[2]Vienna Biocenter Core Facilities, Vienna, Austria
[3]Equal contribution

Inhibitory control, an ability to suppress and cancel prepotent responses is a key element of cognitive control. Impairment in this function underlies both impulsive and compulsive behaviours, which are symptoms of conditions such as: attention deficit/hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), as well as addiction. Maladaptive inhibitory control is highly correlated with increased trait impulsivity, a highly complex behavior, involving incentive salience, attention and fast action response.
In our study we have used the symmetrical Go/No-go task as a model for behavioral inhibition to provide a deeper understanding of evolution of behavioral states in an impulsivity-related setting. In the first step we have determined mouse behavioral states in a data-driven fashion across different learning sessions, as well as in trained animals. For this we have used unsupervised clustering algorithms across several behavioral measures (i.e. distance traveled, position in the cage, immobility state, port visit among others). Lastly, we have investigated how these parameters are affected by pharmacological manipulation using compounds known to affect impulsivity in humans. Our preliminary data suggest that this analysis detects drug induced changes in behavioral patterns that go beyond the classically investigated parameters.

Synaptic contributions to information processing of natural sounds in the VNLL

Michael Rebhan[1], Linda Fischer[3], Felix Felmy[3], Christian Leibold[1,2]

[1]Ludwig-Maximilians-Universität München, Department Biology II

[2]Bernstein Center for Computational Neuroscience, Munich

[3]Stiftung Tierärztliche Hochschule Hannover, Zoologisches Institut

Within the ventral nucleus of the lateral lemniscus (VNLL) exists a population of cells that exhibits a precise onset response to pure tone stimuli. It is believed that these cells play an important role in information processing of amplitude modulated sounds, such as conspecific vocalisation. One major excitatory input to VNLL onset cells arises from the octopus cells of the posterior ventral cochlear nucleus (PVCN), and thus much of the VNLL onset behaviour is presumed to be inherited from octopus cells. We find that glutamatergic synapses at VNLL cells, that presumably convey octopus cell activity, have a double exponential conductance shape combining a fast (sub millisecond) AMPA component and a slow time component, which we would attribute to NMDA receptor activation by pharmacological intervention.

It is unclear how such complex synaptic filtering would affect the processing of octopus cell inputs. To elucidate the effects of such synaptic filtering, we devised a model network with octopus cells feeding into VNLL neurons. Octopus cells are thereby described by a novel phenomenological model that only comprises few well-constrained free parameters and closely emulates the properties of the octopus cell responses to pure tones and amplitude modulated tones. Using this model, we study the effects of synchronous firing of auditory nerve-fibers over a wide frequency band as well as the influence of additional filtering on the response patterns. Furthermore, the VNLL neuron also receives broadband inhibition, assumed to be originating from multiple neurons of the medial nucleus of the trapezoid body (MNTB).

To understand the role of VNLL onset responses in the processing of natural sounds, we used information theory to determine the optimal frequency range and stimulus features most efficiently driving VNLL responses. Preliminary testing with human speech stimuli suggests that the model favours certain phonemes over others depending on the centre frequency of the modelled octopus cells. Applying information theoretical tools to the processing of human speech, we find that most of the information transmission occurs for modulation frequencies below 200 Hz. This suggests that the fine structure of the stimulus is suppressed and only the low frequency amplitude modulations of complex sounds are preserved. For conspecific vocalisation in general the responses seem to be keyed to strong transients and sounds with wide power spectra.

Temperature-induced heart arrhythmias - a mathematical modeling perspective

Pia Rose[1,2], Jan-Hendrik Schleimer[1,2], Susanne Schreiber[1,2]

[1]Humboldt-Universität zu Berlin, Institute for Theoretical Biology, Philippstr. 13, Haus 4, 10115 Berlin, Germany
[2]Bernstein Center for Computational Neuroscience, 10115 Berlin, Germany

The heart is the first functioning organ in the human embryo. Starting around 22 days after fertilization, its rhythmic contraction continues reliably for our whole life [1]. Some changes in physiological parameters, however, can challenge the proper functioning of this vitally important rhythm. Among these, alterations in body core temperature are known to constitute a risk factor for heart arrhythmias: exposure of the heart to both too cold (hypothermia) and too warm (hyperthermia) temperatures can cause life-threatening aberrations of the beating rhythm [2,3].

On short timescales, the primary effect of temperature on electrical signaling of the heart is the modulation of ion channel gating rates, peak conductances, and equilibrium potentials. Accordingly, different ion channel mutations have been associated with temperature-induced heart arrhythmias [4]. For example, some sodium channel mutations can lead to the Brugada syndrome, in which patients are more likely to suffer from heart arrhythmia during fever. To better understand the temperature-mediated effects of ion channels on the rhythmicity of the electrical activity in the heart, we here employ conductance-based mathematical neuron models. We aim for a systematic investigation of the temperature effects on a cardiac cell's temporal firing patterns, enabling us to single out those biophysical consequences of temperature changes that are responsible for the induction of pathological heart dynamics. Accordingly, we predict the impact of changes in channel inactivation over changes in channel kinetics. The elucidated mechanisms may help to guide future treatment options in pathological conditions like the Brugada syndrome.

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Full rescue of an inactive olfactory receptor mutant by elimination of an allosteric ligand-gating site

Kanika Sharma[1], Sabine Balfanz[2], Arnd Baumann[2], Sigrun Korsching[1]

[1]Institute of Genetics, Biocenter, University at Cologne, Zülpicherstrasse 47a, 50674, Cologne, Germany
[2]Institute of Complex Systems (ICS-4), Research Center Jülich, 52428, Jülich, Germany

Olfaction poses one of the most complex ligand-receptor matching problems in biology due to the unparalleled multitude of odor molecules facing a large number of cognate olfactory receptors. We have recently deorphanized an olfactory receptor, TAAR13c, as a specific receptor for the death-associated odor cadaverine. It appears to possess an external allosteric binding site for cadaverine, which was assumed to block progress of the ligand towards the internal orthosteric binding-and-activation site. Here we have challenged the suggested gating mechanism by modeling the entry tunnel for the ligand as well as the ligand path inside the receptor. We report an entry tunnel, whose opening is blocked by occupation of the external binding site by cadaverine, confirming the hypothesized gating mechanism. Furthermore we have combined a gain-of-function gating site mutation and a loss-of-function internal binding site mutation in one recombinant receptor. This receptor had almost wildtype ligand affinities, consistent with modeling results that showed localized effects for each mutation. A novel mutation of the suggested gating site resulted in increased receptor ligand affinity. In summary both the experimental and the modeling results provide further evidence for the proposed gating mechanism, which surprisingly exhibits pronounced similarity to processes described for some metabotropic neurotransmitter receptors.

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SWAN: A tool to track single units across consecutive electrophysiological recordings

Shashwat Sridhar[1], Alper Yegenoglu[1], Nicole Voges[1], Thomas Brochier[2], Alexa Riehle[2,1], Sonja Grün[1,3], Michael Denker[1]

[1]Institute of Neuroscience and Medicine (INM-6) and Institute for Advanced Simulation (IAS-6) and JARA Institute Brain Structure-Function Relationships (INM-10), Jülich Research Centre, Jülich, Germany

[2]Institut de Neurosciences de la Timone (INT), CNRS-Aix-Marseille University, UMR 7289, Marseille, France

[3]Theoretical Systems Neurobiology, RWTH Aachen University, Germany

Electrophysiological experiments often involve the measurement of extracellular analog voltage signals from brain tissue using implanted microelectrodes. The spiking activity of neurons in the direct vicinity of an electrode is captured as short-lasting voltage deflections. Some of the deflections are action potentials (spikes) of neurons close to the electrode tip [1]. A crucial first step in the analysis of such data is the extraction of spikes and their assortment into clusters corresponding to contributions from different putative single neurons, or in short 'units' (spike sorting) [1, 2]. The sorting compares the shape of waveforms and the spiking characteristics. With chronically implanted electrodes, spiking activity is recorded for several months over multiple sessions. It is not clear if each electrode detects identical units over the entire course of an experiment. However, this knowledge would help to monitor long-term changes of neural activity during training for a task. In practice, some single units disappear, (re-)appear or progressively change their spike shape, likely due to small movements of the electrode and/or tissue growth. Thus, it becomes a challenge to keep track of identical units across consecutive chronic recordings [3, 4]. In the absence of such a tracking of neurons across sessions, detected units in one session are assumed to be independent of those in other sessions of the experiment. They may thus be considered more than once in analyses of several sessions, and bias statistics across sessions.

Here we present the Sequential Waveform Analyzer (SWAN) - an open-source tool developed to track individual units across sessions, but also to identify units that are different. It provides a graphical user interface (GUI) to visualize and relate spike-sorted data across multiple sessions. The configurable user interface is divided into several windows (see Figure). In each window, a certain set of features (e.g., mean waveforms, inter-spike interval histograms, principal component analysis of mean waveforms, firing rate profiles) are compared between different units and across multiple sessions. Each set of similar units across sessions is then assigned one global unit ID, represented by one common color across all windows. Thus, we visualize the tracking of a certain unit across consecutive sessions. The assignment of units to global unit IDs is performed by published [3,4] and newly developed automatic algorithms, and can be easily edited by the experimenter in the GUI. We demonstrate the capabilities of SWAN and practically illustrate its application on large-scale recordings from macaque monkey motor cortex [5].

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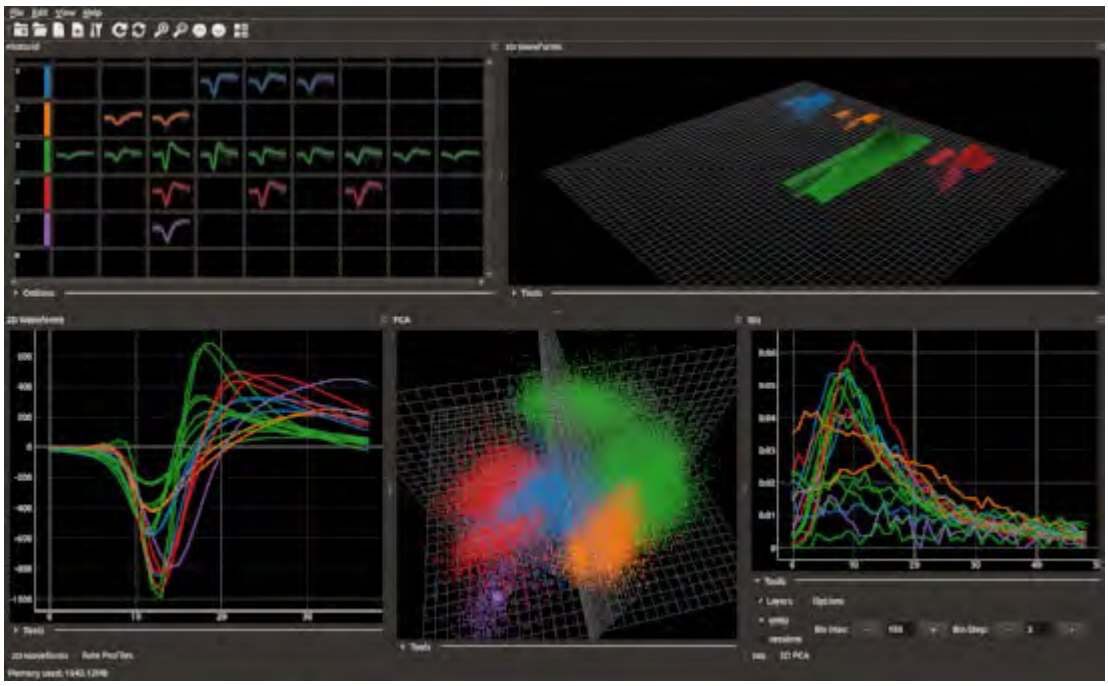

A practical guide for using Poisson GLMs on the basis of simulation studies for predicting neural spiking activity

Valentina A. Unakafova[1], Alexander Gail[1,2,3,4]

[1]Cognitive Neuroscience Laboratory, German Primate Center – Leibniz Institute for Primate Research, Kellnerweg 4, 37077, Goettingen, Germany

[2]Bernstein Center for Computational Neuroscience, Am Fassberg 17, 37077, Goettingen, Germany

[3]Georg-Elias-Müller-Institute of Psychology, University of Goettingen, Gosslerstrasse 14, 37073, Goettingen, Germany

[4]Leibniz ScienceCampus Primate Cognition, Kellnerweg 4, 37077, Goettingen, Germany

Recent technological advances allow to record neural spiking activity from several brain areas and several channels simultaneously over extended time periods [1]. This opens new possibilities for systems neuroscience and makes the problem of data-driven modeling of large scale spiking activity especially relevant. One of the often used techniques for this is Poisson Generalized Linear Models [2]. Poisson GLMs have been recently employed to investigate predictability of spiking activity with the aim to explain neural mechanisms of cognition and adaptive behavior [3], to reveal external stimuli influencing spiking activity in various brain areas under different experimental conditions [4], to track connectivity changes in neural population within or between brain areas in order to explain neural mechanisms underlying the performed task [1,5]. As a technique for predicting spiking activity, Poisson GLM has several advantages. First, it provides a transparent statistical framework that allows clear interpretability of the results compared to novel machine-learning methods. Second, Poisson GLM method allows to investigate predictability within neural population as a whole [5], compared to pairwise connectivity methods such as correlation, Granger causality or mutual information.

However, when applying Poisson GLMs to spiking data, there are many questions one should answer. For instance, what happens if Poisson GLM assumptions do not hold and which of them can be relaxed? It is often unclear which predictors one should consider, what width of the time bins and what overlap between time bins is reasonable to use, or what preprocessing to employ before applying Poisson GLM. How to deal with correlated covariates? Is cross-validation necessary and which technique to use? Which goodness-of-fit measure to employ? And, finally, is Poisson GLM a good model for the considered spiking data at all, or do other techniques provide a better alternative.

In order to answer these questions, we consider several types of simulated and real spiking data satisfying assumptions of Poisson GLM to different extent, and apply different preprocessing to them. We perform computational experiments for different parameters of Poisson GLM (bin size, predictors' number and type, number of observation points etc.). We also consider several measures for goodness-of-fit evaluation. As a result, we provide a practical guideline for the choice of Poisson GLM parameters and suggest a generic algorithmic framework describing the sequence of steps for using Poisson GLM for predicting spike counts. We consider possible problems of applying Poisson GLMs to real-world spiking data such as bad model fit, over-fitting, over-dispersion, multicollinearity, illustrate these problems for simulated and real data, and discuss possible solutions to these problems. Finally, we compare performance of Poisson GLM models with machine learning alternatives.

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A multi-compartment model based on expression patterns of structural and ion channel proteins in a multimodal cell type of the avian optic tectum

Stefan Weigel[1], Thomas Künzel[2], Katharina Lischka[1], Harald Luksch[1]

[1]Technical University of Munich, Zoology, Liesel-Beckmann Strasse 4, 85354 Freising, Germany
[2]RWTH Aachen University, Department of Chemosensation, Worringer Weg 3, 52074 Aachen, Germany

The vertebrate midbrain is an important subcortical area involved in different functions such as integrating different sensory modalities, movement initiation and bottom-up attention. Our group is particularly interested in cellular integration of multisensory information. We focus on the visual part of the avian midbrain, the optic tectum (TeO, homologous to the mammalian superior colliculus). In non-mammalian vertebrates, this area has a complex layered structure with the great advantage of distinct in- and output regions. In chicken, the TeO is organized in 15 layers where visual input targets the superficial layer while auditory input terminates in deeper layers.
One specific cell type, called Shepherd's crook neuron (SCN), has dendrites in both input regions. The characteristic feature of these neurons is the axon that originates up to 120 µm from the soma at the apical dendrite. The molecular identity of this characteristic area and thus the site of action potential generation are of particular importance to understand signal flow and cellular computation. Based on the morphology of the SCN, action potentials at the axon could be evoked either by summation of auditory input at the basal and visual input at the apical dendrite, or maybe just by a strong visual input to the apical dendrites that bypasses the soma. This axopetal information flow was already proposed by Ramon y Cajal. However, despite the involvement of SCN in bottom-up attention little is known about the detailed neuroanatomy and cellular computation.
We confirmed the already supposed morphological subdivision by analyzing expression patterns of structural proteins (NF200, Ankyrin G, and Myelin) and ion channels (Pan-Na_V, $Na_V1.6$ and $K_V3.1b$; Lischka et al. J Comp Neurol 2018). Based on these anatomical data, we built a multi-compartment model in NEURON to understand the signal flow and cellular computation in this neuron. We assumed the same number of active synapses on the apical and basal dendrite mimicking visual and auditory input, respectively. A simultaneous stimulation of both sensory input regions leads to a significantly enhanced number of action potentials on the axon compared to the stimulation of one input region alone. Introducing a delayed onset of one sensory input, the delayed auditory signal increases the spiking rate more than the delayed visual signal. Here, we will further validate the model with physiological data gained in patch-clamp experiments (e.g. I-V relationship, simultaneous and delayed extracellular stimulation) and imaging data of activity spread in single neurons.

Poster Topic

T27: Techniques and Demonstrations

T27-1A Influence of the Individual Factors on Effectiveness of α-tACS: Task Difficulty
Gamze Altas

T27-2A Effects of Anodal tDCS on a Cortical Auditory Learning Task
Gonzalo Arias Gil, Thilo Kaene, Reiner Pielot, Alexander Engler, Anja Oelschlegel, Daniel Vincenz-Zörner, Michael T. Lippert, Jürgen Goldschmidt, Karl-Heinz Smalla, Frank W. Ohl, Kentaroh Takagaki

T27-3A Interrogation of neuronal circuit function using customized optogenetic actuators and silencers
Silvia Rodriguez-Rozada, Jonas Wietek, Johannes Vierock, Mabel Matamala-Petruzzi, Thomas G. Oertner, Peter Hegemann, Peter Soba, J. Simon Wiegert

T27-4A Validation of Payload Delivery to Specific Cell Types Using Fluorescence
Raschel Bouajram, Leonardo A Ancheta, Douglas A Lappi

T27-5A Knockdown of HCN-channel expression in mouse hippocampal neurons by virus delivered gene-interfering tools
Matthias Deutsch, Anne Günther, Arnd Baumann

T27-6A Cryopreservation of Primary Neural Cell Cultures
Annika Haak, Irmgard D. Dietzel

T27-1B Automatic characterization of social communication signals by electrostatic field recordings in honeybee colonies
Karen Haink, Benjamin Paffhausen, Julian Petrasch, Randolf Menzel

T27-2B Inter-individual variability of motor evoked potential as neurophysiological marker in response to continuous Theta Brust Stimulation
Ali Hamza, Shahid Bashir

T27-3B Two-photon Optogenetic Mapping of Excitatory Synaptic Connectivity and Strength
Jan Hirtz, Mercè Izquierdo-Serra, Ben Shababo, Rafael Yuste

T27-4B Temporal changes in brain ferritin level during early postnatal development in C57BL/6 mice
Alina Jaufmann, Nikolaus Bresgen, Hubert H. Kerschbaum

T27-5B Multiple Running Wheels with ID Sensors for Group-housed Mice
Christian Jung, Oliver Janke, Jonas Füner, York Winter

T27-6B Achieving reproducible data workflows: Lightweight tools for safe and efficient data management
Achilleas Koutsou, Michael Sonntag, Christian Garbers, Christian Johannes Kellner, Jan Grewe, Thomas Wachtler

T27-1C Alkyne lipids - a novel tool for tracing
lipid localization and metabolism in the murine brain
Lars Kuerschner

T27-2C A 3D Labeling Approach In Solvent-Cleared Brains To Analyze Axonal Projection Profiles After Cortical Stroke
Christof Kugler, Christian S Thielscher, Cordula Rakers, Gabor C Petzold

T27-3C Basic antidepressant research: the reproducibility project.
Cilene Lino de Oliveira, Rubia Weffort de Oliveira Oliveira, Roberto Andreatini, Samia Joca, Alline Cristina de Campos Campos, Catherine Belzung

T27-4C TRPV4 is the temperature-sensitive ion channel of human sperm
Nadine Mundt, Marc Spehr, Polina Lishko

T27-5C Z-scanning in volumetric 2-photon microscopy with a fast voice coil driven remote focus system
Gert Rapp, Christian Schulze, Thomas Oertner, Florian Huhn

T27-6C Recording of synchronized spikes from the intact cortical surface: a direct means to obtain high decoding value under minimal invasiveness?
Tobias Bockhorst, Florian Pieper, Gerhard Engler, Edgar Galindo-Leon, Andreas K Engel

T27-7C Cerebral open flow microperfusion samples cerebral interstitial fluid and cerebrospinal fluid
Joanna Hummer (Adamczak), Thomas Altendorfer-Kroath, Frank Sinner, Thomas Birngruber

T27-1D A micro-CT based standard atlas of the bumblebee brain
Lisa Rother, Dylan Smith, Farah Ahmed, Richard Gill, Keram Pfeiffer

T27-2D Synapse Locator: A tool for automated registration and segmentation of 3D synaptic activity maps
Christian Schulze, Alberto Perez-Alvarez, Brenna Fearey, Christine E Gee, Thomas G Oertner

T27-3D Toward Optogenetics in Barn Owls: Promising AAV-mediated Opsin Expression
Nadine Thiele, Meike Rogalla, Jannis Hildebrandt, Christine Köppl

T27-4D Visualization of a full body Drosophila larva
Alice Weiglein, Oliver Kobler, Bertram Gerber

T27-5D Drug delivery with polybutylcyanoacrylate nanoparticles to the retina, brain and main organs of rats
Qing You, Maxim Sokolov, Grigartzik Lisa, Werner Hintz, B. van Wachem, Petra Henrich-Noack, Bernhard A. Sabel

T27-6D Integrating neuroscience data into a unified database: accessing individual experiments via a common metadata collection using the Neuroinformatics Platform of the Human Brain Project

Lyuba Zehl, Sara Zafarnia, Stefan Köhnen, Krister Andersson, Milica Markovic, Elodie Legouée, Camilla Hagen Blixhavn, Heidi Kleven, Xiayun Gui, Pavel Chervakov, Oliver Schmid, Simon D. Bell, Tom Gillespie, Mathew B. Abrams, Andrew Davison, Jeffrey C. Muller, Trygve B. Leergaard, Katrin Amunts, Jan G. Bjaalie, Timo Dickscheid

Influence of the Individual Factors on Effectiveness of α-tACS: Task Difficulty

Gamze Altas[1]

[1]Carl von Ossietzky Universitaet Oldenburg, Department of Neurocognitive Psychology, Experimental Psychology Lab.

Transcranial alternating current stimulation (tACS) is a non-invasive brain stimulation technique. It has being used to investigate the relationship between brain oscillations and cognitive functions by modulating endogenous brain oscillations in a frequency specific manner. It has been found that the power of the endogenous oscillations has a critical impact on tACS effectiveness. Under conditions of high endogenous α-power, tACS fails to enhance α-power due to ceiling effect. Stronger event related desynchronization (ERD) of endogenous oscillations is related to higher cognitive performance especially in visual and spatial memory task. It has been proved that it is possible to elicit aftereffects of tACS during tasks interacting with the alpha band. However, it remains unclear whether the effectiveness of stimulation depends on the complexity of task interacting with the stimulated frequency band.
The current study aimed to investigate the influence of task difficulty interacting with α-frequency band. Results indicated that there is no significant effect of task complexity on effectiveness of α-tACS.To be able to make conclusive statements on the impact of task difficulty on effectiveness of α-tACS, sample size should be extended and the other factors have to be taken into account for potential interaction effects.

Effects of Anodal tDCS on a Cortical Auditory Learning Task

Gonzalo Arias Gil[1], Thilo Kaene[1,2], Reiner Pielot[1], Alexander Engler[1], Anja Oelschlegel[1], Daniel Vincenz-Zörner[1], Michael T. Lippert[1,3], Jürgen Goldschmidt[1,2,3], Karl-Heinz Smalla[1,3], Frank W. Ohl[1,2,3], Kentaroh Takagaki[1,2,3]

[1]Leibniz Institute for Neurobiology, Systems Physiology of Learning, Brenneckestraße 6, 39118 Magdeburg, Germany
[2]Institute of Biology, Otto-von-Guericke University, Magdeburg, Germany
[3]Center for Behavioral Brain Sciences (CBBS), Otto-von-Guericke University, Magdeburg, Germany

Transcranial direct-current stimulation (tDCS) is a low cost, non-invasive method that has the potential to actively shape neural activity by sending constant, subthreshold direct current through the skull via electrodes placed over a region of interest. Positive polarity (anodal stimulation) is thought induce excitation of the underlying cortex. However, the mesoscopic extent of such influence is unclear.
We tested the influence of tDCS on learning and memory behavior in a cortex-dependent learning task, discrimination of modulation direction of frequency-modulated tones in a GO/NO-GO shuttlebox paradigm. Animals were divided into two experimental groups, an anodal tDCS stimulated group and a control sham stimulation group.
In order to investigate the mechanisms of such cortical modulation of learning, we also mapped spatial patterns of neuronal activity using in vivo SPECT-imaging of regional blood flow and histochemical detection of the uptake of the K+-probe thallium (Tl+). For a characterization of biochemical changes due to the tDCS, we further collected samples of brain tissue from the animals after behavior and performed mass spectrometry analyses to test for proteomic differences in the expression of critical molecules related to synapse formation and stabilization.
Behaviorally, our analyses show that there is better learning in the anodal tDCS group. Of interest, our effect was only seen in animals which took a particular learning strategy, which emphasizes correct discrimination over impulsive detection. Through SPECT-imaging we see clear activation of the relevant cortical areas caused by our tDCS. Electrophysiology also shows significant activation due to tDCS.

Interrogation of neuronal circuit function using customized optogenetic actuators and silencers

Silvia Rodriguez-Rozada[1], Jonas Wietek[2], Johannes Vierock[2], Mabel Matamala-Petruzzi[3], Thomas G. Oertner[4], Peter Hegemann[2], Peter Soba[3], J. Simon Wiegert[1]

[1]Center for Molecular Neurobiology Hamburg, Research Group Synaptic Wiring and Information Processing, Hamburg, Germany

[2]Humboldt-Universität zu Berlin, Institute for Biology-Experimental Biophysics, Berlin, Germany

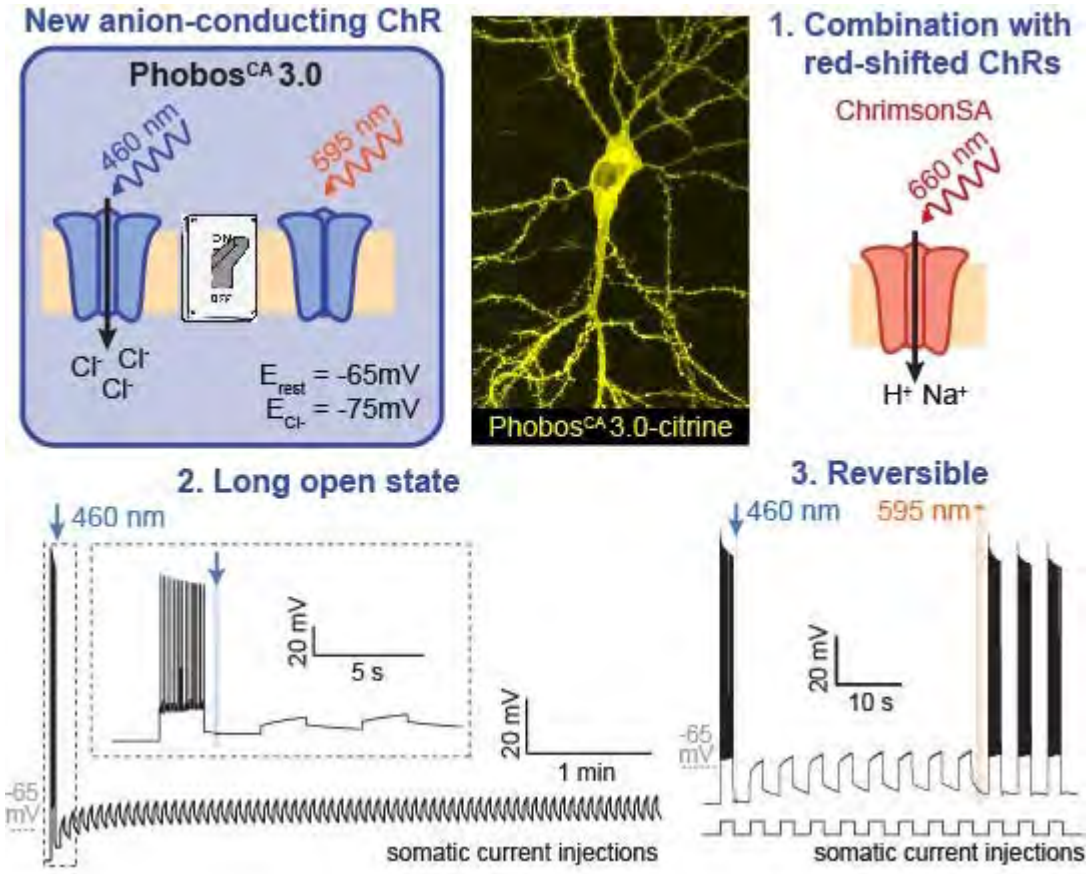
[3]Center for Molecular Neurobiology Hamburg, Research Group Neuronal Patterning and Connectivity, Hamburg, Germany

[4]Center for Molecular Neurobiology Hamburg, Institute for Synaptic Physiology, Hamburg, Germany

Perturbation of neural activity by optogenetic means is a powerful approach to assess the function of defined neuronal populations from the physiological to the behavioral level. Compared to activation, light-induced inhibition of neurons has proven to be technically more challenging. The development of anion conducting channelrhodopsins (ACRs) by targeted mutagenesis of cation conducting ChRs and the discovery of natural ACRs introduced a new class of inhibitory optogenetic tools that overcome some of the limitations of the commonly used ion pumps Halorhodopsin and Archaeorhodopsin. Nonetheless, reversible and temporally precise silencing of neuronal activity for extended periods of time remains a challenge. Our lab has recently developed ACRs with color-tuned action spectra and modified kinetics that efficiently inhibit neuronal activity in hippocampal slice cultures. The introduction of a point mutation (C128A) greatly enhanced the light sensitivity of the engineered ACRs due to a slowed-down photocycle, yielding effective inhibition with reduced light power. The functionality of these ACRs was validated *in vivo* in *Drosophila* larvae, where they showed robust and specific light-dependent inhibition of locomotion and nociception.

Here, we present two new blue-shifted step-function ACRs, termed Phobos^{CA}2.0 and 3.0, with enhanced photocurrents and longer open states, which allow long-lasting silencing of neurons in the absence of light. In order to achieve fine control of neuronal activity, a tool with temporally precise on- and offset is required. Notably, these ACRs can be reversibly toggled between open and closed states using light of different wavelengths, granting termination of silencing with high temporal precision. In addition, due to their blue-shifted activation spectra, Phobos^{CA}2.0 and 3.0 can be combined with red-shifted optogenetic actuators to implement a dual-color excitation/inhibition system, providing an efficient way to dissect neuronal circuits. Combination of the newly developed ACRs and spectrally different excitatory ChRs with genetically defined expression and local illumination allows selective and independent up- and down-regulation of distinct neuronal populations. For instance, driving excitation in CA3 principal neurons, while transiently silencing CA1 interneurons in hippocampal slices, provides an ideal experimental setup to investigate the temporal aspects of feed-forward inhibition during synaptic plasticity of the Schaffer collateral pathway.

In summary, the new color-tuned ACRs with long-lasting, reversible activity broaden the available toolkit of optogenetic silencers in the spectral and temporal domain, thus expanding the possibilities for optical manipulation of neuronal networks.


New anion-conducting ChR
Phobos^CA 3.0
460 nm
595 nm
ON
OFF
Cl- Cl-
Cl-
E_rest = -65mV
E_Cl- = -75mV
Phobos^CA 3.0-citrine
1. Combination with red-shifted ChRs
ChrimsonSA
660 nm
H+ Na+
2. Long open state
460 nm
20 mV
5 s
20 mV
1 min
-65 mV
somatic current injections
3. Reversible
460 nm
595 nm
20 mV
10 s
-65 mV
somatic current injections

Validation of Payload Delivery to Specific Cell Types Using Fluorescence

Raschel Bouajram[1], Leonardo A Ancheta[1], Douglas A Lappi[1]

[1]CytoLogistics, LLC, Research and Development, 10451 Roselle St, Ste 300, 92121 San Diego, USA

A new tool for efficient receptor-mediated delivery of a payload combined with fluorescence validation has been developed. This important tool provides both delivery and validation components to determine the successful delivery of a payload inside a cell via a surface marker. It can be used in many applications where the success of payload delivery is necessary and/or desirable. Examples include: (1) Delivery of payloads to determine cell-specific functions in learning and memory (Gelfo *et al*., 2017; Li *et al*., 2018; Sims *et al*., 2014); and (2) Antibody-drug conjugates designed to target tumor-associated cell surface antigens conjugated to a cytotoxic payload (Damelin *et al*., 2015). The new delivery/validation system described here is a chemical conjugate of the fluorescent reporter fluorescein (FITC) and streptavidin (SA) cross-linked to the ribosome-inactivating protein, Saporin (ZAP). **FITC-SA-ZAP** can be mixed with a biotinylated targeting agent in a 1:1 molar ratio and used in either *in vitro* or *in vivo* applications. The specificity and affinity of the conjugate relies on the targeting agent used. The targeting agent can be any material that is recognized on the cell surface and internalized. The positive binding of the conjugate to the cell surface receptor is then validated by fluorescence. This obviates the difficulty of determining if the payload has been internalized. Previously, *in vivo* experimentation was put on hold while fluorescence staining was performed on samples to determine if the payload was being appropriately delivered (Liu *et al.*, 2015; Mantyh *et al*., 1997). With this new tool it may be possible to use current technologies to view payload delivery *in vivo* (Hong *et al*., 2014). **FITC-SA-ZAP** is not only a tool for specific delivery of a payload but provides fast results to monitor and validate the delivery of a payload within 1 hour of application to cells (Figure 1).

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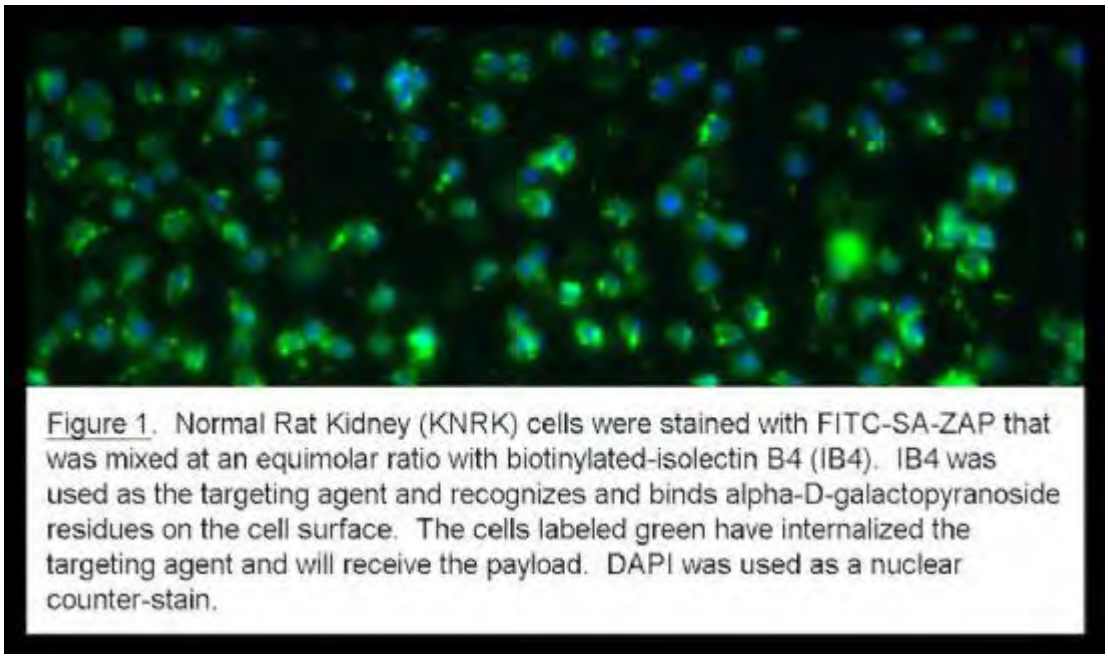

Figure 1. Normal Rat Kidney (KNRK) cells were stained with FITC-SA-ZAP that was mixed at an equimolar ratio with biotinylated-isolectin B4 (IB4). IB4 was used as the targeting agent and recognizes and binds alpha-D-galactopyranoside residues on the cell surface. The cells labeled green have internalized the targeting agent and will receive the payload. DAPI was used as a nuclear counter-stain.

Knockdown of HCN-channel expression in mouse hippocampal neurons by virus delivered gene-interfering tools

Matthias Deutsch[1], Anne Günther[2], Arnd Baumann[1]

[1]Forschungszentrum Jülich, Institute of Complex Systems Zelluläre Biophysik (ICS-4), Wilhelm-Johnen-Straße, 52428 Juelich, Germany

[2]Brain Science Institute, RIKEN, Wako, Japan

Pacemaker ion channels, also known as hyperpolarization and cyclic nucleotide-gated (HCN) ion channels, are abundantly expressed in various neuronal tissues. On the cellular level they contribute to the regulation of the resting membrane potential, integration of synaptic input at dendrites, regulation of presynaptic neurotransmitter release, as well as generation of rhythmic electrical activity. Thus HCN-channel dysfunction or altered gene expression are considered to be involved in several pathologic conditions including epilepsy, neuropathic pain, Parkinson´s disease or age-related decline of the working memory.

To investigate consequences of HCN-channel dysfunction in single neurons and neuronal networks, we aimed to interfere with HCN-channel expression. Here, we specifically targeted the different channel isoforms using two independent gene knockdown techniques. First, we took advantage of a cell-autonomous RNA-interference (RNAi) process. It mediates the breakdown of target mRNA by the application of short-hairpin RNAs. As a second approach, we used an enzymatically inactive Cas9 variant (dCas9). This protein is specifically guided to the transcriptional start regions of the hcn genes, thereby directly interfering with the gene transcription, but without altering the genomic information. Both approaches were delivered to hippocampal neurons by recombinant adeno-associated viruses. We monitored the specificity and efficacy of hcn gene knockdown by immunological, and quantitative PCR assays. Electrophysiological recordings of virus transduced neurons were performed to uncover and evaluate changes of neuronal activity. The knowledge achieved by these experiments provides further insight in HCN-channel functions, in particular their contribution to the activity of neuronal networks.

Cryopreservation of Primary Neural Cell Cultures

Annika Haak[1], Irmgard D. Dietzel[1]

[1]Ruhr-Universität Bochum, Department of Biochemistry II - RG Electrobiochemistry of Neural Cells, Universitätsstraße 150, 44801 Bochum, Germany

Primary cell cultures are preferable to neuronal cell lines because they preserve the receptor expression and signal transduction cascades and thus the developmental potential of native neurons and glial cells. One of the disadvantages of experiments involving primary cultures is the limited availability, limited life span and necessity to obtain cells freshly from animals. This often leads to an excess or shortage of primary neural cells largely reducing the number of cells and rendering timing of experiments difficult. Cryopreservation enables researchers to freeze cells when available and thaw and use them when needed making it unnecessary to adjust experiments to animal life cycles, improving planning of temporal sequence of treatment procedures and ideally allowing shipping of specially treated cells to other laboratories. However, primary neural cells are especially sensitive to the forces that occur during freezing and thawing making it important to apply optimized conditions (cryoprotective agent, media, freezing rate) during cryopreservation. Here we present a first protocol allowing freezing cells to -80 °C and obtaining vital cells upon rethawing. Hippocampi from postnatal rats were dissected and triturated to the cell suspension using standard procedures. The cells were then stored in 70 – 95 % fetal calf serum (FCS) or RPMI supplemented with 10 % FCS and 5 – 30 % cryoprotectant agent such as dimethyl sulfoxide (DMSO) or glycerol. Medium and cells were then frozen at a rate between -0.8 – -1.2 °C/min and finally stored at -80 °C. For thawing, cells were placed for a maximum of 3 min in a 37 °C water bath and then either plated immediately or centrifuged to remove the cryoprotectant agent and plated afterwards. Cells stored at -80 °C for 7 days to 4 weeks were able to grow in culture and differentiate to neurons and glial cells identified by immunohistochemical staining for B-tubulin and GFAP. Neurons preserved their ability to generate Na-currents as shown with patch clamp recordings in the whole cell configuration. We are currently investigating different cryoprotective agents, media and small handling differences to optimize cryopreservation of primary neural cells for longer storage times and higher survival rates.

Automatic characterization of social communication signals by electrostatic field recordings in honeybee colonies

Karen Haink[1], Benjamin Paffhausen[1], Julian Petrasch[2], Randolf Menzel[1]

[1]Freie Universität Berlin, Institut für Biologie, Neurobiologie, Berlin
[2]Freie Universität Berlin, Dahlem Center of Machine Learning and Robotics, Berlin

In honeybee colonies communication between foragers plays a major role for the foraging success of the whole colony, with the overall health of the entire colony being reflected in these multiple communication processes. Besides communication via pheromones, movements of the whole body and the wings are used to encode information. Since the body of foraging honeybees charges up electrostatically during flight it is possible to detect any movements by recording the modulated electrostatic fields emanating from the bees and record it via appropriate electric field sensors. These sensors can be strategically placed so that they do not disrupt natural behavior and the bee activity in the colony's dance area can be recorded over long periods of time, as the bee's bodies do not discharge significantly. Here we show long term recordings of 35 colonies. The different social signals were characterized by their frequency components and their time courses. These characteristics were used for automatic quantification of their occurrence under natural environmental and in-hive conditions. Additional parameters (temperature, humidity, weight of the colony, flight activity) were collected in order to correlate the social signals to environmental and in-hive conditions. Characteristic signals were detected for a whole range of colony conditions (e.g. high vs low foraging activity, weather conditions, colony development, exposure to insecticides, Varroa treatment).

Inter-individual variability of motor evoked potential as neurophysiological marker in response to continuous Theta Brust Stimulation

Ali Hamza[1], Shahid Bashir[1]

[1]Department of Neurophysiology, Neuroscience Center, King Fahad Specialist Hospital Dammam, Dammam, Saudi Arabia.

Objective: Non-invasive Brain Stimulation (NIBS) paradigms considered out on top of methods to modulate cortical plasticity for experimental or therapeutic applications safely. One of the most important paradigms is continuous theta burst stimulation (cTBS) protocol. In this study we discuss the efficacy and reliability of this protocol.

Methods: 50 healthy volunteers (mean age ±2.74 years; were recruited. cTBS was delivered in a hot spot over M1 at an active motor threshold of 80%. Motor evoked potentials (MEPs) were obtained at 120% of the resting motor threshold before and after cTBS. Grand average analysis was also conducted to classify the subjects as “responders R” and “non-responders NR”.

Results: The relative MEP to baseline was significantly decreased 0 and 10 minutes post-stimulation as compared with the baseline condition.

Conclusion: The understanding of the origin of this variability in plasticity measure will be useful to the development of therapeutic application using TMS.

Two-photon Optogenetic Mapping of Excitatory Synaptic Connectivity and Strength

Jan Hirtz[1], Mercè Izquierdo-Serra[1], Ben Shababo[1], Rafael Yuste[1]

[1]NeuroTechnology Center, Department of Biological Sciences, Columbia University, New York, New York 10027, USA

Decoding the structural and functional principles of microcircuitry appears necessary to understand neural computations. Most studies of connectivity and strength of connections have employed multiple simultaneous patch-clamp recordings. While this method provided great insights, it is limited by the amount of cells that can be recorded in a single experiment. Using optical tools to map neural connections with single cell precision is a promising road to perform physiological experiments to probe connectivity between hundreds or thousands of neurons, and has already enabled systematic mapping of inhibitory connections. In contrast, optical mapping of excitatory connections between pyramidal cells (PCs) has been a major challenge due to their high densities in cortical tissue and their weak and stochastic connectivity. The aim of our study was to develop an optogenetic method to map connectivity between PCs in acute cortical brain slices with single cell precision, employing the non-linear excitation of ultrafast lasers.

We expressed the opsin construct C1V1-p2A-EYFP in excitatory neurons in mouse visual cortex via transcranial AAV injection into neonatal mice. Densely packed expressing neurons could be identified by two-photon imaging of EYFP at 940 nm. To optically activate neurons, we employed a custom-build two-photon microscope capable of consecutively visiting a pre-programmed set of point stimulations. Employing a spiral-like point scanning at 1040 nm excitation, we demonstrate an inverse correlation between expression level of C1V1 and latency of action potentials (APs) generated. In addition, AP latency also increased when moving the laser away from the cell soma, both in the lateral as well as in the axial plane. We took advantage of a parabolic fit of this latency distribution for mapping synaptic connections, recording postsynaptic responses in whole-cell configuration. By moving towards and away from optically targeted cell somata in the axial dimension during the mapping process, and comparing the latency changes of evoked synaptic inputs with those obtained in calibration experiments, we were able to identify connections between PCs. We verified this method by performing targeted patch-clamp recordings of the identified preysynaptically connected neuron and used our mapping protocol to perform a first preliminary study of PC connectivity in the visual cortex of juvenile to young adult mice. Our results can be viewed as an important step in the design of optical methods to decipher neuronal connectivity.

Temporal changes in brain ferritin level during early postnatal development in C57BL/6 mice

Alina Jaufmann[1], Nikolaus Bresgen[1], Hubert H. Kerschbaum[1]

[1]University of Salzburg, Department of Biosciences, Hellbrunnerstraße 34, 5020 Salzburg, Austria

The brain is an iron rich organ. This is not surprising taking into consideration that iron is a co-factor in some enzymes implicated in oxidative metabolism and lipid biosynthesis and that the brain is among the organs having a high rate of oxidative metabolism and is a lipid-rich organ. Thus, failures in recruiting iron to the developing brain may have detrimental consequences on physiological and cognitive processes. Although iron is indispensable in cell metabolism, free iron promotes lipid peroxidation, which leads to oxidative injury and even cell death (Bresgen, Eckl, Biomolecules 2015, 5, 808-847). The iron storage protein, ferritin, covers both functions. It delivers iron to the brain to maintain iron-dependent metabolic processes and it stores iron within the cell to prevent iron-dependent detrimental processes. Ferritin consists of 24 units of H- and L-chains, which form a hollow sphere, where iron is deposited as ferric hydroxide phosphate. Interestingly, oligodendrocytes, which myelinate axons in the central nervous system, take up ferritin by receptor-mediated endocytosis via TIM-2 (T cell immunoglobulin and mucin domain-containingprotein-2), which binds to H-Ferritin (Todorich et al., J. Neurochem. (2008) 107, 1495–1505, Todorich et al., GLIA 59:927–935, 2011). Despite the importance of iron in maintaining cellular metabolic processes, the temporal dynamics that underlie the supply of the brain with iron is poorly understood. Early studies in humans showed that ferritin in the plasma decreases within the first months of life (Finch et al., West J Med,1986, 145:657-663). In the rat brain, ferritin has been localized in microglia, oligodendrocytes, as well as in a subpopulation of astrocytes and neurons (Cheepsunthorn et al., J. Comp. Neurol. 400:73–86, 1998). These authors describe that ferritin is predominantly localized in microglial within the first postnatal days, but at postnatal day 30 oligodendrocytes are the predominant ferritin containing cells. In the present study, we used Western-blot analysis to describe the temporal changes in ferritin level in C57BL/6 mice during the first weeks of their life.

Brains were collected from C57BL/6 mice at 6, 8, 10, 21, 35, 60 days of age and processed for Western blot analysis. Blots were probed with a polyclonal antibody to ferritin (F6136, Sigma). Western blot analysis of brain lysates probed with an antibody against ferritin revealed a strong reaction at 20 kD. When we compared lysates from cerebrum and cerebellum, we observed a ferritin positive protein at 20 kD in both samples, but the ferritin protein level was higher in the cerebellum. Further, the ferritin protein levels showed differences depending on the postnatal day. Whereas we observed a pronounced labelling on day 6, 8, and 10, a comparably fainter labelling was detected on

day 21, 35, and 60. Finch and co-workers described a transient increase in plasma protein during the early postnatal period. We assume that our data indicate that at least a fraction of the ferritin, which we detected in the brain, has been released in the periphery, like the liver, transported via the circulatory system to the brain, and has been endocytosed by neural cells, like oligodendrocytes. Taken together, these results indicate that although ferritin is present at each postnatal stage, its levels shows remarkable temporal differences with the highest levels of the protein during the first ten days.

Multiple Running Wheels with ID Sensors for Group-housed Mice

Christian Jung[1], Oliver Janke[2], Jonas Füner[2], York Winter[1,3]

[1]PhenoSys GmbH, Berlin, Germany
[2]Preclinics GmbH, Potsdam, Germany
[3]Humboldt University, Institute of Biology, Cognitive Neurobiology, Berlin, Germany

Mouse models of neurological disorders, such as multiple sclerosis and Parkinsons disease, and models of pain exhibit marked deviations in several parameters of voluntary wheel running activity. Thus, wheel running assays serve to diagnose disease progression and treatment outcome. Typically, mice are kept singly in their wheel cages even for extended periods of time. This social isolation causes stress that interferes with disease progression and symptom severity. We present a novel system where wheel running cages are mounted in a regular cage rack. The cages are interconnected so that mice can move between them. Mice carry subcutaneous RFID chips for individual identification. ID sensors placed outside the cages but next to a cage‘s wheel detect the identity of the mouse that uses the wheel. Thus, any wheel running can be assigned to an individual mouse. Wheel running of individual mice can be scored irrespective of the specific wheels used by a mouse. This novel system allows keeping mice in groups but still obtain individual records of running activity. We demonstrate the great potential of this novel method with data from a mouse model of muscle paralysis.

Achieving reproducible data workflows: Lightweight tools for safe and efficient data management

Achilleas Koutsou[1], Michael Sonntag[1], Christian Garbers[1], Christian Johannes Kellner[1], Jan Grewe[2], Thomas Wachtler[1]

[1]Ludwig-Maximilian-Universität München, Biologie II, Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany

[2]Institute for Neurobiology, University of Tuebingen, Auf der Morgenstelle 28; 72076 Tübingen, Germany

Maintaining reproducible data workflows while keeping data in sync, backed up, and easily accessible from within and outside the lab is a key challenge in research. To minimize time and effort scientists have to spend on these tasks, we provide a suite of tools designed for comprehensive, reproducible and versioned management of scientific data.

Reproducibility and data re-usability require the presence of metadata also providing information about experimental conditions. The odML[1] metadata format is a simple and convenient format to flexibly collect and organize any kind of metadata. It enables comprehensive collection and automated processing of metadata[2], including conversion to other formats such as RDF to utilize semantic web technologies.

To keep data and metadata organized, the NIX[3] data format enables to effectively link data and corresponding analysis results as well as the associated metadata. It supports a wide range of data types, including electrophysiology and imaging data. NIX uses the odML metadata format and is integrated with the Neo[4] Python package for electrophysiology, enabling Neo users to store their data in a common open format.

The GIN[5] services provide versioned data management and collaborative data sharing. Using established versioning technology [6,7], GIN keeps track of changes and provides secure access, making it convenient to work from multiple workplaces while keeping all data available and in sync. Data can be managed from web and file browsers or a command line client, enabling integration into data acquisition or analysis procedures. The service works with any kind of directory structure and file types, keeping previous versions accessible when datasets are updated. It makes it straightforward to share data within a lab or with off-site collaborators and to work on it together.

The tools presented are easy to use, can be combined with other approaches supporting reproducibility and data sharing [8,9,10], and enable efficient data management that supports the FAIR principles [11]. Combining them for data annotation, organization, and storage allows streamlining data workflows and efficiently sharing well-annotated datasets within the lab, among collaborators, or with the larger scientific community.

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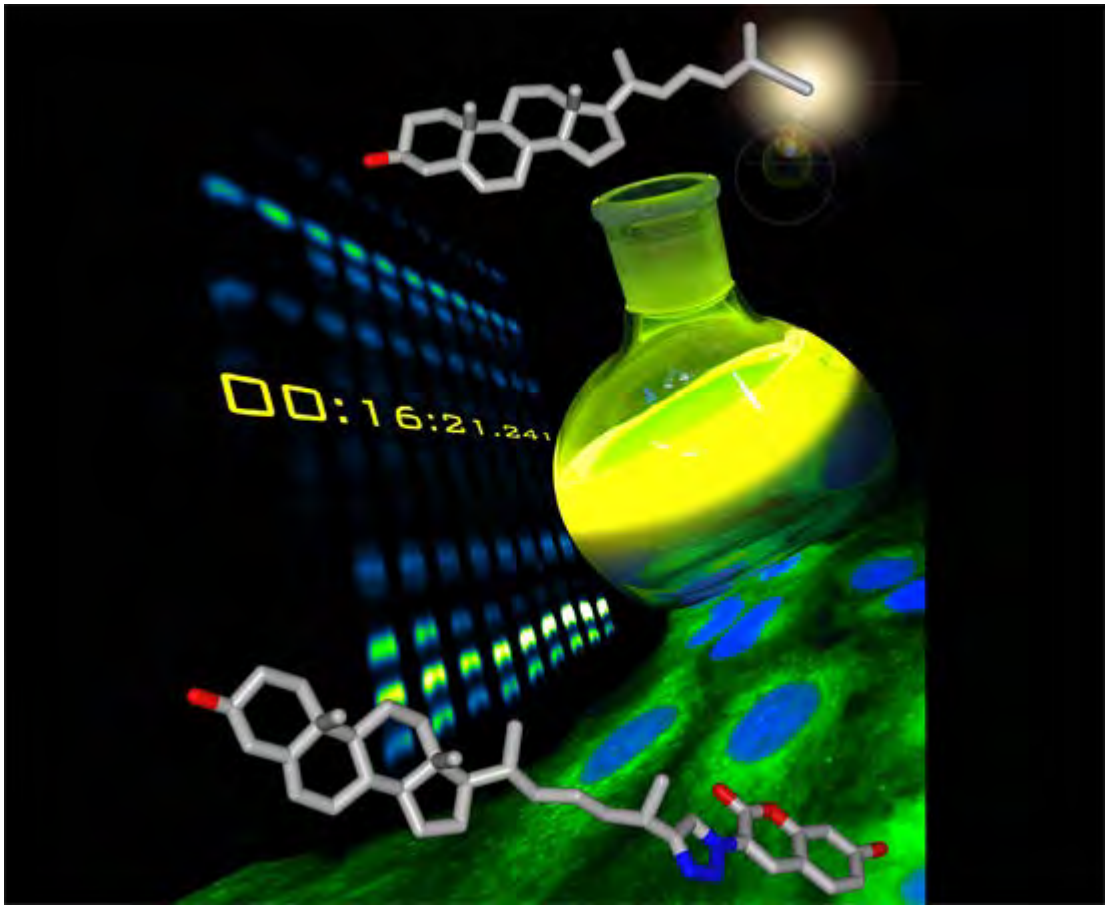
Alkyne lipids - a novel tool for tracing lipid localization and metabolism in the murine brain

Lars Kuerschner[1]

[1]LIMES, Uni Bonn, NeuroBiochemistry of Lipids, Carl-Troll-Str. 31, 53115 Bonn, Germany

Alkyne lipids are supreme tracers that allow for parallel studies of lipid localization and metabolism. A single carbon-carbon triple bond at the terminus of these tracer lipids allows for their detection, while this tiniest tag confers only minimal disturbance to the hydrophobic properties on which the characteristic behavior of lipids is based.

We demonstrate the power of the alkyne lipid tracer technology by applying it to cells, tissues and whole animals. We have developed protocols for the analysis of alkyne lipids from various lipid classes at highest spatial and metabolic resolution and sensitivity using modern lipid analytics including super-resolution microscopy and mass spectrometry. Applying these methods and using *in vitro, in situ,* and *in vivo* models, we are investigating lipid transfer across the blood-brain barrier, across the tanycyte-barrier into the hypothalamus, and uptake into other grey and white matter structures of the murine brain.



A 3D Labeling Approach In Solvent-Cleared Brains To Analyze Axonal Projection Profiles After Cortical Stroke

Christof Kugler[1], Christian S Thielscher[1], Cordula Rakers[1], Gabor C Petzold[1,2]

[1]German Center for Neurodegenerative Diseases, Neurovascular Diseases, Sigmund-Freud-Strasse 27, 53127 Bonn, Germany

[2]Department of Neurology, University Hospital Bonn, Sigmund-Freud-Strasse 25, 53127 Bonn, Germany

Rationale
Stroke is a major cause of worldwide death and disability that warrants development of effective treatments to ameliorate insult burden. In this project, we aimed to establish a labeling approach throughout the mouse cortex to analyze axonal projection profiles and consequently tested possible regenerative compounds to promote regeneration of severed connections.

Methods
Male C57Bl/6N mice receive an injection of Rose Bengal (RB) into the tail vein. The left forelimb motor cortex (M1) is exposed to green laser light. Upon irradiation, RB produces singlet oxygen, resulting in the formation of thrombi in small cortical vessels with minimal or no reperfusion to exposed tissue. Three weeks after stroke, tracer virus and dye molecules are injected: i) AAV9.hSyn.eGFP adjacent to the infarct to anterogradely label axon bundles, and, ii) fluorescently-labeled cholera toxin b (CTb-AF594) into the premotor cortex (PMC) to retrogradely label cell somata. After two weeks of viral transduction and expression and tracer transport, brains are subjected to organic solvent-based clearing (BABB method) followed by 2-photon imaging. Furthermore, mice are assessed on an elevated grid walk to monitor motor deficits throughout our experimental setup.

Results
Preliminary results of our retrograde labeling study indicate a significant reduction of CTb-positive somata (cells that project to the PMC) anterior, medial and lateral to stroke, compared to sham controls. In contrast, no significant reduction is observed in the PMC in stroke animals.
The anterograde labeling approach is currently ongoing in sham and stroke animals, but preliminary data indicate a change in cortical projection profiles in ischemic mice in both premotor and somatosensory cortical areas as compared to sham mice and analyzed by 3D cluster analysis.
In addition, mice perform significantly worse on an elevated grid walk with their contralateral fore- and hindpaws after stroke as compared to both ipsilateral paws and sham controls.
Furthermore, we will present preliminary data on how different drugs affect i) connections to the PMC, ii) cortical axonal projection profiles between M1-PMC and M1-S1, and, iii) fore- and hindpaw motor outcome.

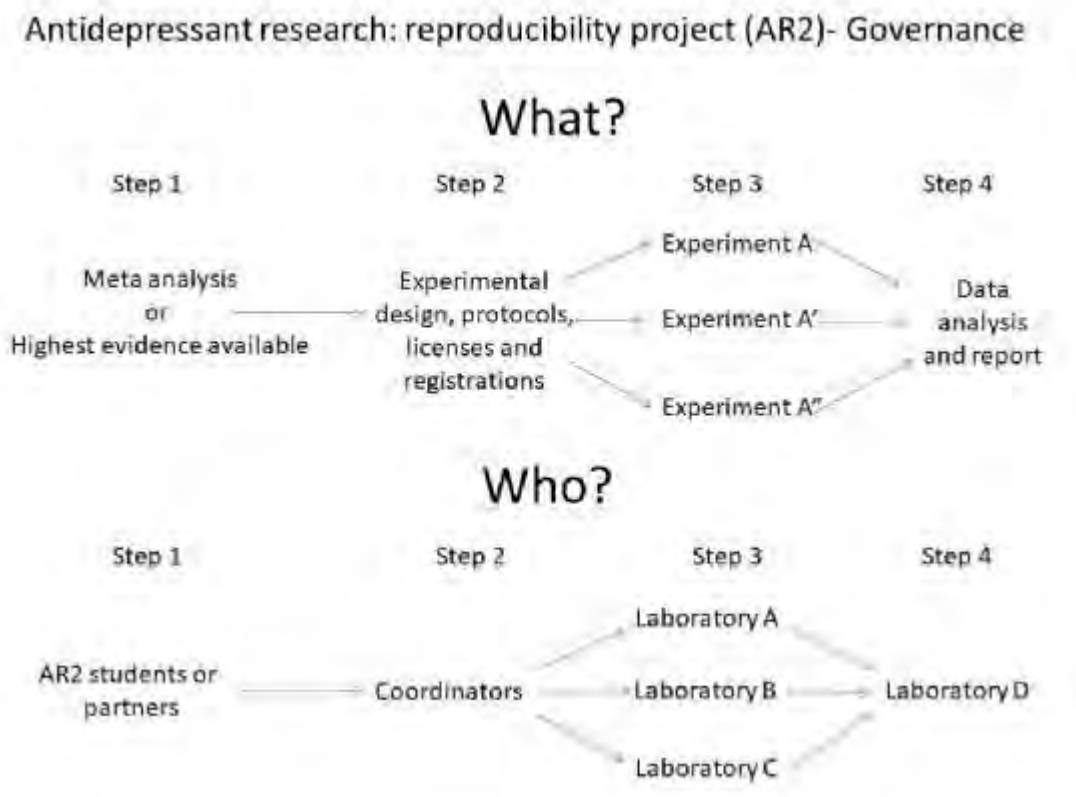
Outlook
In summary, our model and technique allow for comprehensive and in-depth analyses of long-range projections after stroke and their modulation for systemic or local pharmacotherapies aimed at promoting axon sprouting in subsequent translational trials.

Basic antidepressant research: the reproducibility project.

Cilene Lino de Oliveira[1], Rubia Weffort de Oliveira Oliveira[2], Roberto Andreatini[3], Samia Joca[4], Alline Cristina de Campos Campos[5], Catherine Belzung[6]

[61]UFSC, Labnec, Campus Universitário Trindade, 88040900 Florianopolis, Brazil.[2]UEM, Maringá, Paraná, Brazil.[3]UFPR, Curitiba, Paraná, Brazil.[4]FCFRP, Ribeirão Preto, São Paulo, Brazil.[5]FMRP,Ribeirão Preto, São Paulo, Brazil.[6]University of Tours,France.

Reproducibility is a pivotal condition for scientific development, application and innovation. In the last years, scientists worldwide have perceived scientific reproducibility as low and poor originating the notion of a reproduction or replication crisis. The real extension of the putative replication crisis is an unknown but scientific community is developing strategies to estimate and, hopefully, restraint it. Lack of reproducibility seems existent in basic antidepressant research and may underlie poor translation of basic to clinical studies in the field. In the present work, we launch the "Antidepressant research: reproducibility project" (AR2) that aims to assemble a multicenter strategy to estimate the reproducibility of basic antidepressant research. Multicenter strategies should boost reproducibility, according to simulations based on a large number of studies. The AR2 research group will reproduce studies investigating effects of prototypic as well as candidate antidepressants in rats and mice submitted to the forced swimming test. Protocols for testing prototypic antidepressants will rely on meta-analytic evidence. Protocols for testing candidate antidepressants will rely on the highest level of evidence available. Present work is registered in the Open Science Framework under the identifier: DOI 10.17605/OSF.IO/FMKQA



TRPV4 is the temperature-sensitive ion channel of human sperm

Nadine Mundt[1], Marc Spehr[1], Polina Lishko[2]

[1]RWTH Aachen University, Chemosensation, Worringerweg 3, 52074 Aachen, Germany

[2]University of California, Berkeley, 221A LSA, Berkeley, CA 94720-3200, USA

Ion channels control the ability of human sperm to fertilize the egg by triggering hyperactivated motility, which is regulated by membrane potential, intracellular pH, and cytosolic calcium. Previous studies unraveled three essential ion channels that regulate these parameters: (1) the Ca^{2+} channel CatSper, (2) the K^+ channel KSper, and (3) the H^+ channel Hv1. However, the molecular identity of the sperm Na^+ conductance that mediates initial membrane depolarization and, thus, triggers downstream signaling events is yet to be defined. Here, we functionally characterize human DSper, the Depolarizing Channel of Sperm, as the temperature-activated channel TRPV4. It is functionally expressed at both mRNA and protein levels, while other temperature-sensitive TRPV channels are not functional in human sperm. DSper currents are activated by warm temperatures and mediate cation conductance, that shares a pharmacological profile reminiscent of TRPV4. Together, these results suggest that TRPV4 activation triggers initial membrane depolarization, facilitating both CatSper and Hv1 gating and, consequently, sperm hyperactivation.

Z-scanning in volumetric 2-photon microscopy with a fast voice coil driven remote focus system

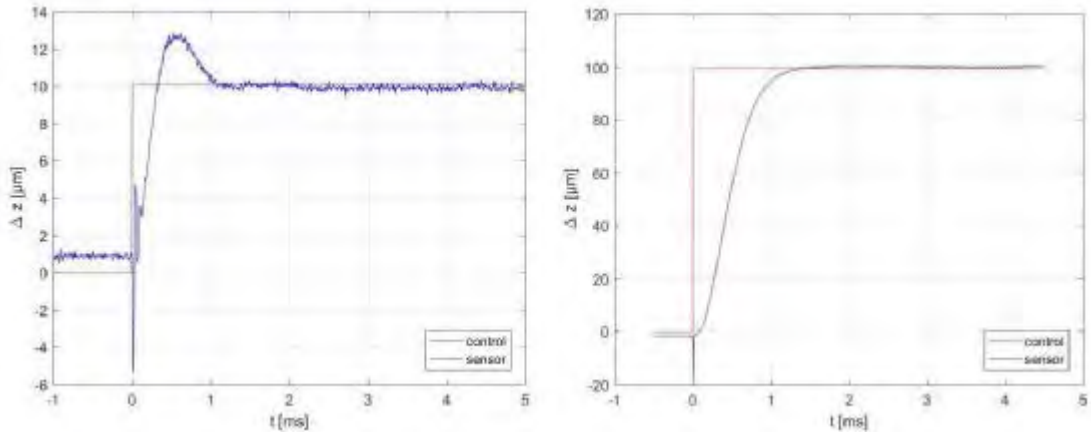
Gert Rapp[1], Christian Schulze[2], Thomas Oertner[2], Florian Huhn[1]

[1]Rapp OptoElectronic GmbH, Kronskamp 110, 22880 Wedel, Germany

[2]Institute for Synaptic Physiology, Center for Molecular Neurobiology Hamburg (ZMNH), Falkenried 94, 20251 Hamburg, Germany

Fast volumetric sampling of biological specimens with laser scanning microscopes is required in many fields of neurobiology, cell biology and medicine. When image acquisition in the lateral (x,y) plane reaches frame rates up to 100Hz by means of resonant scanner systems, a jump between two z-layers in the image stack should also be performed in a few milliseconds, a time scale that cannot be reached by moving the objective or the specimen. Several proposed remote focusing systems only translate a small mirror with a mass below 1g and therefore rely on fast linear positioners.

We present measurements with a fast remote focusing system on a two-photon-microscope using a voice coil driven linear stage that we developed. The voice coil stage has a travel range of 1000µm, a 10µm step response time of 1ms and a position accuracy better than 1 µm (see Fig. 1). We characterize the dynamic properties of the voice coil stage and describe the remote focus system. As proof of concept, we show its applicability for 2-photon calcium imaging of neuronal activity in the CA1 region of rat hippocampus. The remote focusing system is compatible with the open-source software ScanImage (HHMI Janelia Research Campus / Vidrio Technologies, LCC).



Recording of synchronized spikes from the intact cortical surface: a direct means to obtain high decoding value under minimal invasiveness?

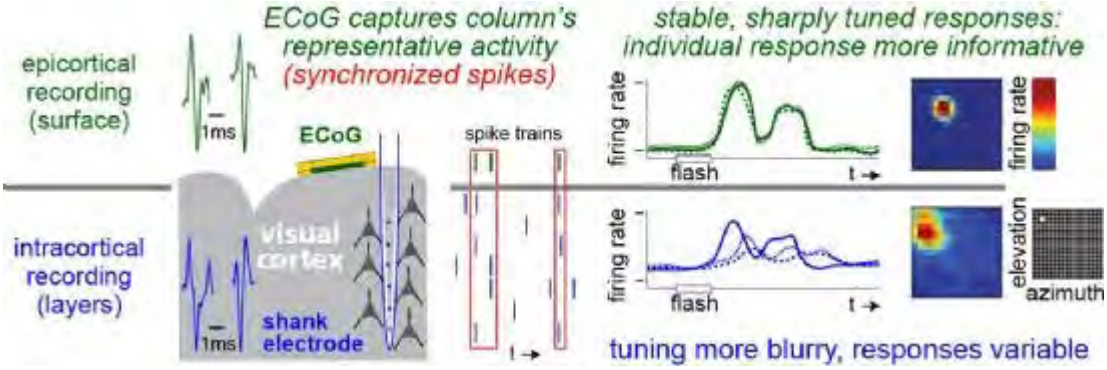
Tobias Bockhorst[1], Florian Pieper[1], Gerhard Engler[1], Edgar Galindo-Leon[1], Andreas K Engel[1]

[1]University Medical Center Hamburg-Eppendorf (UKE), Department of Neurophysiology and Pathophysiology, Martinistrasse 52, 20246 Hamburg, Germany

We introduce an approach for the recording of spikes from the cortical surface (epicortical recording) that builds upon a moderate-density array of 64 electrodes (250 µm diameter) for electrocorticography (ECoG), tested in ferrets. To clarify how epicortical spiking relates to intracortical firing of action potentials, we combined surface recordings from visual and auditory cortices with intracortical recordings via linear probes.

Empirical data and modeling suggest that our surface electrodes captured the synchronized spiking shared by neurons distributed along the depth of the cortical macrocolumn. Accordingly, surface-spiking responses to sensory stimulation (flash/click) were higher in amplitude and less noisy across trials as compared to intracortical single-site or sum activity. The accuracy of sensory tuning was preserved (contour orientation, tonotopy) or increased (retinotopy).

The direct recording of only representative activity should promote insights into assembly coding, may help to increase accuracy of response decoders, and be advantageous to real-time applications such as prosthetic device control.



Cerebral open flow microperfusion samples cerebral interstitial fluid and cerebrospinal fluid

Joanna Hummer (Adamczak)[1], Thomas Altendorfer-Kroath[1], Frank Sinner[1,2], Thomas Birngruber[1]

[1]Joanneum Research, Institute for Biomedicine and Health Sciences, Neue Stiftingtalstrasse 2, 8010 Graz, Austria [2]Medical University of Graz, Division of Endocrinology and Diabetology, Department of Internal Medicine, Austria

Cerebral open flow microperfusion (cOFM) is a membrane-free sampling technique for long term sampling of cerebral interstitial fluid and cerebrospinal fluid. Macroscopic openings in the cOFM probe avoid membrane-related problems such as biofouling, protein clotting, high molecular weight cut-off and the exclusion of large and lipophilic substances. cOFM is thus uniquely suited for unlimited monitoring of any substance in the cerebral interstitial fluid. Similar to other probe-based sampling techniques in the brain, cOFM probe implantation causes capillary rupture and thus disruption of the blood brain barrier (BBB). As an intact BBB is necessary to assess substance transport across the BBB, BBB re-establishment was investigated using Evans blue and sodium fluorescein. BBB was found to be re-established within 14 days after cOFM probe implantation and a 14-day healing period was thus implemented into all cOFM study protocols. Most probe-based sampling techniques in the brain are limited in application time due to the formation of a glial scar that leads to encapsulation of the probes. cOFM probe design and especially the materials used for cOFM probes have been optimized to elicit minimal tissue reaction. Glial scar formation was investigated by qualitative and quantitative histological tissue analysis of microglia and astrocytes and no continuous glial scar was found up to 30 days after cOFM probe implantation.

A micro-CT based standard atlas of the bumblebee brain

Lisa Rother[1], Dylan Smith[2], Farah Ahmed[3], Richard Gill[2], Keram Pfeiffer[1]

[1]University of Würzburg, Department of Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany

[2]Department of Life Sciences, Imperial College London, Silwood Park, Buckhurst Road, Ascot, Berkshire, SL5 7PY, UK

[3]Core Research Laboratories, Natural History Museum, Cromwell Road, London, SW7 5BD, UK

Insect standard brain atlases have become an important tool in insect neuroanatomy. They serve as a common reference frame that allow the comparison of branching areas of neurons obtained from different individuals. Traditionally the datasets implemented in an insect standard brain atlas are obtained through a combination of immunocytochemistry, using antisera that label synapses and therefore neuropils, and confocal microscopy. While this procedure is well established and reliable, it has several drawback: a major source of imprecision can be the need to dissect the nervous tissue from the head capsule to allow for a decent penetration of the antibodies. Especially in larger species, this can lead to a misalignment or distortion of parts of the brain, e.g. the optic lobes. A drawback of confocal microscopy is that the resulting data stack has an unisotropic resolution. This is due to physical limitations leading to a much poorer axial than lateral resolution.

A technique that overcomes both of these problems is micro computed tomography (micro-CT). This technique allows to acquire images that are isotropically resolved from brain tissue that has retained its natural shape, because it is still inside the head capsule. Here we present the first insect standard brain based on micro-CT images.

For our experiments, we used bumblebees (*Bombus terrestris*) from a commercial supplier. To yield high-quality images, we contrasted the tissue using a 5% solution of phosphotungstic acid. Entire heads were scanned at an x/y/z-resolution of 3.8 µm. Data stacks were then imported into the 3D-software Amira. The first segmentation step was to define the outline of the brain tissue in order to discard data from the surrounding tissue. Within the remaining dataset, comprising only the brain, neuropils were manually segmented. We labelled all three optic neuropils, the subunits of the mushroom bodies and the central complex, the anterior optic tubercles, and the antennal lobes. In addition, we were able to label the ocelli. We then applied the iterative shape averaging method to obtain an average standard atlas of the bumblebee brain.

We show that image stacks obtained via micro-CT are suitable for generating high-quality standard brains. It allows to obtain data from undistorted tissue in its natural position. An additional benefit is that tissue, which is very fragile and therefore often left out in standard atlases, such as the lamina, can be integrated just as easy as any other neuropil. In the future it is to be expected that the spatial resolution of the micro-CT technique will improve even more making it a really promising alternative to confocal microscopy for some applications.

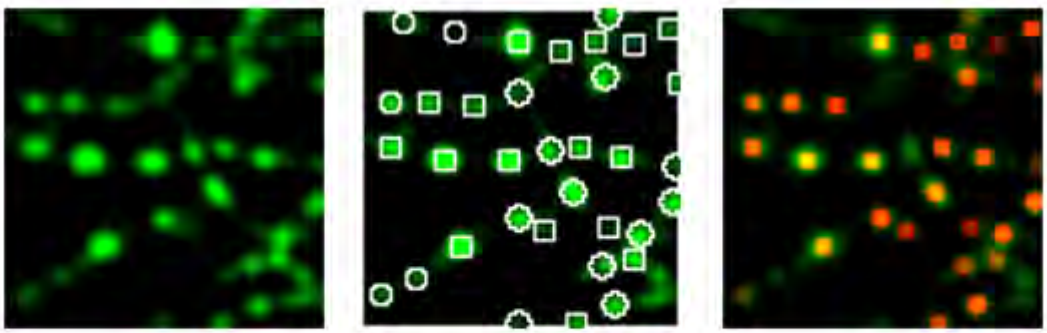
Synapse Locator: A tool for automated registration and segmentation of 3D synaptic activity maps

Christian Schulze[1], Alberto Perez-Alvarez[1], Brenna Fearey[1], Christine E Gee[1], Thomas G Oertner[1]

[1]Center for Molecular Neurobiology Hamburg, Institute of Synaptic Physiology, Falkenried 94, 20251 Hamburg, Germany

Neurons exchange information at synapses. A single neuron contains thousands of input and output sites that are unevenly distributed in 3D across brain tissue. We have developed an optogenetic tool which reports activity at all excitatory synapses impinging on a specific neuron. SynTagMA (Synaptic Tag for Mapping Activity) is a calcium integrator that leaves a lasting fluorescent mark upon calcium elevation by UV-triggered conversion from green to red fluorescence (see also Fearey, B et al. and Perez-Alvarez, A et al.). Repeated imaging at high spatial resolution revealed minute tissue movements and non-concordant positional changes of synapses. These subtle deformations make it difficult to assign corresponding regions of interest (ROI) in related 3D images (time series). Manually drawing ROIs in paired image stacks (i.e., images from pre- and post- activation status) is inefficient, potentially biased, and extremely time consuming when larger volumes containing hundreds of synapses are to be analysed. We set out to create an automated analysis pipeline which controls the necessary steps of image matching, object detection and ratiometric analysis.

Here we introduce Synapse Locator, a versatile tool that combines many steps of image processing into a user-friendly pipeline. The graphical user interface (Matlab) requires minimal user interaction to run an analysis and integrates procedures from various sources (ImageJ, elastix, Weka, Matlab File Exchange). In detail, the workflow makes use of the DeconvolutionLab2 plug-in of ImageJ for image pre-processing (deconvolution). For non-rigid registration, elastix is employed with an optimized parameter set. Object detection uses a supervised machine learning approach (Weka, ImageJ's FeatureJ plug-in), allowing for some flexibility in defining the target structure (i.e. boutons, spines). Coordinates of detected spots as well as intensities from pre- and post-stimulation (two colour channels) are numerically reported and graphically represented as color-coded markers in 3D space. Particular care has been taken to use RAM most efficiently when analysing large datasets. Synapse Locator performance has been tested on simulated 3D image stacks, two-photon images (ScanImage) and sCMOS camera stacks. It was successfully applied to analyse large sets of synapses (>1000) between dissociated hippocampal neurons and in hippocampal slice culture.



Working with Synapse Locator: SynTagMA, unconverted label; Synapse Locator identified spots; scaled converted SynTagMA signal at identified spots (left to right).

Toward Optogenetics in Barn Owls: Promising AAV-mediated Opsin Expression

Nadine Thiele[1], Meike Rogalla[1], Jannis Hildebrandt[1], Christine Köppl[1]

[1]Cluster of Excellence “Hearing4All”, Dept. of Neuroscience, Carl von Ossietzky University, Oldenburg, Germany

Optogenetics is a tool for manipulating neuronal activity with light sensitive proteins. Adeno-associated virus (AAV) vectors are commonly used in mice for delivering optogenetic constructs into cells of interest. However, different AAV serotypes vary in transduction efficiency, depending on the targeted species and cell type. As a first step towards establishing optogenetic manipulation in barn owls, we tested three AAV vectors with different serotypes to identify the most promising one for future experiments on Nucleus laminaris (NL), a large nucleus of the auditory brainstem involved in binaural processing of interaural time differences.

Virus injections were carried out in 6 adult barn owls (*Tyto alba*), anesthetized with ketamine/xylazine and monitored by EKG recordings. To identify the target site, a tungsten recording electrode was first lowered through the cerebellum into the brainstem. Stimuli were noise and tone bursts delivered through a closed, calibrated sound system. Subsequently, 1 to 2 µl of the virus solution was injected at the desired stereotaxic coordinates. Three virus vectors were tested, of different AAV serotypes (AAV2/1, AAV2/9 and AAV5) and with different fluorescent tags (GFP or TdTomato); with a neuronal (CAG) promoter and ArchT opsin in all cases. Both hemispheres were injected, in some cases with different vectors, with one week recovery time between treatments. After one to five weeks expression time, the owls were euthanized and the brains analyzed for ArchT expression patterns, labeled by the expression of the fluorescence protein tag.

The two serotypes AAV2/1 and AAV5 resulted in clear expression three to four weeks after injection, whereas AAV2/9 did not work in our hands within 3 weeks. The expression pattern suggested possible retrograde transport of the vector, with expression in neurons projecting to the injection site.

Our preliminary results suggest that commercially available AAV vectors are suitable to drive virus-mediated expression of target genes in the barn owl brain. However, whereas in another bird species, the zebra finch (Roberts et al., 2012, Nature Neurosci 15:1454-1459), serotype 2/9 was most successful, this serotype did not work in the owl. Our next steps will be to improve the targeting of the expression to NL, and establish light stimulation for optogenetic manipulation of neural activity.

Visualization of a full body Drosophila larva

Alice Weiglein[1,3], Oliver Kobler[2], Bertram Gerber[1,3,4]

[1]Leibniz Institut for Neurobiology, Genetics, Magdeburg, Germany

[2]Leibniz Institut for Neurobiology, Special Lab Electron and Laserscanning Microscopy, Magdeburg, Germany

[3]Institute of Biology, Otto von Guericke University, Magdeburg, Germany

[4]Center for Behavioral Brain Sciences, Magdeburg, Germany

The Drosophila larva depicts a commonly used model organism to study how a behavioural output is brought about by the brain, especially since it possesses a relatively simple and easily accessible brain which can be examined in its entirety by light and electron microscopy techniques. Indeed, research is almost exclusively focused on the brain since like in the vast majority of life science research does not, and often cannot, transgress the boundaries of a given organ system. Thus, the databases documenting the anatomy of the Drosophila nervous systems feature information on exclusively the nervous system, while the rest of the body is literally thrown away during sample preparation. Similarly restricted are the databases documenting the expression of transgenic driver strains which depict the basis for practically all current research in Drosophila. This can lead research badly astray. To overcome this troubling condition we aim at visualizing a full body intact Drosophila larva. For this approach we use solvent-based clearing methods combined with state-of-the-art light-sheet microscopy that allows us the detection of transgenically expressed fluorescent proteins in the context of the complete larval body. Additionally, we adapted the clearing procedure to use nanotags to improve the signal-to-noise ratio and to be able to use different markers for different cells within the same specimen. Anatomical information about a selected number of driver lines could in the longer run be mapped in a full body standard larva.

Drug delivery with polybutylcyanoacrylate nanoparticles to the retina, brain and main organs of rats

Qing You[1], Maxim Sokolov[1], Grigartzik Lisa[1], Werner Hintz[2], B. van Wachem[2], Petra Henrich-Noack[1], Bernhard A. Sabel [1]

[1]Otto-von-Guericke University, Institute of Medical Psychology, Leipziger Str. 44, 39120 Magdeburg, Germany
[2]Otto-von-Guericke University, Institute of Process Engineering, Universitätsplatz 2, 39106 Magdeburg, Germany

Because the blood-brain barrier (BBB) is an obstacle for drug-delivery, carrier systems such as polybutylcyanoacrylate (PBCA) nanoparticles (NPs) have been studied. Yet, little is known of how physiochemical features such as size, surfactants and surface charge influence BBB passage *in vivo*. In our previous study, we used a rat model of *in vivo* imaging of the retina - which is brain tissue and can reflect the situation at the BBB - to study how size and surface charge determine NPs' ability to cross the blood-retina barrier (BRB). The result showed that for poloxamer 188-modified, DEAE-dextran-stabilized, fluorescent PBCA NPs, decreasing the average zeta-size from 272 nm to 172 nm by centrifugation reduced the BRB passage of the NPs substantially. Varying the zeta potential within the narrow range of 0 – 15 mV by adding different amounts of stabilizer revealed that 0 mV and 15 mV were less desirable than 5 mV which facilitated the BRB passage. Now we removed and imaged the retina of the rats *ex vivo* to observe the detailed location of the NPs in retina tissue. Similar as the *in vivo* result, the NPs with larger zeta-size and 5 mV surface charge accumulated more in the vessel wall and in retina ganglion cells. Interestingly, the NPs with 0 mV surface charge accumulated unevenly in vessel wall and some agglomerates attached on the surface of the vessel wall. We also collected blood, brain, heart, kidneys, liver, lungs and spleen of the rats. The biological distribution of NPs in blood and brain is comparable to the results of *in vivo* imaging of blood vessel and retina tissue. Furthermore, over half of the NP dose accumulated in liver, lungs and spleen. Especially the NPs with smaller zeta-size accumulated significantly more in lungs. Thus, minor changes in design of nano-carriers can alter physicochemical parameters such as size or zeta potential, thus substantially influencing NPs' biological distribution *in vivo*, possibly by interactions with blood constituents and peripheral organs.

Integrating neuroscience data into a unified database: accessing individual experiments via a common metadata collection using the Neuroinformatics Platform of the Human Brain Project

Lyuba Zehl[1,2], Sara Zafarnia[1], Stefan Köhnen[1], Krister Andersson[3], Milica Markovic[4], Elodie Legouée[5], Camilla Hagen Blixhavn[3], Heidi Kleven[3], Xiayun Gui[1], Pavel Chervakov[1], Oliver Schmid[4], Simon D. Bell[4], Tom Gillespie[6], Mathew B. Abrams[6], Andrew Davison[5], Jeffrey C. Muller[4], Trygve B. Leergaard[3], Katrin Amunts[1,7], Jan G. Bjaalie[3], Timo Dickscheid[1]

[1]Institute for Neuroscience and Medicine (INM-1), Jülich Research Centre, Jülich, Germany
[2]Department of Neurophysiology, Institute of Biology II, RWTH Aachen University, Aachen, Germany
[3]Institute of Basic Medical Sciences, University of Oslo, Norway
[4]Human Brain Project, École Polytechnique Fédérale de Lausanne (EPFL), Campus Biotech, Geneva, Switzerland
[5]Unité de Neurosciences, Information et Complexité, Centre National de la Recherche Scientifique UPR 3293, Gif-sur-Yvette, France
[6]International Neuroinformatics Coordination Facility (INCF), Karolinska Institutet, Stockholm, Sweden
[7]Cécile and Oskar Vogt Institute of Brain Research, Heinrich Heine University Düsseldorf, University Hospital Düsseldorf, Germany

The Neuroinformatics Platform (NIP) of the Human Brain Project (HBP) provides a novel infrastructure for publicly sharing multimodal neuroscience data in accordance with the FAIR guiding principles [1]. Within this infrastructure, data are made findable, accessible, interoperable, and reusable by organizing relevant metadata into a hybrid semantic-object-graph database system, called Knowledge Graph (KG) [2]. Based on the unified information in the KG, heterogeneous neuroscience data can be mapped to common reference spaces allowing users not only to discover data in text-based queries, but also via interactive visual exploration through multilevel 3D atlas viewers [3,4]. In addition, the unified information in the KG facilitates reuse of data via HBP developed analysis toolkits.

A prerequisite for a FAIR neuroscience data sharing service is obviously the availability of standardized high quality metadata that encompass a multitude of data modalities hosted within the same platform. This is particularly challenging to accomplish for the neuroscience community, because data are produced by numerous laboratories in highly diverse experiments for different species, ranging from structural and functional imaging, electrophysiology and omics, to modeling and simulation studies (cf. figure 1).

The HBP curation team addresses this challenge by guiding data producers through a 3-tiered data integration process (cf. figure 1): Tier 1 curation ensures that data are accessible from a secure data storage, and domain-independent basic metadata (Minimum Information of Neuroscience Data Sets: MINDS) are properly integrated into the KG providing the means for querying the HBP registered data sets. Tier 2 curation supports and validates the assignment of anatomical location to data (spatial metadata) in the KG in order to present data in common brain reference spaces using spatial coordinates, or semantically link data to one of the HBP supported anatomical parcellation schemes. Finally, tier 3 curation additionally enriches the KG with standardized in-depth, domain-specific metadata that are essential for meaningfully analyzing and interpreting the available data sets.

With this 3-tiered data integration process into the HBP KG, the NIP establishes guidelines for data organization and standards for metadata, and thereby implements the FAIR guiding principles for neuroscience data. This novel infrastructure will allow scientists to easily share, discover, and reuse neuroscience data of diverse origin through a unified system.

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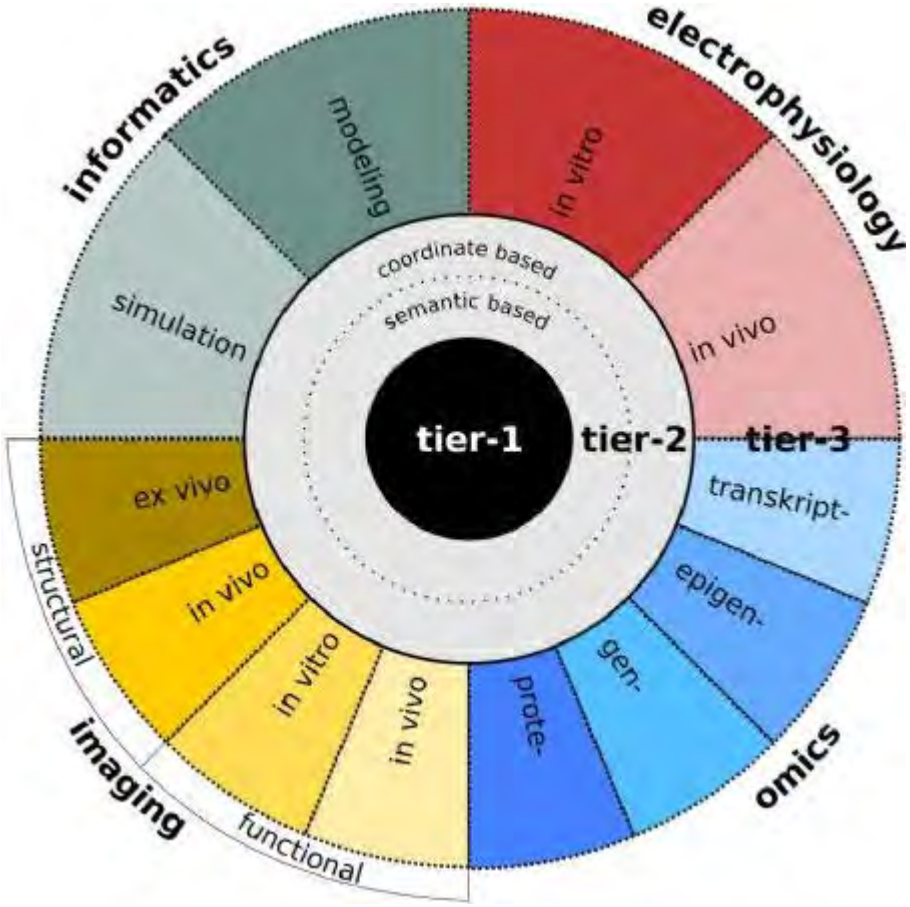

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