

Proceedings

**12th Göttingen Meeting of the
German Neuroscience Society**

Picture: Marina Mayhew, MDC Berlin

March 22–25, 2017

This is a Supplement to
Neuroforum May 2017 (2)
Band 23
eISSN 1868-856X
March 22–25, 2017

**36th GÖTTINGEN
NEUROBIOLOGY CONFERENCE**



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Hidden hearing loss: primary neural degeneration in the noise-damaged and aging cochlea.

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The classic view of sensorineural hearing loss (SNHL) is that the “primary” targets are hair cells, and that cochlear-nerve loss occurs only “secondary” to hair cell degeneration. Our work has challenged that view. In noise-induced hearing loss, exposures causing only reversible threshold shifts (and no hair cell loss) nevertheless cause permanent loss of >50% of cochlear-nerve / hair-cell synapses. Similarly, in age-related hearing loss, degeneration of cochlear synapses precedes both hair cell loss and threshold elevation. This primary neural degeneration has remained hidden for two reasons: 1) the cochlear-nerve cell bodies, the neural elements commonly assessed in studies of SNHL, survive for years despite loss of their synaptic connection with hair cells, and 2) the degeneration is selective for cochlear-nerve fibers with high thresholds. Although not required for threshold detection in quiet (e.g. threshold audiometry or auditory brainstem response threshold), these high-threshold fibers are critical for hearing in noisy environments. Our research suggests that 1) primary neural degeneration is an important contributor to the perceptual handicap in SNHL, 2) it may be key to the generation of tinnitus and hyperacusis, and 3) in cases where the hair cells survive, neurotrophin therapies can elicit neurite outgrowth from spiral ganglion neurons and re-establishment of their peripheral synapses.

Visualizing synapse structural dynamics *in vivo*

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The introduction of two-photon microscopy for *in vivo* imaging has opened the door to chronic monitoring of individual neurons in the adult brain and the study of structural plasticity mechanisms at a very fine scale. Perhaps the biggest contribution of this modern anatomical method has been the discovery that even across the stable excitatory dendritic scaffold there is significant capacity for synaptic remodeling, and that synaptic structural rearrangements are a key mechanism mediating neural circuit adaptation and behavioral plasticity in the adult.

To monitor the extent and nature of excitatory and inhibitory synapse dynamics on individual L2/3 pyramidal neurons in mouse neocortex *in vivo*, we labeled these neurons with a fluorescent cell fill as well as the fluorescently tagged synaptic scaffolding molecules, Teal-Gephyrin to label inhibitory synapses, and mCherry-PSD-95 to label excitatory synapses. We simultaneously tracked the daily dynamics of both synapse types using spectrally resolved two-photon microscopy. We found that aside from the lower magnitude of excitatory synaptic changes in the adult, as compared to inhibitory ones, excitatory synapse dynamics appear to follow a different logic than inhibitory dynamics. Excitatory synapses are generally very stable once established in the naive animal, but many spines are added and removed all along the dendritic branch on a relatively rapid timescale. These short-lived transient spines potentially represent a sampling strategy to search for and create connections with new presynaptic partners, and most of these attempts fail. In contrast, many inhibitory synapses are added and removed at the same location on a rapid timescale and likely represent input-specific regulation at particular dendritic locales.

Synapses. Memories. On the fly.

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Information storage in the brain requires neural circuits that allow for meaningful computations and modes of plasticity that can change the routing and processing of activity patterns. These changes require certain constraints in order to retain information specificity. It is therefore widely hypothesized that plasticity will take place at identified synapses of higher order information-carrying neurons during memory formation. I here address aspects of how synapses are organized at the molecular level and how they can dynamically assemble, change and mature during development - mechanisms that potentially also play a role after plasticity induction. I will then present work identifying a site of memory-relevant plasticity and how aspects of this plasticity are modulated at the network level. For the first part we make use of live imaging of tagged synaptic proteins at a *Drosophila* larval peripheral model synapse; the second part focuses on the *Drosophila* adult mushroom bodies, in vivo calcium imaging and olfactory learning paradigms. For both approaches I draw on the intricate genetics available in *Drosophila*.

Towards a complete parts list: multimodal data science in the retina

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The retina in the eye performs complex computations, to transmit only behaviourally relevant information about our visual environment to the brain. These computations are implemented by numerous different cell types that form complex circuits. New experimental and computational methods make it possible to study the cellular diversity of the retina in detail – the goal of obtaining a complete list of all the cell types in the retina and, thus, its “building blocks”, is within reach. I will review our recent contributions in this area, showing how analyzing multimodal datasets from electronmicroscopy and functional imaging can yield insights into the cellular organization of retinal circuits.

The dual face vision's inroad into the social brain

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Humans, like all primates, take great interest in faces. Faces, by structure and dynamics, display a plethora of social information for a visual system that can extract it. The primate visual system does this through specialized hardware. The functional organization of this hardware, a network of tightly interconnected areas packed with face cells, each tuned to a different dimension of facial information, provides us with a unique model system to understand the computational principles and neural mechanisms of high-level visual object recognition. Yet among objects faces are special: faces are more than passive displays of social information; they evoke emotions, activate memories, invite thought about others' mental states, draw and direct attention, and elicit communicative reactions in the perceiver. Faces trigger these processes in an automatic fashion, suggesting that just as the perceptual analysis of faces is supported by specialized hardware, these diverse cognitive functions may be as well. In my talk, I will describe how the face-processing system encodes, transforms, and packages facial information, and how it integrates facial information with other social stimuli. I will discuss how the network is embedded in the social brain in ways to suggest specific pathways for social information processing and a deep evolutionary heritage of high-level social cognition in humans.

Neurobiology of sky compass orientation in insects

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Insects are highly mobile animals and, depending on species, show excellent performance in flight, walking, swimming, and jumping. Both by running and in flight, insects can cover considerable distances in short time. Seasonal migrations as well as precision in homing require sophisticated mechanisms for spatial orientation. Across a range of species, landmark orientation and landmark memory have been demonstrated, as well as the use of sky compass cues for path integration and long-range migrations. In contrast to behavioral data, considerable progress on the neural mechanisms underlying spatial representation and navigation has been achieved only in recent years.

Many insects use a specialized polarization-sensitive dorsal rim area in their compound eyes to exploit the polarization pattern of the blue sky for sun compass orientation. We have taken advantage of this capability to identify and characterize navigational centers in the brain. Desert locusts are long-range migratory insects with a particularly well developed dorsal rim area. Tracing studies from the dorsal eye region to the central brain showed that pathways of polarization-sensitive neurons converge in the central complex, an unpaired neuropil in the middle of the brain. The central complex can be regarded as a highly ordered matrix of layers intersected by arrays of columnar neurons crossing the brain midline in a highly regular fashion. Neurons sensitive to the plane of polarized light make up a considerable proportion of this network. Columnar neurons of the central complex form a topographic representation of celestial polarization orientation, suited to code for spatial directions around the animal. These neurons are not only sensitive to celestial polarization but apparently also code directly for the position of the sun. This system of sky compass coding may, therefore, fulfill a function equivalent to mammalian head direction cells, which likewise code for orientation of the animal in space. Recent data show that other sensory signals, and probably feedback from behavioral activity, strongly influence signaling properties in the central complex. Work in other species supports a general role of the central complex as an internal sky compass in insects and shows that it might serve additional navigational roles in landmark orientation and memory.

Natural products as probes of the pain pathway: from physiology to atomic structure

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We are interested in determining the molecular basis of somatosensation - the process whereby we experience touch and temperature - with an emphasis on identifying molecules that detect noxious (pain-producing) stimuli. We are also interested in understanding how somatosensation is altered in response to tissue or nerve injury. Our approach has been to identify molecular targets for natural products that mimic the psychophysical effects of commonly encountered somatosensory stimuli, such as heat or cold, and to then ask how these molecules are activated or modulated by noxious stimuli or injury.

Evolution of neurons and nervous systems: a cell type perspective

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How animals progressed from a simple nerve net, as observed in basal metazoans, to the most complex centralized nervous system, as found in human, remains one of the most exciting and unsolved questions in animal evolution. Two major strategies contribute to solving this grand puzzle: the comparison of neurodevelopment and the comparison of neural cell types in animals that live today. Both approaches benefit tremendously from recent technical progress - in CRISPR-Cas9 genetic engineering and single cell genomics, respectively.

Encoding synaptic signals into gene expression: a role in brain physiology and diseases

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Despite key progress in the understanding of synapse formation, synaptic transmission and plasticity, much remains to be determined with respect to the molecular and cellular mechanisms that could lead to therapies for brain diseases. To develop effective treatments, deciphering the synaptic functioning between the thousands of molecules present in a single synapse represents a fundamental need. Knowing better the normal functioning will help in the understanding of disorders by comparison with pathological situations.

Based on these considerations, in the last decade our research activity dealt with the study of synapses and extends from proteins and macromolecular complexes to morphological function and diseases. In particular, we investigated the molecular mechanisms leading to synaptic retention of NMDA-type glutamate receptors (NMDARs) as well as NMDAR-dependent reshaping of dendritic spines following induction of synaptic plasticity. NMDAR composition and synaptic retention represent pivotal features in the physiology and pathology of excitatory synapses. Several studies have proposed that abnormal GluN2-type subunits trafficking, resulting in the modification of the NMDAR subunit composition at synapses, has a major role in the pathogenesis of several brain disorders. We recently identified Rabphilin 3A (Rph3A) as a new NMDA receptor-binding partner. In hippocampal neurons, Rph3A modulates the surface localization/stabilization of synaptic containing NMDARs and NMDAR currents through the formation of a ternary complex with the GluN2A subunit and the scaffolding protein PSD-95. Understanding how local synaptic events at the glutamatergic synapse are translated into changes in gene expression is an additional crucial question in neuroscience. Synapses and nuclei are efficiently connected by bidirectional communication routes that enable transfer of information. In this framework, we investigated how the activation of these synaptic NMDARs is linked to long-lasting structural plasticity thanks to the activation of genes at nuclear level. NMDAR complex is a very rich source of protein messengers that are capable of trafficking to the nucleus. In this context, we discovered that Ring Finger Protein 10 (RNF10) represents a novel synaptonuclear protein messenger responsible for long-lasting re-shaping of dendritic spines as observed after specific synaptic stimuli and required for postsynaptic modifications needed to convey synaptic plasticity induction.

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Symposium

S1: Olfactory processing and behavior across the vertebrate/insect divide: communalities and differences

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Lisa Stowers

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Matthew DeGennaro, Joshua Raji, Babak Ebrahimi, Sheyla Gonzalez, Valeria Saldana

Leveraging olfaction to study social behavior in the mouse

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Survival behaviors such as aggression, fear, and mating are highly conserved across evolution. Knowing when and how to display survival behavior is essential for fitness and requires neural activity from known brain regions such as the amygdala and hypothalamus. However, the identity of the precise neurons and circuits that generate these survival behaviors remains largely unknown and therefore unstudied. In the mouse, all of these essential behaviors can be robustly initiated by olfactory cues. We have identified specific bioactive odors that now enable us to precisely stimulate and identify the neural mechanisms across the brain that generate behavior. We are creating and assessing novel tools to be able to identify and manipulate the subsets of neurons that translate a sensory signal into innate behavior. In addition, we are studying how the sensory information elicits variable responses depending on state, gender, or the complexity of the environment.

Olfactory control of behavior

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Olfaction is one of our five basic external senses, and a principal mechanism by which we perceive the external world. The mouse olfactory system detects odors and pheromones using large families of G Protein-Coupled Receptors (GPCRs). We recently identified additional mammalian olfactory receptors, including a family of GPCRs named trace amine-associated receptors (TAARs). Using the TAARs as a model system, we characterized ligands, receptors, and neural circuits underlying various odor-driven behaviors. We identified the first ligands for many TAARs, with each detecting different volatile amines, and obtained structural insights into how ligand recognition is achieved. Interestingly, TAARs detect natural products that evoke strong aversion or attraction responses. Ethological odors that activate TAARs include an aversive predator odor that repels mice and rats, a sexually dimorphic mouse odor that evokes species-specific attraction responses, and a repugnant carrion odor. Our work provides a foundation for understanding neuronal mechanisms by which sensory circuits generate perceptions and behaviors.

Functional properties of feedback projections from the anterior olfactory nucleus to the mouse olfactory bulb

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The brain conducts extensive filtering processes in order to extract relevant sensory information from a complex environment. Even at early processing stages sensory information is filtered before being transmitted to higher order centers. Modulations of sensory information processing are mainly mediated by so called top-down inputs, which can be divided into neuromodulatory and cortical feedback projections. Here, we aim to shed light on the role of cortical feedback projections in early sensory processing, using the mouse olfactory system as a model.

The olfactory bulb (OB) receives cortical feedback projections mainly from the piriform cortex (PC) and the anterior olfactory nucleus (AON) with the AON being the largest cortical feedback source. Both PC and AON receive direct input from the OB, are strongly interconnected and sent information to higher olfactory areas. However, there are also some marked differences in the way the PC and AON are organized: in contrast to the PC, receiving OB input mainly from mitral cells, the AON is strongly innervated by tufted cells (Nagayama et al., 2010). Moreover, part of the AON is topographically organized whereas the PC lacks a specific spatial organization (Miyamichi et al., 2011).

We recently demonstrated strong top-down feedback activity from AON fibers in response to odorant stimulation in the anesthetized as well as in the awake animal (Rothermel and Wachowiak, 2014). This odorant-evoked activity was also found in top-down projections originating in PC (Boyd et al., 2015, Otazu et al., 2015). These studies showed broad odor-evoked activation patterns in fibers originating in PC with no apparent spatial organization. In contrast, we observed that feedback projections from AON showed some odorant-specificity in the spatial as well as the temporal domain.

In order to further investigate the spatial organization of AON feedback projections to the OB we used optogenetics in combination with in vivo 2-Photon microscopy. Thereby we were able to selectively activate a single glomerulus by stimulating only olfactory receptor neurons (ORN) expressing a specific OR while simultaneously imaging top-down projections from the AON in the OB. In comparison to odor stimulation, AON fibers in the OB showed a rather local activation profile during optogenetic stimulation. These preliminary results indicate that AON top-down projections might be capable of modulating homotopic OB areas. The spatial difference between sensory evoked feedback projections from PC and AON suggests that they might have differential effects on OB circuitry: broadly tuned top-down activity from the PC could be used for gain control, whereas more spatially restricted feedback from the AON might be important for experience-dependent changes.

Our group also investigates the specific cell types affected by AON feedback projections. Our current experiments focus on electrical and optical AON stimulation experiments in anesthetized mice, while simultaneously recording spontaneous as well as odor-evoked responses from defined cells types in the OB. We predict that activating AON will strongly influence OB output in vivo, with inhibition being the most dominant feature. Future projects will focus on the connections between top-down activity and different behavioral states.

Mapping Circuits for flexible behavior using *Drosophila* Chemosensation

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When interacting with their environment animals constantly have to make decisions. These decisions aim at maximizing reward while avoiding negative consequences such as energy costs, pain, or long-term disadvantages. Faced with a choice animals consider and integrate several parameters such as their internal state as well as other external stimuli. While it is generally accepted that such contexts influence behavior, our knowledge of the neural mechanisms of how internal states alter behavioral outcomes is scarce. The problem can be broken down into several aspects: (i) behavior: how does context alter behavior, (ii) circuits: how does context change neural processing, and (iii) genes: which molecules modulate behavior in a context-dependent manner? The goal of our research is to provide a comprehensive understanding of the neuronal and molecular basis of context-specific behavior. In particular, we are interested in understanding how physiological states influence chemosensory (taste and odor) processing and food choice behavior. We use mainly the genetically tractable model organism *Drosophila* and combine state-of-the-art technologies such as *in vivo* 2-photon microscopy, optogenetic control of behavior, and physiological recordings in living and behaving animals. In my talk, I will present some recent and ongoing work of my lab.

Genetic analysis of *Aedes aegypti*'s attraction to plant and human hosts

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Aedes aegypti represents a threat to humans as it spreads dengue fever, chikungunya, and Zika. There is currently no effective treatment or preventative vaccine for these illnesses. Molecularly informed strategies to prevent mosquito host-seeking behavior are needed to block disease transmission. Mosquitoes use olfaction as a primary means of detecting their human and plant hosts, but our understanding of this sensory modality is far from complete. I have previously identified a family of 131 olfactory receptors, the odorant receptors (ORs), which are necessary for mosquitoes to detect human odor by eliminating the function of their obligate co-receptor Orco. However, loss of these receptors was not sufficient to eliminate human host-seeking, but did reduce mosquito attraction to nectar volatiles. This suggests the olfactory receptors that remain intact in *orco* mutants, the ionotropic receptors (IRs), are playing a significant role in human host detection. A functional IR odor-gated ion channel consists of an odor-tuned IR and one or more IR co-receptors. To address the role of IRs in mosquito host detection, my lab has pursued a genome editing strategy using the CRISPR/Cas9 system to disrupt IR co-receptors. We have found a role for these receptors in human host detection. We are also cataloging the expression of the IRs in the *Aedes* olfactory system. Using a combination of behavioral genetics and expression analysis, we are validating which IRs are suitable molecular targets for vector control.

Symposium

S2: Mechanisms of neuronal and synaptic plasticity in epilepsy

- [S2-1](#) Mechanism of Nogo-A actions in regulating functional and structural synaptic plasticity
Marta Zagrebelsky, Steffen Fricke, Niklas Lonnemann, Kristin Metzdorf, Stella Kramer, Martin E Schwab, Martin Korte
- [S2-2](#) Gephyrin-dependent epilepsies
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Felix Beinlich, Raul Guzman, Christoph Fahlke, Thomas Gensch
- [S2-5](#) The role of stress in seizures and epilepsy
Nicola Maggio
- [S2-6](#) Disrupting neuronal nitric oxide synthase PDZ - interactions results in schizophrenia-like behavior
Esin Candemir, Aet O`Leary, Lena Grünewald, Andreas Reif, Florian Freudenberg

Mechanism of Nogo-A actions in regulating functional and structural synaptic plasticity

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The functions of neural circuits in the adult brain are determined by the architecture of axons and dendrites and of the synapses connecting them. Changes in synaptic connectivity - structural plasticity - have been correlated to functional changes at synapses - functional plasticity - and are thought to underlie learning and memory processes. Yet, long-term in vivo imaging in the adult brain reveals that the neuronal network is remarkably stable providing a substrate for long-term memory storage. These observations suggest the need for a set of molecules regulating the balance between stability and plasticity of mature neuronal networks, ensuring the spatial and temporal specificity of plastic changes and preventing interference between different memory events. The myelin-associated neurite growth inhibitor Nogo-A and its receptors, Nogo-66 receptor 1 (NgR1) and sphingosine 1-phosphate receptor 2 (S1PR2) are expressed pre- and post-synaptically and have been shown to negatively modulate neuronal architecture and activity-dependent synaptic plasticity. Moreover, we recently showed that Nogo-A signalling regulates structural plasticity at a fast time scale by modulating actin polymerization within spines via the RhoA-ROCK pathway. Also, Nogo-A acutely restricts AMPA receptor (AMPA) insertion and formation of new AMPAR clusters as well as synaptic strength and the number of active synapses at spines, as shown by calcium imaging. Finally, we could show that Nogo-A modulates spatial learning in the Morris water maze, possibly by negatively regulating excitation within the hippocampus. On the other hand, Nogo-A is highly expressed especially in Parvalbumin (PV)-positive interneurons within the hippocampus suggesting the possibility that it might modulate plasticity levels and learning processes also by controlling the activity of the inhibitory network and thus, the balance between excitation and inhibition. To address this question, we started by analysing changes in the amplitude and frequency of miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs). Interestingly, in CA3 pyramidal neurons while the amplitude of mEPSCs increases within minutes after the neutralization of Nogo-A, both the amplitude and the frequency of mIPSCs are decreased at a similar time scale. These results indicate that Nogo-A indeed regulates both excitation and inhibition at a fast time scale. To assess the relevance in vivo of these findings, changes in Nogo-A and PV expression levels within the hippocampus are currently analysed upon spatial learning by training in the Morris water maze. Our data so far provide a cellular and molecular mechanism mediating the role of Nogo-A signalling in controlling activity-dependent synaptic plasticity thereby maintaining the balance between the plasticity and stability of the neuronal circuitry in the mature central nervous system.

Founded by the DFG (ZA 554/3-1)

Gephyrin-dependent epilepsies

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Gephyrin directly interacts and clusters GABA type A and glycine receptors (GABAARs and GlyRs) and is thereby indispensable for normal functioning of inhibitory synapses. Additionally, gephyrin catalyzes the synthesis of the molybdenum cofactor (Moco) in peripheral tissue. We identified a de novo missense mutation (G375D) in the gephyrin gene (GPHN) in a patient with epileptic encephalopathy resembling Dravet syndrome. We showed that gephyrin-G375D acts dominant-negatively on postsynaptic gephyrin clustering leading to decreased cell-surface expression of GABAARs in hippocampal neurons. Our molecular analysis revealed a decreased binding between gephyrin-G375D and receptors and identifies novel regions of gephyrin critical for GABAAR clustering. Furthermore, our results suggest a reciprocal regulation of gephyrin-receptor clustering. Gephyrin-G375D was also unable to synthesize Moco and to activate Moco-dependent enzymes. Thus, we describe a missense mutation that affects both functions of gephyrin and suggest that the identified defect at GABAergic synapses is the mechanism underlying the patient's severe phenotype

RNA editing and neuron type specific effects on neuropsychiatric symptoms in epilepsy

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C-to-U RNA editing of glycine receptors (GlyRs) may play an important role in mesial temporal lobe epilepsy (mTLE) as it is upregulated and can contribute to neuropsychiatric symptoms in a cell type specific way. It will be shown that, in a mouse model, presynaptic expression of these gain-of-function receptor variants in hippocampus facilitates glutamatergic and GABAergic synaptic transmission, resulting in cognitive dysfunction or persistence of contextual fear memory, respectively (Winkelmann et al., 2014 and Caliskan et al., 2016). However, until recently, detection of neuronal endogenous expression of RNA-edited GlyR mRNA or protein at the single cell level was a challenging task. New agonist and antagonists specifically acting on RNA-edited GlyRs will be presented, and neuronal endogenous expression of these proteins in hippocampal neurons will be demonstrated for the first time. Furthermore, a novel fluorescence-based C-to-U RNA editing sensor tool and new fluorescent probes to detect RNA-edited GlyR mRNA in single cells will be introduced. Together, these new tools and novel approaches shall help us understanding regulatory mechanisms of GlyR C-to-U RNA editing and develop alternative treatment options for patients with mTLE.

Monitoring the contribution of ClC-3 on the acidification of glutamatergic synaptic vesicles with fluorescence lifetime imaging microscopy

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Question: Glutamate uptake into synaptic vesicles (SV) is mediated by vesicular glutamate transporters (VGLUTs) that utilize the electrochemical gradient of protons for vesicular glutamate accumulation. Moreover, vesicular glutamate uptake is stimulated by low millimolar cytosolic Cl⁻ concentrations and inhibited by high millimolar cytosolic Cl⁻ concentrations. We recently reported that removal of the chloride/proton exchanger ClC-3 inhibits glutamate loading into SVs, indicating that Cl⁻/H⁺ antiport in the SV decreases the driving force for vesicular glutamate transport. At present, it is not understood whether Cl⁻ acts as a cofactor for VGLUT activation or whether VGLUT is a membrane-potential driven Cl⁻/glutamate antiporter. To determine the mechanisms underlying glutamate accumulation in SVs, and the role of ClC-3 in its regulation, it is necessary to determine pH and Cl⁻ concentrations in this compartment.

Methods: The resting pH of glutamatergic SVs is thought to be around 5.7. Previous studies have worked with the intensity-based fluorescent dyes pHluorin (pK_a 7.1) or mOrange2 (pK_a 6.5). However, these fluorescent dyes are not very precise at low pH. Their usefulness is limited by fluorescence quenching in an acid environment and by the requirement for ratiometric measurement to obtain absolute concentrations. To overcome these limitations, we employed two-photon lifetime fluorescence imaging microscopy (FLIM) for the non-invasive determination of absolute pH during SV re-acidification in cultured primary hippocampal neurons. To distinguish glutamatergic from GABAergic SVs neurons were labelled with an anti-VGAT antibody. The remaining surface pool of Cerulean at the plasma membrane was proteolytically cleaved by inducing a TEV protease cleaving site between VAMP-2 and Cerulean.

Results: We calibrated the fluorescence lifetime of Cerulean fused to the SNARE protein VAMP-2 in SVs by clamping primary hippocampal neurons to pHs between pH 4 and pH 7.4 in high potassium buffer supplemented with ionophores. From the calibration curve a pK_a of 5.7 is determined for Cerulean. The fluorescence lifetime changes by a factor of 2.1 in the range of pH 4.5 to pH 7. Thus, Cerulean provides a higher accuracy in measuring luminal pH during re-acidification than mOrange2 because of its higher sensitivity. We are currently analyzing long-time (80 sec acquisition time) FLIM recordings of VAMP1-Cerulean expressed in primary hippocampal neurons from WT and Clcn3^{-/-} KO mice under physiological and blocking conditions.

Conclusion: Fluorescence lifetime imaging microscopy might provide a tool to investigate the relation between pH and Cl⁻ concentration in synaptic vesicles and other cell organelles.

The role of stress in seizures and epilepsy

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In this presentation I'll show how life stress affects synaptic, cellular and network mechanisms of epilepsy.

Disrupting neuronal nitric oxide synthase PDZ - interactions results in schizophrenia-like behavior

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Neuronal nitric oxide synthase (NOS-I) and its adaptor protein (NOS1AP) have been associated with schizophrenia in multiple genetic association and expression studies [1]. NOS1AP directly competes with PSD-95 and -93 for interaction with the NOS-I PDZ domain. We have recently shown that disruption of NOS-I PDZ interactions results in schizophrenia-related dendritic alterations [2]. Here, we further investigated whether disturbed integrity of this interaction leads to schizophrenia-like behavior.

Recombinant adeno-associated virus expressing full length murine NOS1AP (NOS1AP), residues 396-503 of NOS1AP (NOS1AP₃₉₆₋₅₀₃) encoding the NOS-I PDZ binding motif, N-terminal 133 amino acids of NOS-I (NOS-I₁₋₁₃₃) containing the NOS-I PDZ domain and an mCherry control vector [2] were stereotaxically delivered to the dorsal hippocampus of 7-week-old male C57Bl/6J mice (n=9/group). After 4 weeks, mice were tested for spatial working memory (SWM) in the T-maze, locomotor activity in the open field, prepulse inhibition (PPI) of the acoustic startle response (ASR) and nesting behavior.

Spatial working memory dysfunction is an important endophenotypic marker for the cognitive deficits observed in patients with schizophrenia. Therefore, mice were tested in the rewarded alternation paradigm in a T-maze. Disturbed NOS-I PDZ interactions (NOS1AP, p=0.004; NOS1AP₃₉₆₋₅₀₃, p=0.001; NOS-I₁₋₁₃₃, p<0.001) resulted in significantly impaired SWM (Figure 1A). To investigate the positive symptoms of schizophrenia (i.e. psychomotor agitation, stereotypic behaviors), we tested mice for locomotor activity in the open field for 10 min. Mice overexpressing NOS-I₁₋₁₃₃ displayed significantly increased horizontal activity (Figure 1B), suggesting that overexpression of the NOS-I PDZ domain results in hyperactivity. Another indicator of positive symptoms observed in patients with schizophrenia is deficiencies in the processing of information (i.e. gating sensorimotor stimuli). To test this, PPI of ASR was measured using startle pulses with 120 dB intensity and prepulses at 4, 8, 12 or 16 dB above background (65 dB). In all treatment groups ASR was comparable to that of mCherry mice, suggesting normal startle sensitivity. Only mice overexpressing NOS1AP showed significantly reduced PPI levels especially when using pre-pulse intensities 16dB above background (16 dB, p=0.043; 12 dB, p=0.055; 8 dB, p=0.4; 4 dB, p=0.06), suggesting impaired sensorimotor gating (Figure 1C). Mice overexpressing NOS1AP₃₉₆₋₅₀₃ had lower scores in nest building test (Figure 1D) as an indicator of self-neglect, however this effect was not significant (p=0.089).

In a nutshell, increased psychomotor agitation, impaired memory and sensorimotor gating in mice indicate translational relevance to the positive and cognitive symptoms observed in patients with schizophrenia and support our hypothesis that disturbed integrity of the NOS-I PDZ interactions may contribute to the development of this disorder. Our findings may eventually aid to understand the molecular mechanisms involved in schizophrenia and to develop more direct treatment strategies.

References

- [1] Brzustowicz LM. 2008. NOS1AP in schizophrenia. *Curr. Psychiatry Rep.* 10:158–63.
- [2] Candemir E. et al. 2016. Interaction of NOS1AP with the NOS-I PDZ domain: Implications for schizophrenia-related alterations in dendritic morphology. *Eur. Neuropsychophar.* 26, 741–755.

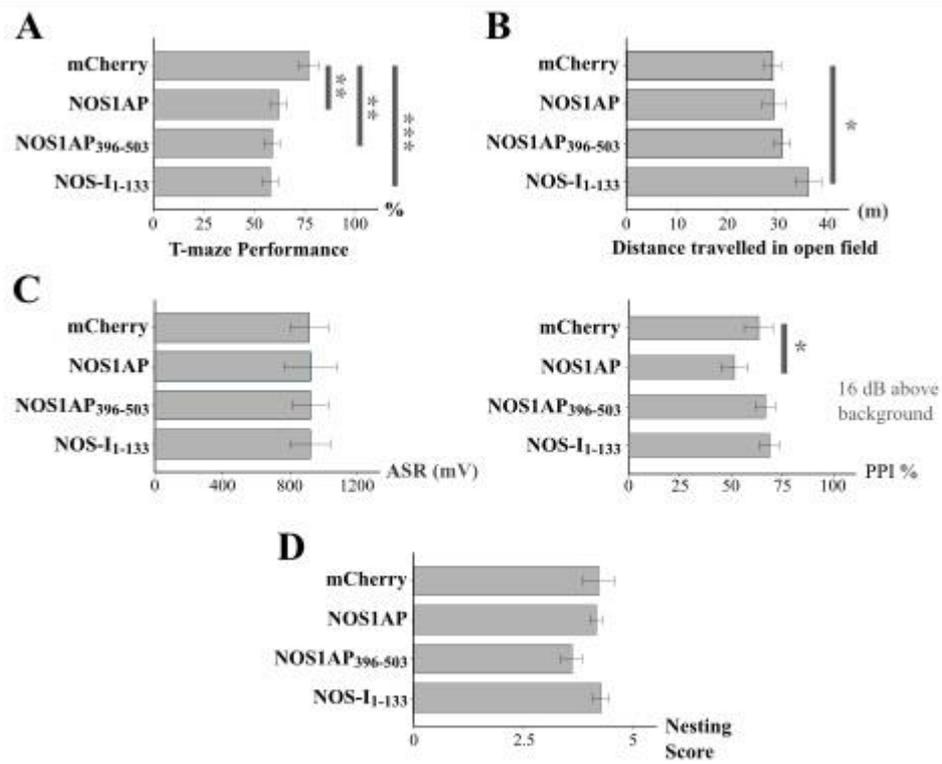


Figure 1. Disruption of NOS-I PDZ interactions contributes to the behavioral phenotypes related to schizophrenia. **A)** Mice with disturbed NOS-I PDZ interactions showed strongly impaired spatial working memory in T-maze. **B)** Locomotor activity of mice was measured as distance travelled in open field. **C)** Prepulse inhibition (PPI) of the acoustic startle response (ASR) is impaired in mice overexpressing NOS1AP. **D)** Mean score for nest building. * $p \leq 0.05$, ** $p \leq 0.005$, *** $p < 0.001$. Data is shown as Mean \pm SEM. $n=9$ /group

Symposium

S3: Molecular mechanisms of cargo and organelle transport in neurons

- [S3-1](#) Regulation of axonal trafficking of signaling endosomes
Giampietro Schiavo, Ione Meyer, Sunaina Surana, Deniz Tiknaz, Andrew Tosolini
- [S3-2](#) Unconventional myosins as regulators of synaptic function and development
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Sindhuja Gowrisankaran, Vicky Steubler, Sebastien Houy, Monika Tichy, Jakob Balslev Sørensen, Ira Milosevic
- [S3-6](#) The ADP-sensitive P2Y₁₃ receptor attenuates progenitor cell proliferation, new neuron formation, and neuronal activity in the dentate gyrus of adult mice
Jennifer Stefani, Herbert Zimmermann, Klaus Hammer, Peter Brendel, Olga Tschesnokowa, Bernard Robaye, Jean-Marie Boeynaems, Amparo Acker-Palmer, Kristine Gampe

Regulation of axonal trafficking of signaling endosomes

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Tetanus neurotoxin (TeNT) and botulinum neurotoxins (BoNTs) are amongst the most poisonous molecules on Earth. TeNT is a major cause of neonatal death in non-vaccinated areas, whilst BoNTs are responsible for botulism in humans and animals. TeNT and BoNTs block neurotransmitter release by cleaving SNARE proteins controlling the fusion of synaptic vesicles with the presynaptic membrane, yet their clinical symptoms are very different. BoNTs enters neuromuscular junctions (NMJs) and halt synaptic vesicle fusion mainly at this location, thus inducing a flaccid paralysis. In contrast, TeNT is sorted to the axonal retrograde transport pathway upon entry at the NMJ, and it is targeted to the plasma membrane of spinal cord motor neurons. It is then internalised into inhibitory interneurons, causing an irreversible block the release of inhibitory neurotransmitters, which leads to a persistent spastic paralysis.

TeNT targets the NMJ with high affinity, yet the nature of the TeNT receptor complex at this site was lacking. We showed that nidogens are the main determinant for TeNT binding at the NMJ. Inhibition of TeNT-nidogen interaction using small nidogen-derived peptides or genetic ablation of nidogens, prevented the binding of TeNT to neurons. Furthermore, a nidogen-derived peptide specifically protected mice from TeNT-induced spastic paralysis.

Our findings demonstrated the direct involvement of a protein receptor for TeNT at the NMJ and suggest that TeNT is endocytosed via an efficient capture mechanism at specialised NMJ sites, which could concentrate TeNT as well as physiological ligands, such as neurotrophic factors, for their sorting to axonal transport organelles. Molecular details about this mechanism will be presented together with novel tools to better understand ligand targeting at the NMJ. In this regard, the results for a novel screen to isolate enhancers of axonal transport using a tracer engineered on the TeNT backbone will be shown, which offer new possible avenues of therapeutic intervention in neurodegenerative conditions.

Unconventional myosins as regulators of synaptic function and development

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Myosins are a large family of functionally diverse cytoskeletal motors that use actin filaments as tracks and that produce movement and force. Actin filaments are highly enriched, for example, in the postsynaptic specializations called dendritic spines. We have investigated the roles of two unconventional myosins (myosin Va, myosin VI) using cerebellar Purkinje cells (PCs) as a model system. In mice that lack myosin Va, long-term depression at parallel fiber to Purkinje cell synapses (PF-PC LTD) is disrupted due to the absence of endoplasmic reticulum (ER) from the PC dendritic spines. We dissected how the myosin allows for ER targeting to spines via time-lapse live cell microscopy of cultured PCs. We found that myosin Va accumulates at the ER tip as the organelle moves into spines. In the absence of the myosin, ER insertional movement into spines is almost entirely absent. Furthermore, replacement of the myosin with "slow walking" versions leads to a corresponding reduction of the maximum velocity of ER movement into spines. Thus, our data show that myosin Va acts as a point-to-point organelle transporter that pulls ER as cargo into PC spines. In contrast to myosin Va and all other myosins examined to date, myosin VI moves towards the minus end of the actin filament. Myosin VI is known to interact with AMPA receptors (AMPA receptors) and has been implicated in their intracellular trafficking. Our data show that myosin VI localizes to PC spines and that AMPAR-mediated synaptic transmission is impaired in cultured PCs of *Snell's waltzer* mice that lack myosin VI. Moreover, PF-PC LTD is severely reduced in acute cerebellar slices of *Snell's waltzer* mice. This suggests that myosin VI-dependent AMPAR trafficking in PCs is crucial for postsynaptic function and plasticity. We currently analyze the molecular mechanism via which the myosin acts in AMPAR trafficking. Moreover, to test whether myosin VI in PCs is required for motor coordination and motor learning, we generated a conditional knock mouse that lacks the myosin specifically in PCs. Despite the myosin's role for postsynaptic function in PCs, no severe impairments were detected in tasks that assay cerebellum-dependent motor learning such as vestibulo-ocular reflex adaptation. In conclusion, using cerebellar PCs as a model system, we provide novel insights into the molecular mechanism and physiological significance of unconventional myosins.

Synaptic control of dendritic secretory organelle transport and positioning

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Secretory trafficking is essential for many neuronal processes including development, homeostasis and synaptic plasticity. Membrane proteins and lipids are processed through the ER, the Golgi complex and the endosomal system on their way to the plasma membrane from where they can be recycled back or targeted for subsequent degradation. In addition to the cell body, all these components are found in dendrites and are believed to serve as supply stations for the local dendritic arbors and spines. During secretory trafficking coordinated interaction of organelles could ensure a proper passage of proteins from one compartment to the other. Here we describe a widespread microsecretory Golgi satellite system (GS) that is in contrast to Golgi outposts present throughout basal and apical dendrites of all pyramidal neurons. GS contains glycosylation machinery and is localized in between ERGIC and retromer. Synaptic activity restraints lateral movement of ERGIC, GS and retromer in close proximity to each other, allowing for confined processing of secretory cargo. Several synaptic transmembrane proteins pass through and recycle back to GS. Thus, the presence of an ER-ERGIC-GS-retromer microsecretory system in all neuronal dendrites enables autonomous local control of transmembrane protein synthesis and processing.

Light induced transport to study neuronal polarity *in vivo*

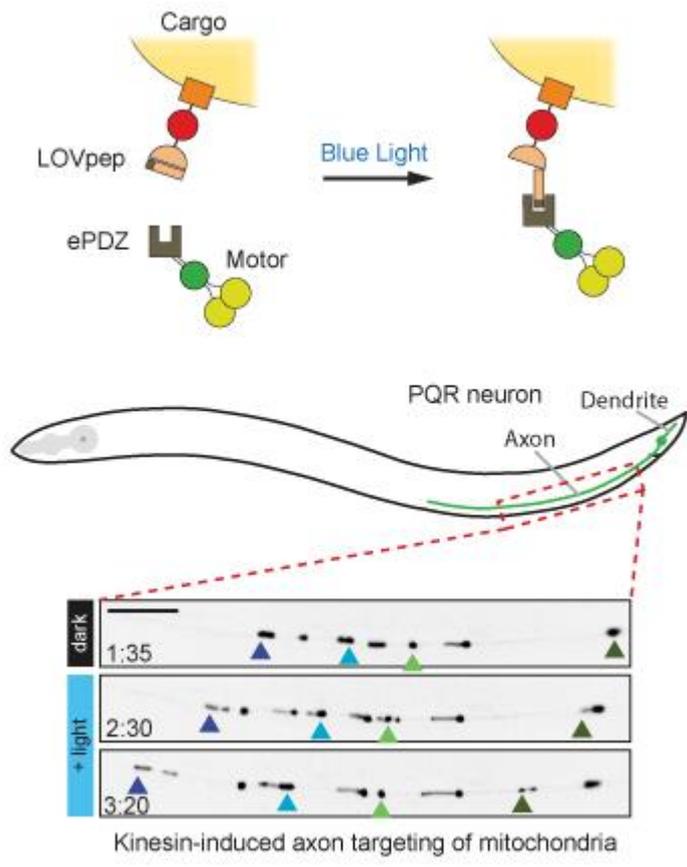
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To establish and maintain their complex morphology and function, neurons and other polarized cells exploit cytoskeletal motor proteins to distribute cargoes to specific compartments. Recent studies in cultured cells have used inducible motor protein recruitment to explore how different motors contribute to polarized transport and to control the subcellular positioning of organelles. To extend these studies *in vivo* we adapted these for use in the nematode, *C. elegans*. Since it has relatively simple neurons which develop in a highly reproducible fashion it is an ideal model to study the basics of polarized transport *in vivo*.

Previously drug induced motor recruitment was used, however this system misses (sub-)cellular specificity and due to drug penetration problems would be hard to use *in C. elegans*. Therefore we switched to an optogenetic approach using the TULIPs heterodimerization system (Strickland et al., 2012). This system is based on a synthetic interaction between the photosensitive LOV2 domain from *Avena sativa* phototropin 1 and an engineered PDZ domain. Upon blue light exposure we are able to couple mitochondria to kinesins or dynein to induce their transport into axons and dendrites respectively, highlighting the basic trafficking rules that govern polarized sorting in neurons. We will present preliminary results to highlight how the TULIP system can be further exploited to modulate intracellular transport in a controlled manner.



Endophilin-A stimulates priming of secretory vesicles

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The endophilins-A constitute a family of evolutionarily conserved endocytic adaptors with membrane curvature-sensing and curvature-inducing properties. Given that endophilin-A has also been found on secretory vesicles, we aimed to inspect whether endophilin-A has a role in exocytosis, and we used adrenal chromaffin cell as a model system.

Secretion in endophilin A1-A3 triple knock out (TKO) chromaffin cells was studied by fast capacitance measurements. Simultaneously, the catecholamine release was quantified using amperometry. The ultrastructure of chromaffin cells was checked by confocal and electron microscopy.

We found that the secretory vesicle priming is impaired in the chromaffin cells without endophilins-A, although the number of secretory vesicles is not altered, as seen by two independent morphological analysis (electron microscopy and immunostaining). Expression of endophilins A1 (neurons-specific) and A2 (ubiquitous) in endophilin TKO chromaffin cells can rescue exocytosis by stimulating priming. The stimulation-of-priming effect is dependent on SH3 domain of endophilin-A, since expression of BAR-domain only constructs was not able to rescue the reduced burst size in chromaffin cells without endophilins. Most interestingly, endophilin with two point mutations that disrupt endophilin-intersectin interaction was not able to rescue the secretion.

We report that in addition to its well established role in endocytosis, endophilin-A also plays a role in exocytosis of secretory vesicles.

The ADP-sensitive P2Y₁₃ receptor attenuates progenitor cell proliferation, new neuron formation, and neuronal activity in the dentate gyrus of adult mice

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The adult mammalian brain retains the capacity for lifelong de novo generation of neurons from stem cells within defined neurogenic niches. A multitude of hormones, growth factors, cytokines, and transcription factors govern adult hippocampal neurogenesis. Extracellular purine and pyrimidine nucleotides are involved in the control of both embryonic and adult neurogenesis¹. Nucleotides are released from various cell types in the nervous system and act via purinergic receptors including ionotropic P2X or metabotropic P2Y and P1 receptors. Studies of the adult subventricular zone^{2,3} and the dentate gyrus⁴ provide strong evidence that ATP promotes progenitor cell proliferation in these stem cell rich regions. We have previously shown that the extracellular nucleotide-hydrolyzing enzyme NTPDase2, catalyzing the dephosphorylation of extracellular ATP to ADP and modulating extracellular nucleotide ligand availability and purinergic signaling, is highly expressed by adult neural stem and progenitor cells within both neurogenic niches⁵. Its deletion increases progenitor cell proliferation and expansion of the hippocampal stem and progenitor cell pool in situ. In situ hybridization data allocates high expression levels of the ADP-sensitive P2Y₁₃ receptor to cell populations within the dentate gyrus. Here we report that deletion of the receptor increased progenitor cell proliferation and long-term progeny survival as well as new neuron formation in the dorsal but not in the ventral dentate gyrus. Increased neuron formation was further paralleled by thickening of the granule cell layer, increased CREB phosphorylation in immature neurons, and enriched expression of the neuronal activity marker c-Fos. Although neurogenesis declines during aging, increased progenitor cell proliferation and survival persisted in aged *P2ry13* knockout animals.

We suggest that extracellular nucleotide signaling plays a pivotal role in the control of hippocampal neurogenesis in the adult rodent brain. Activation of the P2Y₁₃ receptor reduces progenitor cell proliferation, new neuron formation, and neuronal activity in the hippocampal neurogenic niche.

¹Zimmermann H (2011) Semin Cell Dev Biol 22: 194–204

²Suyama S, Sunabori T et al (2012) J Neurosci 32: 9238-9247

³Boccazzi M, Rolando C et al (2014) Glia 6: 428-439

⁴Cao X, Li LP et al (2013) Stem Cells 31: 1633–1643

⁵Gampe K, Stefani J et al (2015) Stem Cells 33: 253-264

Symposium

S4: Neuronal circuit wiring in development

[S4-1](#) Simple Rules in Brain Wiring: A Fly Perspective
Peter Robin Hiesinger

[S4-2](#) Characterization of spinal cord motor circuitry
Samuel Lawrence Pfaff

[S4-3](#) Necortical circuits: how do we build them in development?
Victor Tarabykin

[S4-4](#) Regulation of myelination as part of neuronal circuit development
Michael Wegner

[S4-5](#) Disruption of mouse *Mtss1* causes abnormal ciliary patterning and congenital hydrocephalus
Barbara Wieners, Maksym Vasyukov, Vera C. Keil, Stephan L. Baader, Gregor Kirfel, Elke Hattingen, Hans H. Schild, Karl Schilling, Britta Eiberger

[S4-6](#) Semaphorin7a rescues migration and axon growth in *Satb2* deficient neurons
Paraskevi Bessa, Victor Tarabykin

Simple Rules in Brain Wiring: A Fly Perspective

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In this symposium presentation, I will offer a view through the fly's eye at design principles that ensure robust brain wiring.

Characterization of spinal cord motor circuitry

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Even basic motor behaviors, such as walking or withdrawing the limb from a painful stimulus, rely upon integrative multimodal sensory circuitry to generate appropriate muscle activation patterns. Classical studies of movement and the influence of sensory stimuli have found that the spinal cord contains circuitry for generating motor commands that are modulated by sensory information from pain, touch, and muscle (proprioception) to produce limb movements that are appropriate for the environmental conditions. However, both the cellular components and the molecular mechanisms that instruct the assembly of the integrative circuit nodes for motor commands are poorly understood. Here we describe a discrete sub-population of inhibitory spinal relay neurons transiently marked by *Satb2* (ISRSatb2) that receive inputs from multiple streams of sensory information and relay their outputs to motor command layers of the spinal cord. *Satb2* encodes a special AT-rich sequence binding protein that is mutated in Glass Syndrome, a human genetic disorder associated with intellectual disability. Targeted deletion of *Satb2* from developing ISRSatb2 cells perturbs their position, molecular profile, and pre- and post-synaptic connectivity. Accordingly, the influence of painful mechanical, chemical, and thermal signals on limb positioning is perturbed in *Satb2* mutants. Our findings indicate that *Satb2* is necessary for establishing sensorimotor circuitry that governs how multimodal sensory commands influence motor behaviors.

Necortical circuits: how do we build them in development?

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Cortico-cortical connections are comprised of the commissural axons that connect one half of the neocortex with the other half, hence crossing the midline. It includes the Corpus Callosum (CC) and the Anterior Commissure (AC). In the mouse, mainly axons from layer II and layer III and to a lesser extent layer V neurons project through the commissural path. Abnormal development of the Corpus Callosum can cause a wide spectrum of cognitive impairments in humans. We are interested in molecular mechanisms underlying development of cortico-cortical connections. There are three distinct steps in callosal development. First, cortical axons have to make a decision whether to grow medially and from Corpus Callosum or to travel laterally and form cortico-subcortical tracts. Second, they have to reach the midline and do not re-enter the cortical plate. Finally, callosal axons have to cross the midline and reach their target region. Here we describe the role of NeuroD 2/6 transcription factors and their targets in the development of the Corpus Callosum. Without Neurod2 and Neurod6, callosal axons fail to reach the midline. We identify EphrinA4 as a major transcriptional target of Neurod2/6 that is required for Corpus Callosum development. We also provide evidence that EfnA4 forms co-receptor complexes with TrkB (Ntrk2) to activate AKT signaling.

Regulation of myelination as part of neuronal circuit development

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Myelinating glia are an integral part of neural circuits. As a consequence, their development and differentiation has to be tightly controlled and coordinated on multiple regulatory levels. To ensure proper stage-specific gene-expression and lineage progression, a regulatory network has to be established that is both sufficiently stable and at the same time amenable to defined transitions. This is achieved by sets of interacting transcription factors in combination with epigenetic machinery and microRNAs. A prominent transcription factor in myelinating glia of the central and peripheral nervous systems is the HMG-box containing Sox10. By analyzing its mode of action on the molecular level (including functional redundancy, cross-regulation, antagonism and cooperativity with other transcription factors, interaction with chromatin remodeling machinery, crosstalk with microRNAs), several modules of the glial regulatory network will be delineated and interpreted in the context of glial development and myelination.

Disruption of mouse *Mtss1* causes abnormal ciliary patterning and congenital hydrocephalus

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Congenital hydrocephalus, the accumulation of excess cerebrospinal fluid (CSF) in the ventricles of the brain, affects one in every 1000 children born today, making it one of the most common human developmental disorders. Hydrocephalus is classified as either communicating, in which there is no physical blockage to CSF flow, or noncommunicating, in which CSF flow is blocked, usually at the aqueduct of Sylvius. Beating of motile cilia on the ependymal lining of the ventricles is thought to facilitate intraventricular CSF circulation, as well as increase laminar flow across the ependymal surface. Animal models have implicated damage to or loss of the ependymal layer, reduction in number or loss of its cilia, impaired ependymal cilia motility, and aqueduct stenosis in the development of hydrocephalus, suggesting a broad genetic program underlying the regulation of CSF balance.

Mtss1, a multifunctional protein acting at the interface between cell membranes and the actin cytoskeleton, has also been shown to be associated with basal bodies of primary cilia. In this study, histological and MRI-based analyses of *Mtss1* deficient mice revealed dilated lateral ventricles and an open aqueduct indicative of communicating hydrocephalus. Ependymal flow analysis using fluorescent microbeads on live preparations of the lateral ventricle walls showed decreased speed and directionality of fluid transport in *Mtss1* null vs. wildtype mice. Furthermore, morphological abnormalities of basal bodies and cilia were visible in *Mtss1* deficient primary ependymal cell cultures and wholmount preparations of lateral ventricle walls.

Together, our results provide a mechanistic perspective on *Mtss1* function for ependymal cilia patterning and present a so far unknown candidate involved in the development of congenital hydrocephalus.

Semaphorin7a rescues migration and axon growth in Satb2 deficient neurons

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Satb2 is an important transcription factor that regulates several aspects of embryonic development. Mutations in the human SATB2 gene correspond with intellectual disability and delayed speech development, facial dysmorphism and Rett-syndrome-like phenotypes in children. In the embryonic mouse brain, homozygous loss of the Satb2 gene results in agenesis of the Corpus Callosum (CC), the largest fiber tract of the brain that facilitates transmission of information between the two hemispheres. Upon targeted deletion of Satb2 from post-mitotic cortical neurons, we found that the Upper Layer (UL) neocortical projection neurons require Satb2 cell-autonomously to migrate and to extend axons to the contralateral hemisphere. A large screen of axon guidance molecules revealed that Semaphorin 7A can rescue cell migration as well as the extension and interhemispheric projection of callosal axons due to a mechanism that appears to occur mainly in cis. Here we examine the spatiotemporal expression of this guidance molecule, in addition to probing its mechanism of action that allows for cell-autonomous rescue of the corpus callosum in Satb2 deficient neurons.

Symposium

S5: Trends in small-animal neuroimaging: assessing functional connectivity of the whole brain

- [S5-1](#) Checking plasticity: Functional connectivity imaging of the brain
Mathias Hoehn, Claudia Green, Anuka Minassian, Adrien Riou, Michael Diedenhofen, Dirk Wiedermann
- [S5-2](#) fMRI of the mouse brain - pseudostatic and dynamic functional networks under physiological and pathological conditions
David Bühlmann, Aileen Schroeter, Joanes Grandjean
- [S5-3](#) Small-animal SPECT in neuroscience - principles and applications
Jürgen Goldschmidt
- [S5-4](#) Translational fMRI from mouse to man: validation of antinociceptive drug therapy in dynamic functional brain networks
Andreas Hess
- [S5-5](#) Understanding microglia activity in the stroked brain using in vivo imaging
Franziska Melanie Collmann, Rory Pijnenburg, Cordula Schäfer, Gabriele Schneider, Somayyeh Hamzei Taj, Andreas Beyrau, Markus Aswendt, Kathryn Folz-Donahue, Christian Kukat, Mathias Hoehn
- [S5-6](#) Food as a Modulator of Functional Connectivity in Rodents and Humans.
Andrea Mendez Torrijos, Silke Kreitz, Laura Konerth, Stefanie Horndasch, Monika Pischetsrieder, Arnd Dörfler, Andy Hess

Checking plasticity: Functional connectivity imaging of the brain

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Introduction: The understanding of cerebral diseases has moved from the focal attention on the lesion itself to more global concepts of far ranging effects in neuronal networks during the past years. Invasive approaches are either limited by a few local recordings such as electrophysiology or permit only a description of a single time point because of invasiveness of the method. Modern noninvasive molecular imaging modalities recently have opened new doors to generate temporal profiles of dynamic processes relating to both structural and functional connectivity networks in the brain. Animal studies in rodent models can now be investigated with impressive sensitivity and resolution, thus unravelling mechanisms and interactions between various components such as different cell types during lesion development and (therapeutically intervened) outcome.

In the present contribution we discuss the functional network changes during stroke, analyzed with high field resting state fMRI in the mouse brain. The role of purely cortical ischemic lesions and of cortico-striatal lesion is compared for the derangement of the functional network, using the sensorimotor network as a suitable read-out which can be correlated with behavioral monitoring of functional deficit and improvement. Stem cells have become a therapeutically attractive approach for the treatment of cerebral diseases. With molecular imaging of gene expression reporters, the neuronal differentiation is followed after stem cell grafting into the rodent brain, thus providing for the first time the direct temporal correlation between neuronal differentiation stages and behavioral improvement after grafting. The effect of the stem cell treatment on the functional network is critically assessed with functional connectivity imaging.

With this approach, the modulatory role of stem cell grafts on the functional connectivity network can be unraveled. Further, we will present data indicating the role of endogenous neurogenesis upregulation for the spontaneous response to stroke but also discuss a novel role of neurogenesis for the stabilization of the functional connectivity homeostasis.

fMRI of the mouse brain - pseudostatic and dynamic functional networks under physiological and pathological conditions

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Functional magnetic resonance imaging (fMRI) in rodents is attractive in many regards. Mechanistic information on the link of the hemodynamic response to the underlying neural activity can be obtained by combining fMRI with established invasive readouts of neuronal function. Use of genetically engineered mouse lines allows assessing the impact of specific molecular entities involved in signal processing. The relatively simple (cortical) morphology enables detailed analyses of the functional topology and its rearrangements following focal CNS injury. Modern fMRI techniques allow for full three-dimensional coverage of the brain essential for the elucidation of large scale networks involved in specific task, pharmacological activation or during rest.

Challenges in rodent fMRI are linked to the small dimensions and correspondingly the high demands on spatial resolution, to the animal physiology, which should be stable enough to allow for detection of percent changes in signal intensity, and to potential interference by anesthesia. Yet, technical solutions are available and rodent (mouse) fMRI is becoming a commodity.

Different aspects of mouse fMRI will be addressed: 1) Mechanistic information on the neurovascular coupling obtained by combined fMRI/fiber-optics measurement of the bulk Ca signal using a fluorescent ligand illustrating a potential role of glia in determining the vascular signal has been implicated, 2) structural and functional connectivity in mouse brain, 3) dynamic aspects of functional connectivity, 4) and assessing alterations in connectivity under pathological conditions such as a mouse model of cerebral amyloidosis mimicking aspects of Alzheimer's disease, model of chronic psychosocial stress (CPS) in mice and alterations in serotonergic signaling in a mouse model of early life stress.

Small-animal SPECT in neuroscience - principles and applications

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Single-photon emission computed tomography (SPECT) is an in vivo imaging modality that provides 3d images of the body or brain distribution of suitable, gamma-emitting radionuclides or molecules labeled with these radionuclides. In small animals the maximum spatial resolution of SPECT-imaging is higher than that of positron emission tomography (PET) - imaging.

Following a short introduction to the principles underlying small-animal SPECT this presentation will focus on imaging rodent brain activation patterns using regional cerebral blood flow (rCBF) as a proxy.

Data will be presented illustrating how SPECT can be used to obtain images from global, brain-wide spatial patterns of neural activity in awake behaving rodents.

Unrestrained animals are continuously intravenously injected, during a time span of a few minutes of ongoing stimulation outside the scanner, with the blood-flow tracer ^{99m}Tc-HMPAO. After flow-dependent wash-in into the brain the tracer is converted to a hydrophilic compound that remains trapped in the brain and shows no redistribution. The tracer distribution, representing the average blood flow during stimulation, can be mapped in anesthetized animals after stimulation.

Images will be shown from activity-dependent changes in rCBF in awake behaving animals upon optogenetic stimulation and in different behavioral settings.

Changes in rCBF can also be used to image pathological alterations in rodent brains. In addition, using the lipophilic thallium (Tl⁺) compound ²⁰¹TlDDC it is also possible to map changes in regional cerebral potassium metabolism. It will be shown how dynamic SPECT-imaging of the uptake and redistribution of the K⁺-probe Tl⁺ in the brain can be used for monitoring disease progression in neurodegenerative diseases.

Translational fMRI from mouse to man: validation of antinociceptive drug therapy in dynamic functional brain networks

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Pain is the key symptom in patients with inflammatory diseases like rheumatoid arthritis (RA) or Crohn's disease (CD). Pain is reflected in the central nervous system by a complex activity of different brain structures which led to the term "pain matrix" which can be objectively measured using BOLD fMRI. It is well known, that TNF-alpha is the primary molecule in pathological processes of RA and CD and TNF-alpha overexpression leads to increased pain sensitivity, called hyperalgesia. Several anti-bodies against TNF are already clinically approved and highly beneficial for patients. However, it remains unclear which patient benefits from which anti-TNF. Major advancement in understanding how nociceptive signals are processed at the level of the CNS processes was provided by functional MRI studies in animals and humans over the last decade. Aimed at a deeper mechanistic insight and investigation of new treatment options, here particular anti-TNF treatments, animal experiments play a major role because mice allow for specific genetic modifications affecting molecular key players like TNF. We therefore adapted functional BOLD MRI imaging to mice overexpressing human TNF (hTNFtg) allowing us to measure 3D brain activity maps of heat-induced nociception in this model of rheumatoid arthritis (1). We first could demonstrate the pathophysiological changes going along with the chronic pain in hTNFtg mice compared to wild-type mice and second how successful treatment with anti-TNF Infliximab results in unexpected fast changes in brain activity. Graph theoretical connectivity analysis data showed rewiring within the pain matrix under chronic pain conditions i.e. tight clustering of brain activity particular between thalamus and periaqueductal grey. Neutralization TNF by antibodies reversed rapidly this hyper-nociception. This was reflected by an overall decrease of the functional activity in the brain pain matrix and by dissociation of the tight clustering. These dynamic changes in the brain happened long before anti-inflammatory effects were evident. Due to the non-invasiveness of BOLD fMRI we were able to translate these findings to the human brain of rheumatoid and Crohn's disease patients. For both diseases after treatment with an anti-TNF drug a reduction of the hypernociception (reduced activated brain volume) in brain areas activated by the painful stimulation was found (2,3). For RA and CD this happened as fast as 24 h after the first drug application which is weeks before any established clinical score indicates an improvement. Most intriguingly based on the BOLD results therapy responders and non-responders, again for both diseases could retrospectively be differentiated. These results suggest 1) profound functional connectivity changes of pain processing in the brain for long lasting inflammatory diseases like RA or MC, which 2) normalizes upon hTNF blockade very fast and 3) are indicative of therapy success by clearly separating responders from non-responders and 4) will strongly contribute to improve treatment protocols for the patients.

References:

- 1) Hess, A. et al. Proc Natl Acad Sci U S A. (2011); 108, 3731-3736
- 2) Hess, A. et al. Gastroenterology. 2015;149(4):864-6
- 3) Rech, J. et al. Arthritis Rheum. (2013); 65, 325-333

Understanding microglia activity in the stroked brain using in vivo imaging

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Introduction

Microglia/macrophages switch from an anti-inflammatory (M2-like) phenotype in the early onset towards a more pronounced pro-inflammatory (M1-like) phenotype during stroke development.¹ Here, we aim to unravel the temporal profile of this polarisation shift in vivo using Bioluminescence imaging (BLI).

Methods

We generated lentiviral (LV) particles with the optical imaging reporters Luc2 (bioluminescence) and eGFP (fluorescence) under control of cell-specific promoters representing M1- (iNOS, Fcgr3) or M2-skewed (Ym1) microglia. For in vitro validation of promoter specificity and reporter reliability, a microglia cell line (BV-2) was transduced with the LV particles and then shifted into M1- or M2-skewed state: Cells were sorted using a BD FACSAria, and reporter protein expressions studied after LPS+IFN- γ ; or IL-4 stimulation using a plate reader.

For the in vivo application, we injected 2 μ l of concentrated LV-EF1 α -Luc2-T2A-eGFP (n=8) into the striatum of nude mice. After assessment of stable Luc2 expression, we injected 2 μ l of LV-iNOS-Luc2-T2A-eGFP (n=6) and LV-Ym1-Luc2-T2A-eGFP (n=12), and induced stroke by middle cerebral artery occlusion (MCAO) 21 days (d) later. Magnetic resonance imaging (MRI) allowed monitoring of lesion progression. We followed the BLI signal up to 14 d (iNOS) or 21 d (Ym1) post stroke (dps) using a Perkin Elmer IVIS Spectrum CT system.

Results

In vitro studies showed successful monitoring of microglia cell line response to stimuli by up- and down-regulating M1- and M2- reporter expression. M1-like iNOS promoter activity was up-regulated by LPS+IFN- γ ; , while M2-like Ym1 promoter activity was activated by IL-4 stimulation but down-regulated by LPS+IFN- γ ; at 24 hours.

After in vivo transduction, the BLI signal continuously increased and reached steady state at 14 d.

Based on T2-weighted MRI, 3 out of 5 LV-iNOS injected and 5 out of 7 LV-Ym1 injected animals revealed a cortico-striatal lesion.

LV-iNOS injected animals showed a diverse signal distribution after MCAO: BLI signal peaked at 3 d in two animals, while the third animal revealed a signal increase until d 11. In one of the animals with an early peak however, the signal steadily increased even before MCAO.

Interestingly, for the Ym1 promoter, we observed a steady signal increase for more than 8 dps. Further, a lack of a cortico-striatal lesion positively correlated with a lacking BLI signal increase.

Conclusions

Polarisation for anti- and pro-inflammatory phenotypes of microglia is appropriately monitored with selected markers (iNOS, Fcgr3, Ym1). The imaging reporters (Luc2; eGFP) sensitively reflect the

stimulation condition. Thus, a reliable strategy for in vivo monitoring is achieved.

BLI signal reached steady state 14 d after LV injection, which remained pronounced 35 d after injection. Thus, monitoring of promoter activity after MCAO covering the relevant time window of acute inflammatory response is assured.

M1-like phenotype: The signal in stroked LV-iNOS injected animals is likely contaminated by iNOS expression in other cell types such as neurons and astrocytes, diluting the expected effect.

M2-like phenotype: In LV-Ym1 injected animals with cortico-striatal lesions, BLI signal increased up to at least 8 d, which is contrary to previous post mortem reports. This observation shows the importance of in vivo studies covering the acute time window.

Brains of LV-iNOS and LV-Ym1 injected animals are prepared for histology at 14 d and 21 dps, respectively.

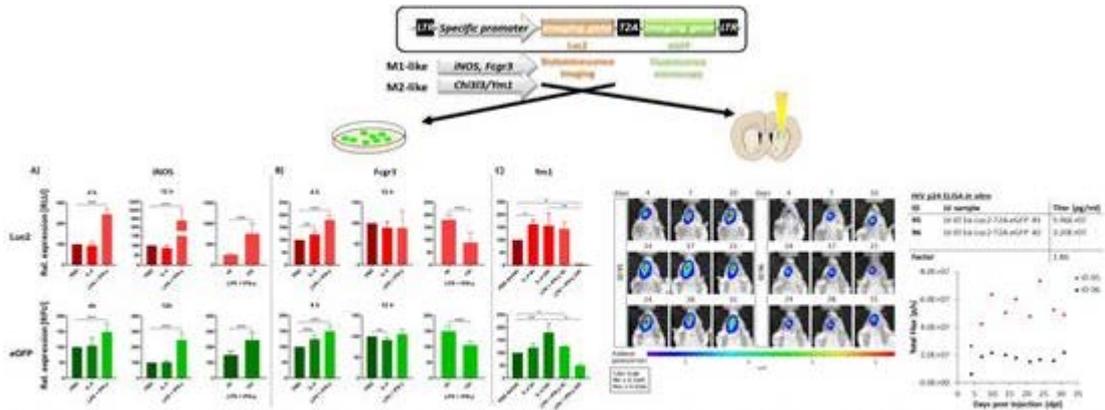


Figure 1. Strategy of monitoring the temporal profile of microglia polarization. The schematic on top shows the expression constructs, with reporter proteins Luc2 and eGFP under control of M1- and M2-like cell-specific promoters, which were packed into lentiviral (LV) particles. For in vitro validation, BV-2 microglia cells were transduced with LV particles containing the highlighted promoters and stimulated (4h). A, B) M1-like iNOS and FcγR3 promoters were activated by LPS + IFNγ with a significant increase after 4 hours (4h) and 12h, and after 4h, respectively (n = 6 wells; 3 independent experiments). C) M2-like Ym1 promoter was activated by LPS + IFNγ at 24h (n = 3 wells). Luc2 and eGFP levels were measured using a Mithras[®] LB943 plate reader. Second, LV particles were injected into the striatum of mice in order to follow promoter activity over time by BLI (right). BLI signal was followed over 32 days and reached steady state after 14 days. For each time point, maximum (max) photon emission per second (pps) is shown. The injected liver correlates with the signal intensity. Abbreviations: BLI, bioluminescence imaging; ChREBP/Ym1, Chinese 3-like 3, FcγR3, Fc gamma receptor 3; iNOS, induced nitric oxide synthase; Luc2, luciferase2.

Food as a Modulator of Functional Connectivity in Rodents and Humans.

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Non-homeostatic hyperphagia is a major contributor to obesity's hyperalimentation. Excessive intake is associated with the diet's molecular composition, for example, the energy content. As a result, specific food items such as snack food may induce food intake independently from the state of satiety. To elucidate the mechanisms of how snack foods may induce non-homeostatic food intake, we tested using a rat animal model, whether there is a tendency to consume snack foods such as chips vs. standard chow while additionally controlling the fat/carbohydrate content. Manganese-enhanced magnetic resonance imaging (MEMRI) was used for mapping the whole brain activity related standard chow and chips intake over one week. Results showed that the intake of potato chips manganese accumulation increased in certain areas related to the reward system as well as to locomotor activity indicating higher neural activity in these areas[1]. Furthermore a specific mixture of 35% fat and 50% carbohydrate in food but not the pure energy content leads to this hedonic hyperphagia[2].

These results on rats cannot be directly translated to humans as MEMRI is toxic. For this reason, Resting State functional Magnetic Resonance Imaging (RS-fMRI) was considered as an interesting alternative, as it has been proven that resting state networks can individually adapt to experience after short time exposures to a stimulus. Seventeen healthy human subjects underwent two different fMRI sessions where an initial resting state scan was acquired, followed by visual presentation of different chips and zucchini images. There was then a 5 minutes pause to ingest food (Day 1=Chips, Day 3=Zucchini), followed by a second resting state scan.

Human data analysis relied on Graph theory [3] and network based statistic (NBS)[4]. NBS evidenced chips consumption induces dynamic modulation of resting state connectivity, this is characterized by significant changes in connectivity strength (decreased connectivity patterns for frontal and visual areas in comparison to zucchini, indicating less efficient information flow)(Fig 1a) and node degree (Fig 1b). Additional graph theoretical parameters: small world were not found to be significantly depending on the ingested food. Nevertheless, we found a tendency of reduced small world index for chips in contrast to zucchini. The hub score showed higher connectivity in the frontal and secondary somatosensory cortex, middle temporal gyrus and amygdala after zucchini's ingestion (ANOVA, $p=0.007$).

Our human study was able to show specific activation and deactivation patterns of numerous brain structures dependent on the ingested food. Some of the human findings, such as nucleus accumbens connectivity changes (Fig 1b) can be compared to the reward system changes found in the rodent studies. However, some human connectivity differences such as in the visual cortex cannot be found in the rat study, as it had no visual stimulation phase. Furthermore, our results in humans involve higher

order brain functions such as semantic reasoning, which are not present in rats. One single exposure to chips in humans might not be sufficient to generate the same brain changes found in rats exposed to chips for a week. Overall, we propose our human resting state fMRI study design as a way to translate results from rats MEMRI studies.

Acknowledgements: Neurotrition Project by FAU Emerging Fields Initiative.

References:

1. Hoch, T., et al., Manganese-enhanced magnetic resonance imaging for mapping of whole brain activity patterns associated with the intake of snack food in ad libitum fed rats. PLoS One, 2013. 8(2): p. e55354.
2. Hoch, T., M. Pischetsrieder, and A. Hess, Snack food intake in ad libitum fed rats is triggered by the combination of fat and carbohydrates. Frontiers in psychology, 2014. 5: p. 250.
3. Watts, D.J. and S.H. Strogatz, Collective dynamics of 'small-world' networks. Nature, 1998. 393(6684): p. 440-2.
4. Zalesky, A., A. Fornito, and E.T. Bullmore, Network-based statistic: identifying differences in brain networks. Neuroimage, 2010. 53(4): p. 1197-207.

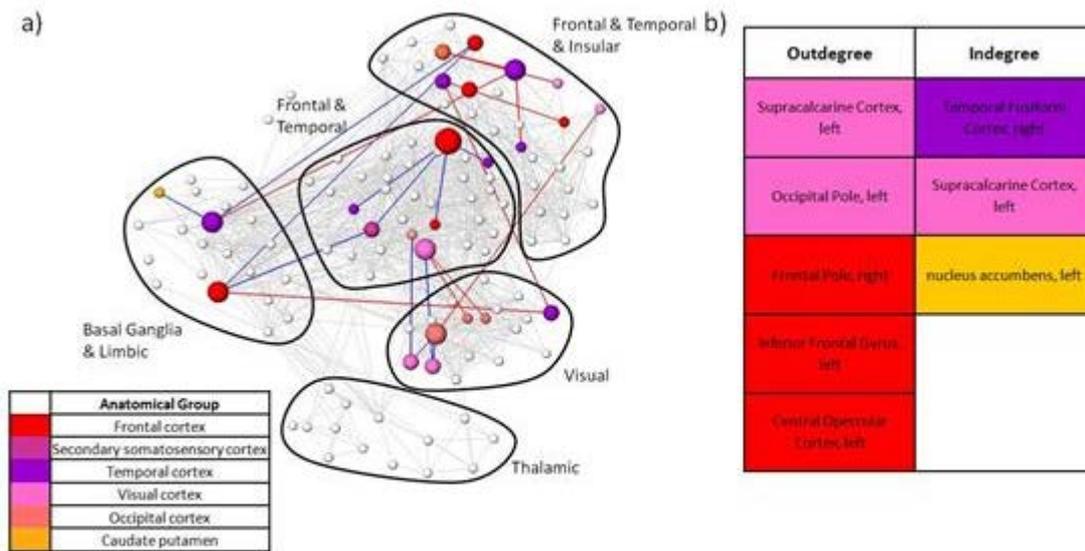


Figure a) Graph visualization of the resting state modulation of chips vs. zucchini. At the background a Kamada-Kawai plot of the Zucchini's resting state network is represented including its communities encircled. Overlaid as colored nodes and edges are significant ($p < 0.05$) different connections from chips to zucchini. Red edges represent increased connectivity for chips compared to zucchini. Blue edges represent decreased connectivity for chips compared to zucchini. Node colors correspond to the anatomical groups in the Harvard-Oxford brain atlas. Node size represents the total degree (total amount of connections that link the node to the rest of the network). The labeled communities contained more than two nodes.
 Table b) Significant different structures after paired t-test ($p < 0.05$) between chips and zucchini for in degree and out degree. Label colors correspond to the anatomical groups in the Harvard-Oxford brain atlas.

Symposium

S6: Facets of spatial information processing

[S6-1](#) Navigating over complex terrain
Kate Jeffery

[S6-2](#) Splitting and lumping: how hippocampal place cells support and constrain spatial cognition.
Emma R Wood, Roddy M Grieves, Bruce Harland, Paul A Dudchenko

[S6-3](#) Encoding of spatial and associative memories through hippocampal synaptic plasticity
Denise Manahan-Vaughan

[S6-4](#) Input-specific theta and gamma oscillations in the lateral septum regulate exploratory and goal-directed locomotion
Franziska Bender, Maria Gorbati, Li Ye, Marta Carus-Cadavieco, Natalia Denisova, Xiaojie Gao, Karl Deisseroth, Tatiana Korotkova, Alexey Ponomarenko

Navigating over complex terrain

Kate Jeffery¹

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How the brain collects and organises spatial information is a critical and not-yet-answered question. Study of the neural encoding of space has revealed several classes of neurons that handle different kinds of spatial information including direction, distance and place. However, experiments to study the properties of these neurons have mostly been conducted in simple, flat environments, whereas the real world is complex and three-dimensional. This talk will introduce some of the complexities introduced by complex terrain, and present neuronal data that shed light on how such environmental complexity may be processed.



Splitting and lumping: how hippocampal place cells support and constrain spatial cognition.

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The hippocampus plays a critical role in spatial navigation and memory. I will describe two features of hippocampal place cell activity - splitting and lumping - and discuss their possible role in supporting and constraining spatial cognition. "Splitting" refers to the observation that, when animals are performing tasks in which they are required to choose between different goal locations, many hippocampal place cells fire at different rates as the animal traverses the cell's place field on its way to different goals. This may provide a mechanism for discriminating among different spatial choices. The second feature of place cell activity - "lumping" - refers to the finding that when animals explore a multi-compartment environment comprising several visually and geometrically similar compartments, place cells fire in equivalent locations across the different compartments (termed place field repetition). I will discuss data suggesting that the ability of place cells to disambiguate among different compartments constrains the ability of animals to differentiate among the rooms at a behavioural level, and that directional information, provided by the head direction cell circuitry, is sufficient to allow disambiguation of otherwise identical compartments.

A representational-hierarchical view of pattern separation

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Pattern separation is a theoretical mechanism involving the transformation of similar inputs into outputs that are less correlated with each other. In the brain, pattern separation is hypothesised to create distinct ensemble neural responses from overlapping input. By transforming similar experiences into discrete representations, pattern separation is postulated to increase the likelihood of accurate memory encoding and subsequent retrieval, which is fundamental to successful episodic memory. In this talk I will present—and provide evidence for—a Representational-Hierarchical perspective on pattern separation, which proposes that pattern separation is not localised to the hippocampus or relevant only to memory processes, but rather a ubiquitous mechanism throughout the brain.

Input-specific theta and gamma oscillations in the lateral septum regulate exploratory and goal-directed locomotion

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Hippocampal theta (5-10 Hz) oscillations support encoding of an animal's position during spatial navigation. Cortical cognitive processing involves gamma oscillations (30-120 Hz), which support memory and attention. Yet longstanding questions about behavioral functions of network oscillations and about their coordination with subcortical regions remain unanswered. Combining optogenetic control of oscillations with electrophysiological recordings in mice, we studied causal impact of hippocampal theta oscillations on locomotion. The regularity of theta oscillations underlied more stable and slower running speeds during exploration (Bender et al., Nat. Commun., 2015). Theta oscillations were coordinated between hippocampus and its main subcortical output, the lateral septum (LS). In contrast, hippocampal gamma oscillations were only weakly coherent with intermittent gamma oscillations in the LS. On the other hand, gamma oscillations were coordinated between medial prefrontal cortex (mPFC) and LS. This coordination was accompanied by the interregional coordination of neuronal activity. To get an intact overview of mPFC projections to LS, we used CLARITY method, which revealed prominent fibers of mPFC neurons in the LS. Inhibition of hippocampus to LS pathway, using chemo- (DREADDs) or optogenetics (eNpHR3.0), revealed its necessity for the hippocampal control of running speed. Gamma-rhythmic stimulation of mPFC to LS pathway modulated goal-directed behavior, increasing performance in the T-maze. These results show that entrainment of the lateral septum by hippocampal theta and mPFC gamma oscillations regulate exploratory and goal-directed behaviors, respectively.

We gratefully acknowledge support by the DFG (Exc 257 NeuroCure, TK and AP, SPP1665, AP), The Human Frontier Science Program (HFSP; RGY0076/2012, TK, DB), and GIF (I-1326-421.13/2015, TK).

Symposium

S7: Calcium homeostasis in neuroinflammation and -degeneration: new targets for therapy of multiple sclerosis?

- [S7-1](#) Regulation of Store-operated Calcium entry (SOCE) in health and disease
Barbara Anne Niemeyer
- [S7-2](#) Source and influence of calcium entry in retinal ganglion cells during the preclinical phase of autoimmune optic neuritis
Richard Fairless, Jovana Bojcevski, Andreas Draguhn, Ricarda Diem
- [S7-3](#) Advanced intravital microscopy of calcium homeostasis and cellular interactions in the CNS: from tumors to inflammation
Frank Winkler
- [S7-4](#) Synaptic communication at photoreceptor ribbon synapses of the retina: relevance for signalling in the retina under normal and pathological conditions
Frank Schmitz
- [S7-5](#) Distinct temporal characteristics of intracellular Ca^{2+} and cAMP/PKA responses upon adrenergic stimulation in single rat astrocytes
Anemari Horvat, Robert Zorec, Nina Vardjan
- [S7-6](#) Computational modeling of Ca^{2+} signals in astrocytes
Franziska Oschmann, Klaus Obermayer

Regulation of Store-operated Calcium entry (SOCE) in health and disease

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Store-operated Ca²⁺ channels (SOCs) are found in the plasma membrane of virtually all cells and are activated through a decrease in the luminal calcium concentration ([Ca²⁺]_{ER}) within the endoplasmic reticulum (ER), which serves as a reservoir for stored calcium. SOCs are widely expressed in both excitable and non-excitable cells and generate Ca²⁺ signals important for gene expression, proliferation, and the secretion of growth factors and inflammatory mediators. STIM proteins (STIM1/STIM2) are the sensor proteins that sense the ER [Ca²⁺]_{ER} and, upon activation, cluster and activate Orai (Orai1-3) channels in the plasma membrane, thereby triggering highly Ca²⁺ selective ICRAC. ICRAC is essential to activate immune cells and its inhibition or gain-of-function can lead to immune dysfunction and a number of other pathologies.

During inflammation and neurodegeneration, cells produce a significant amount of reactive oxygen species (ROS), which by interacting with cysteine residues, can alter protein function. Pretreatment of the Ca²⁺ selective Orai1 with the oxidant H₂O₂ reduces ICRAC with C195, distant to the pore, being its major redox sensor. This inhibition is not seen in the Orai1 paralogue Orai3 and we have shown that combined assemblies of both channel types modify the overall redox sensitivity and have elucidated the molecular mechanism of inhibition. Because CNS infiltration by autoreactive CD4⁺ T cells is a hallmark of early multiple sclerosis we are investigating and characterizing SOCE of in vitro differentiated human T cell subtypes with the aim to find pharmacologically targetable differences between subtypes. Results of SOCE regulation by posttranslational modifiers, splicing and environmental factors will be discussed.

Source and influence of calcium entry in retinal ganglion cells during the preclinical phase of autoimmune optic neuritis

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Under autoimmune conditions, elevated neuronal calcium levels can mediate degeneration of both neuronal cell bodies and their axons, acting as a mediator of various insults and injuries involved in inflammatory attack. These mechanisms appear to be involved in a rat model of autoimmune optic neuritis, where retinal ganglion cells (RGCs) undergo neurodegenerative changes beginning during the early preclinical phase of the disease. We have shown that calcium elevation begins in the retina, where RGC bodies reside, before extending to the optic nerves, where their axons are located, correlating with the development of histopathological alterations. Similarly, this coincided with activation of the calcium-activated enzyme calpain, whose inhibition led to RGC protection, providing evidence that calcium-activated processes contribute to progression of neurodegeneration. In order to determine the source of this toxic calcium accumulation, we are now using a screening approach through calcium-imaging and qPCR analysis to determine the neuronal calcium channels and pumps involved in regulating calcium entry and extrusion that may become dysregulated under auto-inflammatory conditions. So far, we have seen evidence that glutamatergic receptors are involved in early RGC degeneration during the preclinical disease stage, and we are investigating the possible downstream function of the sodium-calcium exchanger. Through pharmacological targeting of these channels and pumps, we wish to further investigate potential strategies for protecting neurons from inflammation-associated death.

Advanced intravital microscopy of calcium homeostasis and cellular interactions in the CNS: from tumors to inflammation

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Intracellular calcium homeostasis, but also intercellular calcium communication is critical for normal CNS function. Recent results point towards an important role in CNS pathology, too. Furthermore, a dynamic interplay of different cells and cell types over time is crucial to understand the development, key players, and therapeutical strategies with respect to CNS diseases. In this talk, intravital microscopy methods using in vivo two-photon microscopy of the brain and retina will be explained that have increased our understanding of CNS diseases, with a focus on glioma progression and neuroinflammation. A novel animal model will be presented that allows to investigate the interplay of microglia, vascular permeability, neuronal survival, and calcium homeostasis in the live mouse retina over the course of optic neuritis during EAE.

Synaptic communication at photoreceptor ribbon synapses of the retina: relevance for signalling in the retina under normal and pathological conditions

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Synaptic communication in presynaptic terminals occurs via Ca²⁺-triggered exocytosis of neurotransmitter-containing synaptic vesicle at the active zone. Ribbon synapses are specialized chemical synapses formed by a limited subset of neurons, e.g. photoreceptors and bipolar cells of the retina, hair cells in the inner ear and cells in the pineal gland. Most remarkably, ribbon synapses are continuously active synapses that faithfully transmit a large quantity of informations over a broad range of stimulus intensities in a versatile manner. These physiological features require molecular and functional specializations of the active zone. The central structural specialization of ribbon synapses is the synaptic ribbon. The synaptic ribbon is anchored to the active zone and is associated with large numbers of release-ready vesicles. The synaptic ribbon is important for both fast and sustained exocytosis at the ribbon synapse. Main component of the synaptic ribbon is the protein RIBEYE. In the presentation, we will discuss molecular networks at the synaptic ribbon and the importance of the synaptic ribbon for signalling at photoreceptor ribbon synapses under normal and pathological conditions. Among the pathological conditions, we will discuss changes of synaptic transmission in Experimental Autoimmune Encephalitis (EAE), a model system for multiple sclerosis.

Distinct temporal characteristics of intracellular Ca²⁺ and cAMP/PKA responses upon adrenergic stimulation in single rat astrocytes

Anemari Horvat, Robert Zorec^{1,2}, Nina Vardjan^{1,2}

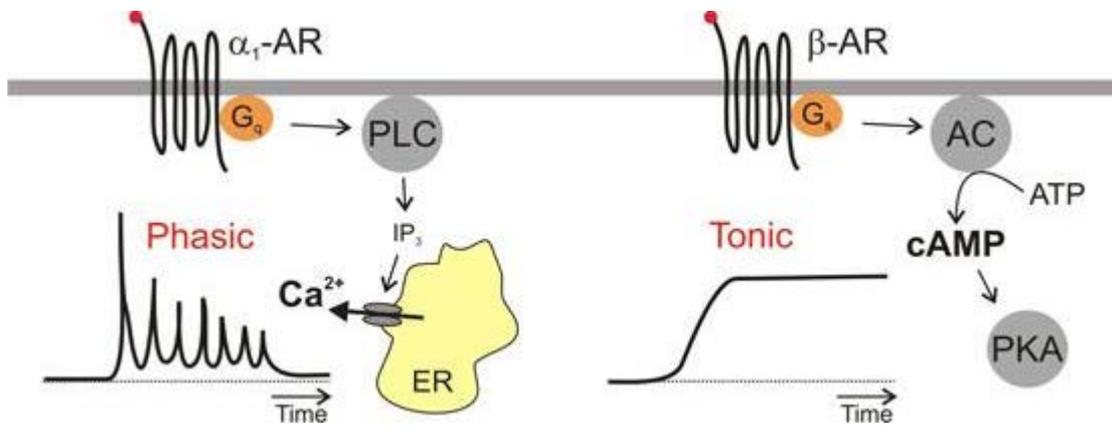
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In the central nervous system (CNS) some neurotransmitters, including noradrenaline (NA), are released from the non-synaptic terminals (varicosities), which are positioned widely in the CNS (*i.e.* volume transmission). NA is released from neurons that originate in the *locus coeruleus*, a small nucleus in the dorsal part of the brainstem, projecting into the brain and spinal cord areas bilaterally. This allows the activation of cell surface G-protein coupled adrenergic receptors (ARs) on neuronal and non-neuronal cells, including astrocytes, causing an increase in arousal, alertness, attention, and memory formation/retrieval.

Astrocytes represent a heterogeneous population of glial cells with many homeostatic functions in the CNS. They express both α - and β -ARs and thus represent an important target for NA. Although devoid of electrical excitability, astrocytes respond to NA by activation of α - and β -ARs and cytoplasmic excitability, *i.e.* changes in intracellular concentration of second messengers Ca²⁺ and cAMP, respectively. AR-activation has been shown to control various downstream cellular processes in astrocytes, including gene transcription, glucose metabolism, cell morphology, and release of gliotransmitters, all of which have distinct temporal characteristics. It is known from biochemical studies that Ca²⁺ and cAMP signals in astrocytes can interact. However it is presently unclear whether the temporal properties of the second messengers are time associated upon AR-activation.

To get a deeper insight into the dynamics of AR agonist-induced intracellular changes in Ca²⁺ and cAMP in single cultured cortical rat astrocytes, we used confocal microscopy and performed real-time monitoring of the Ca²⁺ indicator Fluo4-AM, and the fluorescence resonance energy transfer-based nanosensor A-kinase activity reporter 2 (AKAR2), which reports the activity of cAMP via its downstream effector protein kinase A (PKA). We have observed that temporal profiles of the respective secondary messenger systems are distinct in astrocytes. While the activation of α_1 -ARs by phenylephrine triggers periodic (phasic) Ca²⁺ oscillations within 10 s, the activation of β -ARs by isoprenaline leads to a ~10-fold slower tonic rise to a plateau in cAMP-dependent PKA activity devoid of oscillations. Moreover, the activation of β -ARs increases the incidence and frequency of α_1 -AR-induced Ca²⁺ oscillations and the activation of both α - and β -ARs is needed to achieve Ca²⁺ and cAMP/PKA responses in all cells. The results show that β -AR activation in cultured astrocytes potentiates the α_1 -AR induced Ca²⁺ response and *vice versa*, indicating that the pathways control and tune the activity of each other at the single cell level (Horvat et al., 2016). In this way astrocytes can optimize their key support functions during periods of increased demand, when *locus coeruleus* neurons are activated.



Computational modeling of Ca^{2+} signals in astrocytes

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Astrocytes generate Ca^{2+} signals in response to neurotransmitter (glutamate) release from neighboring synapses. Binding of glutamate to metabotropic glutamate receptors (mGluRs) induces the production of a second messenger (IP_3), which then evokes Ca^{2+} release from internal Ca^{2+} stores, the endoplasmic reticulum (ER) (mGluR-dependent pathway).

However, different experimental results showed not only a clear attenuation of Ca^{2+} signals during an inhibition of the mGluR-dependent pathway, but also during a block of the glutamate transporter (GluT). The glutamate transporter itself does not influence the intracellular Ca^{2+} concentration, but it indirectly activates Ca^{2+} entry over the membrane mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) (GluT-dependent pathway). Therefore, at least two different mechanisms contribute to the generation of astrocytic Ca^{2+} signals. A closer look into Ca^{2+} signalling in different astrocytic compartments revealed a spatial separation of those two pathways. Ca^{2+} signals in the soma are mainly evoked on the mGluR-dependent pathway, whereas in perisynaptic astrocytic processes (PAPs) most Ca^{2+} signals are evoked by Ca^{2+} entry over the plasma membrane (Srinivasan et al., 2015). This assumption is supported by the finding, that PAPs are devoid of internal Ca^{2+} stores and the volume ratio between the internal Ca^{2+} store and the intracellular space increases towards the soma (Patrushev et al., 2013).

Most mathematical models describing Ca^{2+} signals in astrocytes focus on the mGluR-dependent pathway and completely neglect the impact of the GluT and Ca^{2+} entry over the plasma membrane. To fill this gap, we extended a model for mGluR-dependent Ca^{2+} signals in astrocytes with a mechanism including the GluT. Our extension is based on the hypothesis that Na^+ entry via the GluT activates the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) in the reverse mode bringing Ca^{2+} into the intracellular space. In addition we included the volume ratio between the internal Ca^{2+} store and the intracellular compartment into the model in order to analyze Ca^{2+} signals either in the soma or in astrocytic processes.

Our model results confirm that Ca^{2+} signals in the soma mainly depend on the mGluR-dependent pathway, whereas in astrocytic compartments close to the synapse Ca^{2+} signals are evoked by Ca^{2+} entry over the membrane. The model does not only allow to study the binary Ca^{2+} response during a block of either of both pathways, but also the reduction of channel densities and their impact on the interaction of both pathways and on the Ca^{2+} signal.

Thus, the model serves as a description of a single astrocyte compartment with respect to the volume ratio between the internal Ca^{2+} store and the intracellular space, which can be extended to a multi-compartment model describing the spread of Ca^{2+} signals within a single astrocyte or a network of astrocytes.

Symposium

S8: Neuronal circuits underlying biological timekeeping

- [S8-1](#) Sea, moon and seasons: The impact of light on animal physiology and behavior
Kristin Tessmar-Raible
- [S8-2](#) Light resetting of the circadian clock of *Drosophila*
Maite Ogueta Gutierrez, Adam Bradlaugh, Edgar Buhl, James Hodge, Ralf Stanewsky
- [S8-3](#) Neural correlates of circadian behaviour in *Drosophila melanogaster*
Virginie Sabado, Ludovic Vienne, Dorian Latrasse, Emi Nagoshi
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Thordis Arnold, Andreas Arendt, Monika Stengl

Sea, moon and seasons: The impact of light on animal physiology and behavior

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The work in my lab focuses on the impact of light on animal nervous systems, the mechanisms of endogenous clocks and their evolution. These different aspects are partially interconnected as light functions as a zeitgeber for these clocks.

The marine bristle worm *Platynereis dumerilii* harbors a light-entrained circadian, as well as a monthly (circalunar) clock. In order to study the molecular and cellular nature of its circalunar clock, as well as its interaction with the circadian clock, we have established transient and stable transgenesis, inducible specific cell ablations, chemical inhibitors, as well as TALEN-mediated genome engineering. We investigated the extent of transcript change in the brain caused by the circalunar clock and compare this change to other major conditions (sex determination, maturation) occurring during the life of the worm, as well as to the known extent of transcript change caused by the circadian clock. We furthermore follow the question, how the worm's different light receptors sense solar vs. lunar light to entrain its respective circadian and circalunar clocks. For this we investigate light receptors inside and outside (non-visual) the eyes.

Non-visual photoreceptors also exist in vertebrates. In fact, light perception by cells in the inner brain of vertebrates, independent of eyes and pineal organs, was already discovered more than 100 years ago. The responsible encephalic photoreceptors have been thought to be specialized cells, similar to the photoreceptors present in the eye and pineal. Consistently, the expression of several opsins has been described at places harboring such deep brain photoreceptors, and hence these opsins were independently claimed to mediate non-visual light responses, such as seasonality. During recent years, an impressive number of non-visual opsins was identified and shown to be in principle able to function as light receptors. Their complexity is particularly high in teleost fish. In order to obtain a better understanding of this puzzling complexity, we investigate several 'non-visual' Opsins on a functional level. Our particular focus is on the TMT/Enkephalopsin group, since these Opsins exhibit a particularly slow sequence evolution and some members are conserved across all vertebrate phyla.

Light resetting of the circadian clock of *Drosophila*

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The circadian clock of *Drosophila* is well conserved and shares with the human clock the basic gene regulatory mechanisms and most of the clock genes and proteins involved. In mammals and invertebrates the circadian clocks are synchronized to environmental light:dark (LD) cycles via visual and non-visual pathways. In *Drosophila*, the blue light photoreceptor Cryptochrome (Cry), in combination with canonical rhodopsin photoreception operating in the compound eyes, are responsible for the main light-input to the circadian clock. Cry undergoes a light-induced conformational change, followed by binding to and degradation of the clock protein Timeless (Tim). Wild type flies synchronize their behavioural activity to a 12h:12h light-dark (LD) cycle and when exposed to a 6 hour phase delay of this LD cycle re-entrain to the new LD conditions after only one day (i.e. they show the same activity peaks as before the shift). In contrast, mutants lacking both Cryptochrome and the *norpA* gene (encoding Phospholipase C- ; [PLC-]) need 5 to 7 days to resynchronize showing reduced yet sustained light sensitivity of their circadian clock. Additional removal of Rh5 and Rh6 completely abolishes resynchronization of *norpA*^{P41} *cry*^b double mutants.

We found that the main rhodopsin of the compound eyes, Rh1, also contributes via the same pathway. This pathway depends on the activation of a heterotrimeric guanine nucleotide-binding protein (Gq), and induces the degradation of core clock proteins in at least a subset of clock neurons to ultimately mediate clock resetting.

Neural correlates of circadian behaviour in *Drosophila melanogaster*

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Virtually all organisms use an internal clock to tune a plethora of physiological and behavioural processes to a cycle of approximately 24h, thus allowing them to anticipate and prepare for daily and seasonal changes. The circadian rhythms of activity such as locomotion are controlled by the expression or inhibition of circadian genes through complex feedback loops. In the brain of *Drosophila melanogaster*, clusters of neurons express these circadian genes and induce rhythmic outputs. Because they are robust and easily quantifiable, circadian rhythms provide an excellent basis to study the neuronal mechanisms underlying behavioural control. The circadian circuitry is well described and each individual cluster has been linked to a specific role in controlling different behaviours. Notably, neurons producing the neuropeptide pigment-dispersing factor (PDF) are considered the master pacemaker that synchronizes circadian clocks circuit-wide and controls behavioural rhythms. However, how neuronal activity affects the molecular phase of the circadian clock and consequently locomotor behaviour has just started to be investigated.

Here we studied how the firing rate of circadian neurons affects the whole circuitry and therefore the behavioural output using time memory assay in larvae. Optogenetic tools were used to acutely inhibit or activate the neuronal activity of PDF-expressing master pacemaker neurons at different times of the day and the phase of the locomotor activity was assessed during adulthood. We observed time-of-day-dependent behavioural phase delays or advances that were differently induced by acute activation or inhibition. These results indicate that the firing rate rhythms of PDF neurons directly impact behavioural output, which is probably mediated by the time-of-day-dependent neurotransmission.

Computational modeling reveals design principles underlying *robustness and sensitivity* of the master neuronal clock in mammals

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Most physiological processes in mammals, such as blood pressure, sleep, body temperature and alertness, are regulated by the circadian clock. The **master circadian clock** consists of two bilateral nuclei located in the hypothalamus, each containing about 10000 neuronal oscillators. Each neuronal oscillator generates sloppy 24h oscillations in both gene transcription and firing on the basis of a transcriptional-translational feedback loop of certain *clock* genes. A network of neuropeptide (**VIP**, AVP), neurotransmitter (**GABA**) and gap-junction mediated interactions couple and *synchronize* the neuronal oscillators that are themselves heterogeneous in their intrinsic properties. Robustness against noise and genetic perturbation and precision of the rhythms are thus **emergent properties** of this network. Finally, the internal clock time is adapted to the external light-dark environment using light inputs from the eye delivered via the retino-hypothalamic tract in a process termed *entrainment*. The circadian clock thus provides an interesting dynamical system with an *interplay of transcriptional processes in the timescale of minutes and hours and electrophysiological processes in the ms timescale*, although the relationship between the timescales is largely unclear. The goal of my work is to identify the design principles underlying the emergent properties of this multiscale system using **computational modeling**.

It is known from the theory of coupled oscillators that network synchrony occurs when the strength of coupling agent exceeds a threshold. However, we showed that in addition to the strength, the timing (or phase) of coupling plays an equally important role in determining the synchrony and entrainment properties of the network. This led us to an important dichotomy in the clock between being robust to irrelevant environmental perturbations and being responsive to changes in the environment at the same time. While changing the strength and timing of one coupling agent does not allow control of this balance, we propose having two coupling agents, one synchronizing and one desynchronizing, *synergistically allows the clock to balance the two opposing requirements of robust rhythms and flexible entrainment*. We have identified two coupling agents, VIP and GABA, that could play this role. We combine experimental data with dynamical models to test this proposition.

Moreover, these two emergent properties are determined by the topology of the network at all timescales. However, most studies on inferring network properties have been based on bioluminescence recordings of clock gene or proteins in single cells in the network that only reflect processes at longer time scales. Therefore, we analyze firing data from multi-electrode arrays to find the nature of diffusive neuropeptide-based coupling at shorter timescales, thus, allowing us to investigate the link between firing and gene expression in clock. Simultaneously, we also quantify the characteristic firing patterns of different classes of neurons and identify firing patterns that are capable of phase shifting the circadian clock. Ultimately, being able to *manipulate the balance between synchrony and entrainment and to phase-shift the clock* would allow faster recovery from jet-lag and shift-work and combat the deterioration of rhythms with age.

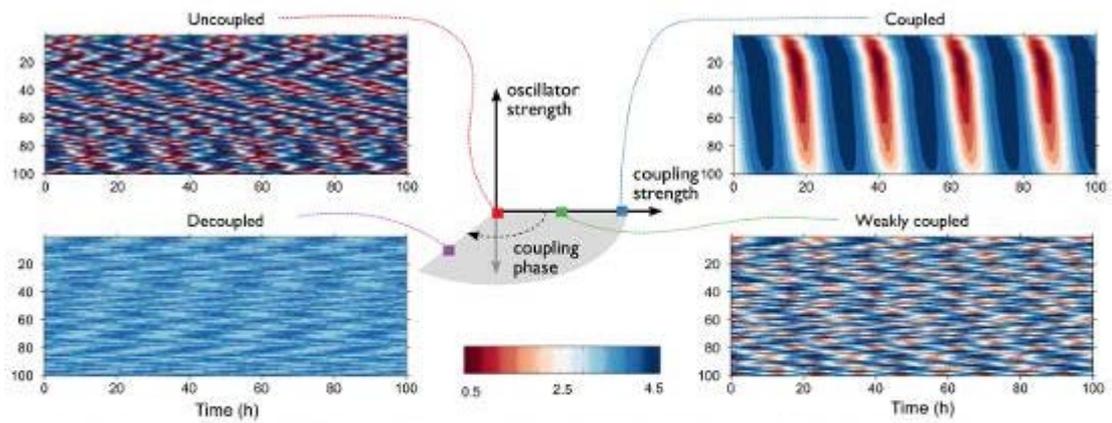


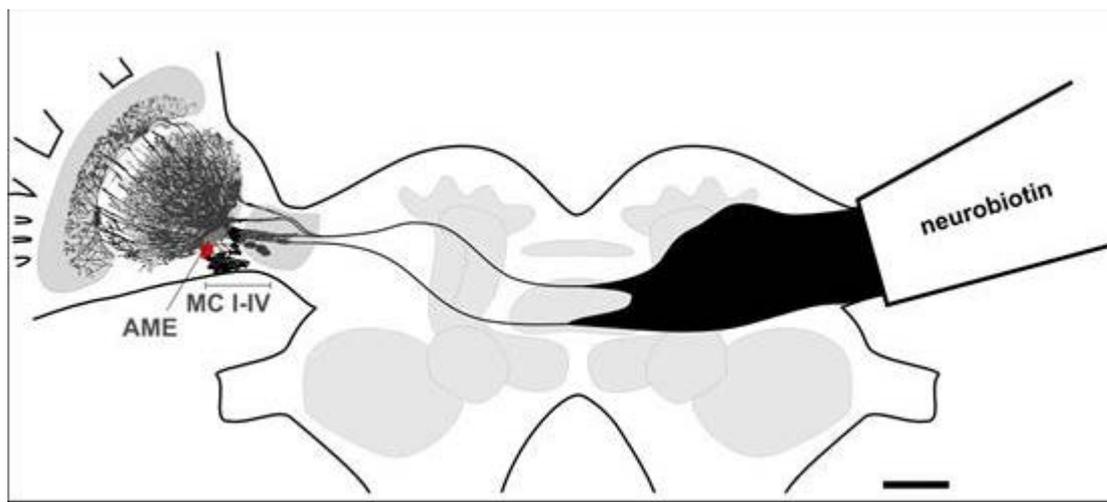
Figure: Raster plots of neurons coupled by a single hypothetical coupling agent released in a circadian manner by each neuron into the intracellular medium. The coupling in each network can be characterized by an amplitude (strength) and phase⁸ (see *middle*). Each row of the raster plot is the 'bioluminescence' trace of one neuron. Neurons are ordered in increasing order of their intrinsic periods. The network behavior was computed using delay-differential equations⁸ (not shown here).

Ipsi- and contralateral light input pathways to the circadian clock of the Madeira cockroach *Rhyparobia maderae*

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In the Madeira cockroach transplantation experiments located the circadian pacemaker center that controls locomotor activity rhythms to the accessory medulla (AME, plural AMAE), a small neuropil in the optic lobes of the brain. Eight soma groups containing ~240 neurons, which are abundant of partly colocalized neuropeptides, innervate the AME. Among them are the pigment-dispersing factor (PDF) expressing clock neurons which were suggested to take part in light entrainment pathways, local clock circuits, as well as clock outputs. While the PDF-neurons in the lamina that partly colocalize FMRFamide and 5HT were implicated in ipsilateral light entrainment, PDF neurons next to the AME might be involved in contralateral light entrainment. Previously, next to PDF, also orckinins (ORCs), and myoinhibitory peptides (MIPs) were suggested to take part in ipsi- as well as contralateral light entrainment pathways forming either light-dependent delaying- or advancing clock input pathways. Here, we concentrated on the characterization of contralateral light entrainment pathways to the circadian clock with neurobiotin backfill experiments combined with multiple-label immunocytochemistry. Previously, four contralaterally projecting soma groups MC I-IV were identified with backfills from the contralateral optic stalk (Fig. 1). Four of the PDF neurons, which arborize in the AME, medulla, and lamina, as well as in different midbrain targets, belong to MC I cells (Fig. 1, red). Here, we found that one of these contralaterally projecting PDF expressing MC I clock neurons co-expresses MIP- and ORC-, and apparently also FMRFamide. We hypothesize that the triple-labeled PDF clock neuron plays a central role in the synchronization of ipsi- and contralateral light entrainment pathways of the bilateral pacemakers. In behavioral experiments combined with RNAi-dependent knockdown of different neuropeptides/neuropeptide receptors we challenge our hypothesis. (Supported by DFG grants STE531/21-1 and STE531/25-1 to MS)



Symposium

S9: Correlating synaptic structure and plasticity at the nanoscale

[S9-1](#) Silent synaptic growth and the augmentation of LTP
Kristen Harris

[S9-2](#) Presynaptic ultrastructure-function relationships resolved by electron tomography
Cordelia Imig, Lydia Bickford, Kwun-nok M. Man, JeongSeop Rhee, Sonja M. Wojcik, Nils Brose, Benjamin H. Cooper

[S9-3](#) Ultrastructural changes in functional vesicle pools accompanying long-term potentiation in hippocampus
Kevin Staras, Stephanie Rey, Catherine Smith

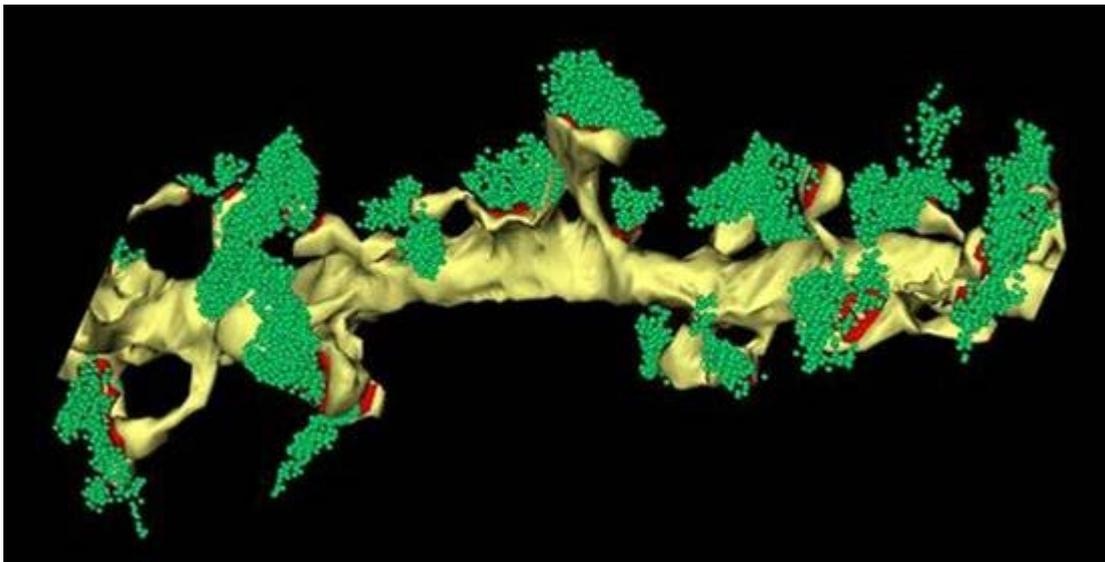
[S9-4](#) Exploring protein interactions involved in vesicle tethering to the active zone cytomatrix
Robert J. Kittel, Nicole Scholz, Nadine Ehmman, Christian Stigloher, Tobias Langenhan

Silent synaptic growth and the augmentation of LTP

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Three-dimensional reconstruction from serial section electron microscopy has revealed several of nature's synaptic secrets. When synapses share the same activation history, then their dimensions are identical in hippocampal area CA1 of the adult rat. When long-term potentiation (LTP) is fully saturated at induction, it requires more than an hour before LTP can be augmented. The induction of LTP halts ongoing spinogenesis in adults, and enlarges existing synapses, both being essentially silent processes as they are expressed after the potentiation has plateaued. Recent findings suggest that coordinated sequestering of pre and postsynaptic components could reduce spinogenesis while silent growth of the postsynaptic density prepares the synapses for subsequent potentiation. Such structural preparation may underlie latent or enhanced learning when episodes are properly spaced.



Presynaptic ultrastructure-function relationships resolved by electron tomography

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Secretory vesicle docking, priming, and Ca²⁺-triggered fusion are orchestrated by a complex and highly conserved molecular machinery. Despite of the similarities in the composition of the molecular release machinery between different neurosecretory systems and synapse types, their functional and morphological characteristics can differ dramatically, for example, with respect to the ultrastructural architecture of release sites, transmitter release properties, and short- and long-term plasticity characteristics. However, it is currently unknown whether ultrastructural differences contribute to or reflect the functional differences between distinct synapse types. The accurate assessment of vesicle docking requires electron microscopy to resolve inter-membrane distances in the nm range, but information on proteins involved in this process has been partly inconclusive. Important reasons for experimental inconsistencies are that diverse preparations, cell types, sample fixation methods, imaging approaches, and docking definitions have been employed. Combining hippocampal organotypic slice cultures from mice lacking key proteins of the presynaptic transmitter release machinery, rapid cryofixation, freeze substitution, and three-dimensional electron tomography of synapses in the CA1 region of the hippocampus, we previously dissected sequential steps in synaptic vesicle (SV) recruitment (tethering) and membrane attachment (docking). We found that SV docking requires Munc13 priming proteins, which mediate the formation of a pool of readily-releasable, fusion-competent SVs as well as presynaptic short-term plasticity processes, and all three neuronal SNAREs (Imig et al., *Neuron*, 84, 416-431, 2014). However, loss of Munc13 proteins in photoreceptor ribbon synapses of the retina or in chromaffin cells of the adrenal medulla had no effect on SV or large dense-core vesicle docking in these secretory systems (Cooper et al. *J. Neurosci.*, 32, 8040-8052, 2012; Man et al., *eLife*, 4:e10635, 2015). Our findings indicate that the molecular requirements of vesicle docking differ between synapse types and neurosecretory systems and that key functional synaptic features could indeed become manifest at the ultrastructural level.

Ultrastructural changes in functional vesicle pools accompanying long-term potentiation in hippocampus

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Presynaptic vesicle pools are attractive potential substrates to support changes in synaptic efficacy associated with forms of plasticity. Synaptic labelling techniques combined with ultrastructural methods offer powerful strategies to read out functional pool properties down to nanoscale resolution. Here, we exploit these approaches to reveal specific changes in vesicle pools that accompany long-term potentiation in acute hippocampal slices. Using FM-dye labelling and photoconversion we demonstrate significant modulation of functional pool size and spatial organization and, in particular, we find key changes in the occupancy of docked vesicle pools offering a possible structural basis for changes in synaptic efficacy. Activation of cAMP/PKA pathways mimic some of these changes, providing insights into the underlying molecular pathways.

Exploring protein interactions involved in vesicle tethering to the active zone cytomatrix

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Bruchpilot (Brp) is a core protein component of the *Drosophila* active zone (AZ) where it promotes calcium channel clustering to ensure adequate transmitter release probability. In addition, the very C-terminal region of Brp supports vesicle tethering to the AZ cytomatrix. In a C-terminally truncated allele, *brp^{nude}* (lacking the last 17 amino acids), impaired vesicle tethering is accompanied by short-term synaptic depression, impaired sustained transmitter release, and a slowed recovery phase.

We set out to test the hypothesis that neuronal expression of a peptide containing the Brp C-term would deliver a synaptic phenocopy of *brp^{nude}* by competitively binding the putative vesicular interaction partner(s) of Brp. Our electrophysiological analysis of larval neuromuscular synapses supports this hypothesis and sets the basis for a subsequent *in vivo* screen to identify the interacting protein(s). To this end, a membrane-bound Brp C-term was neuronally expressed to enrich synaptic vesicles at ectopic locations. Different RNA_i lines against vesicle-associated proteins were then screened and scored for their ability to revert the ectopic vesicle localisation.

Symposium

S10: How single neuron properties determine network dynamics

- [S10-1](#) Shaping network dynamics via single-neuron activation functions
Tatjana Tchumatchenko, Nataliya Kraynyukova
- [S10-2](#) Too hot to function properly? On the temperature dependence of network synchronization.
Susanne Schreiber
- [S10-3](#) An adaptive behavior requires a mixed network representation generated by active dendritic integration
Jeffrey C. Magee, Gayathri N. Ranganathan
- [S10-4](#) Hippocampal nonlinear dendrites, memory and high frequency oscillations
Raoul-Martin Memmesheimer, Sven Jahnke, Marc Timme
- [S10-5](#) Infralow Intrinsic Rhythmogenesis in a Subset of Mitral Cells Entrain Oscillatory Microcircuits in the Accessory Olfactory Bulb of Mice
Chryssanthi Tsitoura, Julia Mohrhardt, Kira Gerhold, Katja Watznauer, Monika Gorin, Marc Spehr
- [S10-6](#) Inferring collective network dynamics from the activity of few neurons
Jens Wilting, Viola Priesemann

Shaping network dynamics via single-neuron activation functions

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Despite recent technological advances in the development of experimental methods, understanding how connectivity and single neuron properties interact in neural circuits remains one of the major challenges in neurobiology. Neural firing rates and their dependence on external inputs can be measured in individual neurons or neural populations. However, how exactly neural firing rates are determined by recurrent interactions with local surrounding neurons and feed-forward inputs from distant upstream cortical areas is difficult to investigate with the state-of-the-art experimental methods. The inhibition stabilized network model put forward by previous studies [1-5] offers an attractive alternative to balanced networks and describes networks in which neurons have low to medium firing rates that are consistent with experimental measurements [3,6]. In such networks the firing rates of excitatory and inhibitory neurons are described by two coupled nonlinear differential equations and include a power law activation function for individual neurons. Here, we present the first complete set of closed-form solutions showing that such the single-neuron non-linearity in such networks can lead to oscillations, persistent states and bistability as well as other forms of nonlinear computation.

[1] D. Hansel, C. Van Vreeswijk. How noise contributes to contrast invariance of orientation tuning in cat visual cortex. *The Journal of neuroscience* 22(12), pp. 5118-5128, 2002.

[2] E. Persi, D. Hansel, L. Nowak, P. Barone, C. Van Vreeswijk. Power-law input-output transfer functions explain the contrast-response and tuning properties of neurons in visual cortex. *PLoS comp. biology*, 7(2), e1001078, 2011.

[3] N. J. Priebe, D. Ferster. Inhibition, spike threshold, and stimulus selectivity in primary visual cortex. *Neuron* 57(4), pp. 482-497, 2008.

[4] D. B. Rubin, S. D. Van Hooser, K. D. Miller. The stabilized supralinear network: A unifying circuit motif underlying multi-input integration in sensory cortex. *Neuron* 85, pp. 402-417, 2015.

[5] Y. Ahmadian, D. B. Rubin, K. D. Miller. Analysis of the stabilized supralinear network, *Neural Computation* 25, pp. 1994-2037, 2013.

[6] T. W. Margrie, M. Brecht, and B. Sakmann. In vivo, low-resistance, whole-cell recordings from neurons in the anaesthetized and awake mammalian brain. *Pflugers Arch*, 444(4):491-498, 2002.

Too hot to function properly? On the temperature dependence of network synchronization.

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Temperature? While being a well-known and relevant parameter for most invertebrate neurobiologists, many researchers of mammalian systems seem to get around quite well without its consideration due to the formidable abilities of their research organisms to centrally control body heat. Nevertheless, also the brain temperature of so-called homeotherms is known to vary substantially. The hippocampus of rodents, for example, warms up by 2°C when the animal explores an environment at physiological speed compared to periods of time when the animal sits still.

Temperature strongly affects ion channel kinetics and, as I will argue, consequently even small changes in temperature are sufficient to drastically modify single-neuron computation. The neurons' altered dynamics, in turn, can lead to sudden increases in excitability and synchronization when embedded in networks. Theoretical analysis allows us to understand the underlying critical transition from a mathematical perspective and to derive experimentally testable predictions. Altogether, the temperature-induced boost in network synchronization constitutes a possible mechanism for the induction of febrile seizures or hot-water epilepsy and could generalize to other pathologies, including the onset of other forms of epileptic seizures.

This work was supported by the German Federal Ministry of Education and Research (01GQ0901, 01GQ1403).

An adaptive behavior requires a mixed network representation generated by active dendritic integration

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Active sensing engages both the sensory and motor systems in concert and therefore requires a complex transformation of information from the sensory space on to motor commands and vice versa. Where and how such transformations are implemented within the mammalian nervous system remains an important area of investigation. One powerful and general solution to this problem is for the brain areas performing these computations to build a neuronal representation composed of nonlinearly mixed features that are relevant to the current environment. Although evidence for such mixed representations has been reported for several cortical brain regions, their prevalence and direct link with behavior has not been established. Furthermore the neuronal mechanisms involved in generating complex representations are unknown. Here, we examined these issues in layer 5 (L5) of mouse vibrissae cortex as it has been reported to possess many of the elements necessary to generate the appropriate network activity. These mechanisms include an active form of dendritic integration that multiplicatively combines sensory and motor inputs in the tuft dendrites of layer 5 pyramidal neurons during an active sensing task in mice. Such dendritic processing could therefore act as a nonlinear mixing mechanism capable of producing the type of network representation that is useful in guiding sensory-motor behavior. In summary, we report that a specific network representation in L5 of vibrissae cortex is used (probably by downstream regions) to mediate a sensory-driven motor adaptation of whisking. This representation is composed of individual L5 neurons that each express unique selectivity for a relatively complex feature, sensed object location. We also found that a nonlinear mixing of whisker touch and whisker angular position produces this complex feature selectivity. A diversity of such location selective neurons spread throughout the various columns of the vibrissae cortex could produce a basis network of neurons that encode sensed-object location independent of the whisker frame of reference. This type of network representation is particularly capable of supporting the coordinate transformations required for sensorymotor behaviors. In terms of anatomy, connectivity and physiology, the pyramidal neuron-based microcircuit motif is ubiquitous across the various cortical regions of the mammalian brain. In addition, there is a growing appreciation for the potential role of active dendritic processing in top-down, bottom-up input associations, various forms of learning and behavioral state modulation in a variety of cortical areas. Given this, we suggest that active dendritic integration in pyramidal neurons supports a fundamental microcircuit computation, the non-linear combination of multi-modal information that generates mixed-selectivity and high dimensional network representations. Such a general computation could allow various cortical areas to produce plastic nonlinear associations that yield easily decoded multi-sensory representations and accurate sensorymotor transformations for use in adaptive behaviors.

Hippocampal nonlinear dendrites, memory and high frequency oscillations

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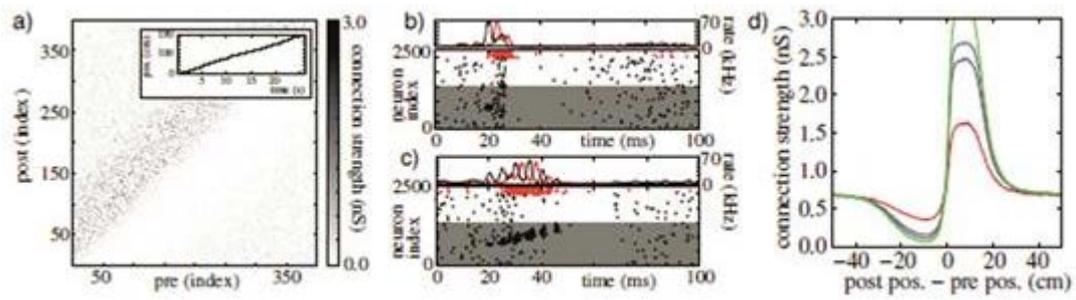
The talk will review recent theoretical insights into the impact of dendritic nonlinearities as prominently found in the hippocampus brain area, on the dynamics and memory Performance of neural networks. We have shown that the coupling nonlinearities can facilitate or enable propagation of synchronous activity and thus Information transmission, in very sparse networks containing weakly enhanced layered feedforward structures. High frequency oscillations promote the propagation. This allows for a novel self-amplifying mechanism based on highly interconnected hub-neurons. It also suggests a specific dynamical role for sharp-wave/ripple (SPW/R) complexes short episodes of increased activity with superimposed high-frequency oscillations, namely that they foster associated replay of spike sequences. I will present a unified model how experience such as traversing a trajectory in space may be stored and thereafter replayed in association with SPW/Rs. We propose that replay and SPW/Rs are tightly interconnected as they mutually generate and support each other. Further, our simulations indicate that for often assumed, standard spike-timing dependent plasticity the SPW/R and replay events may rather erase than enhance learned hippocampal network structures.

References:

- S. Jahnke, M. Timme, R.-M. Memmesheimer. A unified dynamic model for learning, replay and sharp-wave/ripples. *J. Neurosci.*, 35:16236-16258 (2015).
- S. Jahnke, R.-M. Memmesheimer, and M. Timme. Oscillation-induced signal transmission and gating in neural circuits. *PLoS Comput. Biol.*, 10:e1003940 (2014).
- S. Jahnke, R.-M. Memmesheimer, and M. Timme. Hub-activated signal transmission in complex networks. *Phys. Rev. E*, 89:030701 (2014).
- S. Jahnke, R.-M. Memmesheimer, and M. Timme. Propagating synchrony in feed-forward networks. *Front. Comput. Neurosci.*, 7:153 (2013).
- S. Jahnke, M. Timme, and R.-M. Memmesheimer. Guiding synchrony through random networks. *Phys. Rev. X*, 2:041016 (2012).

Figure 1: Model for SPW/Rs and replay. (a) Part of the coupling matrix after repeated running in a linear track (inset: single simulated run). The coupling matrix has a stripe-like structure with stronger coupling between cells with subsequent place fields. (b) Example of unstructured network recall before learning the track. Upper panel: ring rate; lower panel: spiking activity of the excitatory (black) and inhibitory (red) neuron populations. Gray shaded: Neurons with place fields on the track. (c) Same as (b) after several runs. The precise spiking activity consisting of SPW/R and replay is clearly visible. Note that the replay is

structured into pulses despite the absence of groups in the coupling matrix. (d) The structure of the coupling matrix (stronger coupling between cells with subsequent place fields) decays for increasing number of SPW/R and replay events (green: 0, blue 500, Magenta 1000, red 4000 SPW/Rs).



Infraslow Intrinsic Rhythmogenesis in a Subset of Mitral Cells Entrain Oscillatory Microcircuits in the Accessory Olfactory Bulb of Mice

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The accessory olfactory system (AOS) is a key component in rodent conspecific chemical communication. Despite its fundamental function, however, sensory coding in the accessory olfactory bulb (AOB), the first stage of information processing in the AOS, is poorly understood. Here, mitral cells (MCs) receive sensory input from peripheral vomeronasal neurons and project to the vomeronasal amygdala and the hypothalamus. Recently, we demonstrated that a subpopulation of mouse MCs is intrinsically rhythmogenic and exhibits slow stereotypical oscillatory discharge triggered by cyclic activation of three interdependent ionic conductances: subthreshold persistent Na⁺ current, R-type Ca²⁺ current, and Ca²⁺-activated big conductance K⁺ current. Here, we identify a second oscillatory MC subpopulation that is entrained by an excitatory circuit within the AOB network. Using a battery of physiological techniques in acute AOB tissue slices we show that several oscillatory MCs are often organized into synchronized microcircuits. Furthermore, entrained MCs display periodically increased excitatory synaptic input that correlates with their respective rhythmic discharge patterns. Ongoing experiments aim to identify the detailed mechanisms of oscillatory entrainment and synchronization, and the role of slow rhythmic activity in AOB information processing.

Inferring collective network dynamics from the activity of few neurons

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Collective spiking dynamics can reflect the functional properties of neuronal networks (NN). However, to date two contradictory hypotheses prevail: The first proposed an asynchronous irregular (AI) state [1], which minimizes redundancy [2] and promotes fast network responses. The second proposed a critical state [3], which is characterized by long range correlations in space and time, because it optimizes performance in tasks that profit from long reverberation of the network activity in models. Distinguishing between these two states is straight forward when the activity of all neurons is known. However under subsampling [4,5], classic approaches can mistake a network close to critical as AI: We showed that single neurons exhibit exponential inter-spike-interval distributions, and the Fano factor of single neurons is always close to unity.

Using the framework of branching networks (BN), we derived a novel estimator, which can infer the dynamical state even under strong subsampling, in principle from the activity of a single neuron (Fig. A). In this framework, the dynamical state is characterized by the number m of postsynaptic spikes triggered by one presynaptic spike on average. In contrast to previous estimators [6], the novel estimator is unbiased, i.e. it is invariant under subsampling.

We applied this novel estimator to spiking activity in monkey prefrontal cortex, cat visual cortex, and rat hippocampus. Consistently, *in vivo* dynamics is situated in a narrow regime (median $m=0.984$) between AI ($m=0$) and critical ($m=1$). A model with the same m as *in vivo* (*in vivo*-like BN) could predict both, the bin size dependent spike count cross correlations between neurons (Fig. B) and the avalanche size distributions of the *in vivo* recordings (Fig. C). The latter clearly differed from power-laws, which have been used as marker of criticality.

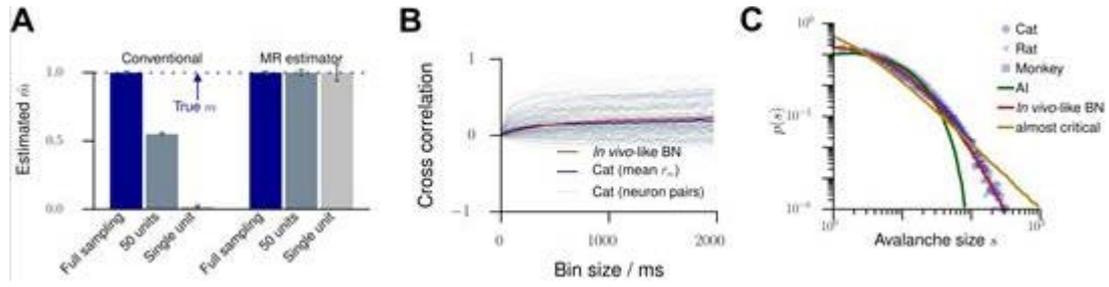
Our results offer a parsimonious solution to the competing hypotheses: activity appeared AI-like, as under subsampling correlations are underestimated. In contrast, coarse measures potentially overestimated correlations, making a network appear critical. Instead, *in vivo* spiking dynamics reflects a specific regime around $m=0.984$. This regime may combine the computational benefits of both states by allowing integration of information without the risk of instability or slowing down associated with criticality.

References

1. C. v. Vreeswijk, H. Sompolinsky, Chaos in Neuronal Networks with Balanced Excitatory and Inhibitory Activity. *Science* 80:274, 1724–1726 (1996).
2. A. Hyvärinen, E. Oja, Independent component analysis: Algorithms and applications. *Neural Networks* 13, 411–430 (2000).
3. J. M. Beggs, D. Plenz, Neuronal avalanches in neocortical circuits. *J. Neurosci.* 23, 11167–11177 (2003).
4. V. Priesemann, M. H. J. Munk, M. Wibral, Subsampling effects in neuronal avalanche distributions recorded *in vivo*. *BMC Neurosci.* 10, 40 (2009).
5. V. Priesemann et al., Spike avalanches *in vivo* suggest a driven, slightly subcritical brain state. *Front.*

Syst. Neurosci. 8, 108 (2014).

6. C. Z. Wei, J. Winnicki, Estimation of the Means in the Branching Process with Immigration. Ann. Stat. 18, 1757–1773 (1990).



Symposium

S11: How hearing happens: speed, precision and sensitivity

- [S11-1](#) Clues to the molecular identity of the hair-cell mechano-electrical transducer channel from experiments with pore blockers
Corné Kros, Laura Corns, Terri Desmonds, Nerissa Kirkwood, Stuart Johnson, Guy Richardson, Walter Marcotti
- [S11-2](#) The Calcium Channel Subunit $\alpha_2\delta_2$ in Inner Hair Cells is Essential for Sensitivity and Temporal Precision in Hearing
Jutta Engel
- [S11-3](#) The Nanoscale Connectome of Bushy Cell Networks in Mouse Cochlear Nucleus
George A Spirou, Michael Morehead, Nathan Spencer, Mariah Dawson, Paul Holcomb, Thomas Deerinck, Mark Ellisman, Paul Manis
- [S11-4](#) Synaptic performance in the superior olivary complex: reliability and precision
Eckhard Friauf
- [S11-5](#) Role of Piccolo in high frequency signal transmission at a central auditory synapse
Tanvi Butola, Tobias Moser
- [S11-6](#) Maturation and heterogeneity of ribbon synapses evaluated by high-resolution microscopic techniques
Susann Michanski, Rituparna Chakrabarti, Christian Fischer, Carolin Wichmann

Clues to the molecular identity of the hair-cell mechano-electrical transducer channel from experiments with pore blockers

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Mechanical stimulation of hair cells in the inner ear modulates the open probability of mechano-electrical transducer (MET) channels located in their hair bundles. The molecular identity of the MET channels is still being debated. Evidence is emerging that two isoforms of the transmembrane channel-like (TMC) family, TMC1 and TMC2, are involved, and may contribute to the permeation pore of the MET channel (1). We sought to investigate this by studying the interaction of dihydrostreptomycin (DHS), an aminoglycoside antibiotic, with MET channels of mouse outer hair cells (OHCs). DHS, a divalent cation, is a permeant blocker of the MET channel, which enters through open channels at negative membrane potentials (2).

Using *Beethoven* mice homozygous for a point mutation in TMC1 (M412K), we found that the affinity of the MET channels for block by extracellular DHS was reduced nearly 8-fold compared to wild-type controls on the same background (3). The entry rate of DHS into the hair cells was similarly reduced. Intracellularly applied DHS was 3 times less effective than in the controls. Using a two-barrier one binding-site model(2), we concluded that the site of the *Beethoven* mutation is at or near the permeation pore of the MET channel (3).

During the first postnatal week, mouse OHCs express both TMC1 and TMC2 (4). At this developmental stage, MET currents of homozygous *Tmc2* knock-out mice had a higher affinity for DHS. Binding of DHS to its binding site in the pore was stronger in the absence of TMC2, and the Ca²⁺ permeability of the MET channel was reduced, showing that TMC2 also contributes to the properties of the permeation pore. Anomalous MET currents with a mostly opposite response polarity and slower activation kinetics can be observed under various conditions in which the normal MET current is not evident, for example if the tip links do not function (5) or in the absence of both TMC1 and TMC2 (6). Block by extracellular DHS of the anomalous MET currents is two orders of magnitude less effective, and the block is non-permeant. These channels, located on the apical surface of the hair cells below the hair bundle (7), are either unrelated to the normal MET channels or may be incomplete precursors. Overall, our findings offer strong support for the idea that TMC1 and TMC2 are pore-forming subunits of normal MET channels.

1. Pan et al (2013) *Neuron* 79:504-515.
2. Marcotti et al (2005) *J Physiol* 567:505-521.
3. Corns et al (2016) *J Neurosci* 36:336-349.
4. Kawashima et al (2011) *J Clin Invest* 121:4796-4809.
5. Marcotti et al (2014) *J Neurosci* 34:5505-5514.
6. Kim et al (2013) *J Gen Physiol* 142:493-505.
7. Beurg et al (2016) *PNAS* 113:6767-6772.

Supported by Wellcome Trust (102892) & MRC (MR/K005561/1)

The Calcium Channel Subunit $\alpha_2\delta_2$ in Inner Hair Cells is Essential for Sensitivity and Temporal Precision in Hearing

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Voltage-gated calcium channels (VGCCs) are protein complexes composed of an α_1 pore-forming subunit and auxiliary subunits α_2 and β . VGCCs of cochlear inner hair cells (IHCs) are mainly composed of the subunits $Ca_v1.3$ (Platzer et al., Cell 2000) and α_2 (Neef et al., J. Neurosci. 2009), and lack of either $Ca_v1.3$ or α_2 causes deafness. So far, expression and contribution of the four β subunits β_1-4 , which assist in channel trafficking, increase the amplitude and modulate gating properties of I_{Ca} , were unknown.

Inner hair cells (IHCs) expressed $\alpha_2\delta_2$ mRNA at pre-hearing and hearing age as shown by quantitative RT-PCR. Therefore we analysed hearing and IHCs of ducky mice ($\alpha_2\delta_2^{du/du}$), a mouse line with a mutation in the *Cacna2d2* gene encoding a non-functional $\alpha_2\delta_2$ protein. Ducky mice had elevated auditory brainstem response (ABR) click and frequency-dependent hearing thresholds with the largest threshold shift in the range of best hearing. Moreover, averaged ABR waveform 40 dB over click threshold showed a significant delay of wave I (discharge of auditory nerve fibers) by 0.3 ms. Otoacoustic emissions were not impaired. IHC presynaptic $Ca_v1.3$ and Ca_v2 were normally clustered at synaptic ribbons. However, peak Ca^{2+} and Ba^{2+} currents of mature du/du inner hair cells (IHCs) were reduced by 30 – 40 %, and gating properties such as voltage of half-maximum activation and voltage sensitivity were altered, indicating that $Ca_v1.3$ channels normally co-assemble with $\alpha_2\delta_2$ at the IHC synapse. Exocytosis of IHCs was reduced in ducky mice but displayed unaltered Ca^{2+} efficiency. These results are in accordance with the classical role of β subunits in surface expression and gating modulation of VGCCs. Quantification of the pairing of double-immunolabeled presynaptic $Ca_v1.3$ clusters with postsynaptic glutamate receptor 4 or with PSD-95 complexes revealed impaired trans-synaptic coupling of pre- and postsynaptic proteins at the IHC synapse in ducky mice. We here show that $\alpha_2\delta_2$ plays a crucial role in the composition of IHC Ca^{2+} channels and forms VGCC complexes at an excitatory synapse together with $Ca_v1.3$ and α_2 . Our findings implicate a novel role for the $\alpha_2\delta_2$ subunit, which is localized extracellularly and contains protein-protein interaction domains, for optimal positioning of the presynaptic release site and the postsynaptic receptor complexes, thereby shortening reaction times. The auxiliary $\alpha_2\delta_2$ subunit of VGCCs therefore contributes to speed and sensitivity in ultrafast transmission at the IHC synapse.

Supported by the DFG, SFB 894 and SFB 1027.

The Nanoscale Connectome of Bushy Cell Networks in Mouse Cochlear Nucleus

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Animals employ amplitude modulation (AM) as a fundamental acoustic property to communicate with and provide localization cues to conspecific or allospecific individuals. Bushy cells (BC) of the ventral cochlear nucleus (VCN) define important early processing CNS elements that encode information inherent in AM features of sound.

BCs encode AM both in the timing and rate of generating action potentials. Temporal coding of AM derives from two general mechanisms. The first is convergence of multiple auditory nerve fibers via large nerve terminals, called end bulbs of Held, which require synchrony of a subset of fibers to drive neural activity. The second is a complement of ion channels that facilitate rapid response to changing amplitude and rapid reset of the membrane potential to maintain temporal fidelity throughout the sound duration. Although BCs may be the most studied auditory system neuron, we lack detailed and systematic information about their basic synaptology and dendritic structure.

To address this issue, we employed modern techniques of nanoscale connectomics to reconstruct a cluster of 31 neurons in the auditory nerve root region of the mouse VCN, which contains a high prevalence of BCs. We employed serial blockface electron microscopy (SBEM) to image at 5.7 nm pixel dimension across 1852 sections, each of 50 nm thickness. Cells and inputs were segmented using manual and semi-automated techniques. Segmented objects were visualized using immersive virtual reality, skeletonized and exported as hoc code for simulation in NEURON (syGlass, custom software).

Novel features of cellular structure were discovered. The number of endbulb inputs exceeded estimates from slice physiology experiments, (maximum 8 vs 4 estimated inputs), and these inputs could be binned into 3 groups based upon size (mean 58, 98 and 180 μm^2 apposed surface area). Afferent auditory nerve fibers travel in relatively well-defined fascicles, and we hypothesized that converging somatic inputs would emerge from fibers in the same fascicle. However, it was rare for inputs to travel in the same fascicle.

Dendrites of BCs are known to be complex, having excessive branching and uneven thickness. Reconstruction of most inputs to a single BC revealed a total of 135 nerve terminals, which were preferentially located on dendritic swellings. These input axons were highly branched and were largely unmyelinated within the image volume. Previous descriptions of bushy cell local groups based on intertwined dendrites were confirmed, with additional depiction of closely parallel branches with shared synaptic input.

We have established a pipeline to populate BCs with ion channels and stochastic multisite synaptic inputs, and generate simulations of BC networks in response to acoustic stimuli. Initial NEURON simulations revealed that 5 end bulb inputs could generate peri-stimulus time histograms typical of bushy

cells. Even subthreshold inputs contributed to spike generation, indicating that these cells integrate across multiple auditory nerve fibers and are not dominated by only the strongest inputs. Cells with different convergence patterns showed phase-locking to the envelope of AM stimuli, and were also correlated to each other on the time scale of the envelope. Initial simulations suggest that the strength of the phase-locking depends on the pattern of convergence.

These procedures, from connectome mapping to in silico simulation, provide a new template for all future studies of the VCN, and a modeling framework to test fine-grained hypotheses about structure and function of neural circuits prior to and after experimentation.

Synaptic performance in the superior olivary complex: reliability and precision

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At early stations of the mammalian auditory pathway, information is encoded in the precise signal timing and rate. The participating auditory synapses maintain the relative timing of events with submillisecond precision even during sustained and high-frequency stimulation. Sustained stimulation extends into the minute range, and high-frequency stimulation goes beyond 300 Hz. Central to understanding the features of high-fidelity signaling between neurons is to elucidate the physical, chemical, and biological factors that determine synapse performance. In this presentation, data from slice recordings are presented that concentrate on inhibitory glycinergic and excitatory glutamatergic synapses in the mouse auditory brainstem. I will focus on the lateral superior olive (LSO), a prominent nucleus in the superior olivary complex in which intensity differences between both ears are analyzed. Results from our laboratory indicate that both types of input to the LSO principle neurons are remarkable in several aspects, namely resistance to synaptic fatigue, low failure rate, and exquisite temporal precision. Their high-fidelity performance supports the functional requirements and appears to be due to a high number of readily releasable synaptic vesicles and a high release probability. Together, this results in a high quantal content and thus, large postsynaptic amplitudes. In conjunction with very robust vesicle replenishment mechanisms, these properties provide extremely rapid and temporally precise signaling that is required for neuronal communication at early stations of the auditory system, even during sustained activation in the minute range. The high-fidelity performance appears to be associated with efficient endocytosis of exocytosed vesicles, an energy-expensive task that goes along with high metabolic costs.

Role of Piccolo in high frequency signal transmission at a central auditory synapse

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Piccolo is a cytomatrix of the active zone (CAZ) protein involved in scaffolding and regulating neurotransmitter release at neuronal active zones. Here, we used Piccolo mutant mice to study central auditory synapses of the cochlear nucleus, which are specialized in high throughput synaptic transmission, capable of sustaining signaling frequencies of several hundreds of Hertz. We argue that even subtle deficits in the regulation of vesicle dynamics and neurotransmitter release will be revealed at synapses with such high functional demands. Moreover, the signal this synapse receives is unbiased by the mutation due to the presence of an unaffected, short isoform of Piccolo (i.e. 'Piccolino') upstream at the ribbon synapses of cochlear inner hair cells. Hence, this site of investigation provides a unique opportunity to study the implications of Piccolo deficiency on neuronal synaptic transmission. At the endbulb of Held synapse, we observed faster rise of miniature excitatory postsynaptic currents (mEPSCs) in the mutants, while mEPSC amplitude and frequency were unchanged. Likewise, we found a faster rise of evoked EPSCs in the mutants. Moreover, when stimulated with high frequency train stimulation, the mutant responses showed lower estimates of readily releasable pool size and a slower vesicle replenishment after the pool depletion, while the release probability remained unaltered. Next, we studied the effect of combined manipulation of Piccolo and its homologue, Bassoon, another CAZ protein. The combination of Piccolo disruption with reducing gene dosage of Bassoon aggravated the vesicle replenishment phenotype of Piccolo-deficient synapses. Current experiments are aimed at unveiling the molecular and ultrastructural implications of the aforementioned findings. In order to so, we plan to address changes in the abundance of other CAZ proteins at the Piccolo-deficient endbulbs by immunohistochemistry and employ transmission electron microscopy to study the changes in presynaptic density and vesicle pools.

Maturation and heterogeneity of ribbon synapses evaluated by high-resolution microscopic techniques

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Hearing in mammals requires temporally precise and reliable transmission of sensory information mediated by inner hair cell (IHC) ribbon synapses. Synaptic ribbons are electron-dense structures that tether synaptic vesicles and transmit signals by Ca²⁺-dependent exocytosis of glutamate at the presynaptic active zone (AZ) to spiral ganglion neurons (SGNs) (*Nouvian et al., 2006; Matthews & Fuchs, 2010; Wichmann & Moser, 2015*). Studies in cats showed that the position of individual synapses within a single IHC is of great importance: synapses containing large or multiple ribbons drive low spontaneous rate neurons, localized mainly at the neural side of IHCs, whereas high spontaneous rate neurons, driven by small synapses, are preferentially situated at the abneural side (*Merchan-Perez & Liberman, 1996*). Interestingly, in mouse IHCs, heterogeneous excitatory postsynaptic current (EPSC) shapes and amplitudes (*Glowatzki & Fuchs, 2002*) as well as variances in voltage dependence (*Frank et al., 2009*) are observed. This variability is intensely discussed to originate from multi- or unquantal release of synaptic vesicles (*Glowatzki & Fuchs, 2002; Chapochnikov et al., 2014*). Moreover, morphological differences of synapses and SGNs may contribute to such heterogeneous EPSCs. To date, the correlation between ribbon morphology, position and function, factors that contribute to the synaptic heterogeneity observed within the individual IHC, still remains elusive.

To address the heterogeneity of mouse IHC ribbon synapses, we use serial-block-face scanning electron microscopy (SBF-SEM). This way we can reconstruct whole IHCs and their innervating SGNs in 3-D to determine the location and diameter of SGNs and correlate it with the attributes like ribbon and postsynaptic density (PSD) sizes as well as the ribbon number. In order to understand how the heterogeneity is established we additionally use conventional embedding for transmission-electron-microscopy and electron-tomography, to study ultrastructural changes at the IHC. Parameters like vesicle numbers and diameters were investigated in depth at different developmental stages (E18-P48).

Our findings revealed that synaptic vesicles and ribbons exhibit changes in number and size indicating a developmental refinement of the AZ. Furthermore, we detected fusion events of synaptic ribbons around the onset of hearing at P12, which we interpret as a potential mechanism for synapse assembly and establishment of heterogeneity. However, it is important to note that we still observe multiple ribbons at the mature AZs (P48), possibly the structural correlate of a large complex synapse.

Therefore, we conclude that our observed morphological differences might play a crucial role in contributing to the functional heterogeneity in murine IHCs.

Symposium

S12: Structural and functional implementation of bottom-up and top-down influences in the primate brain

- [S12-1](#) Oscillation-based predictive mechanisms in speech processing.
Anne-Lise Giraud, Luc Arnal, Lorenzo Fontolan, Sevada Hovsepyan, Itsaso Olasagasti
- [S12-2](#) The Rhythms of Hierarchy
Pascal Fries
- [S12-3](#) The Spatially-Embedded Brain
Henry Kennedy
- [S12-4](#) Repetition-induced changes in gamma-band synchronization are stimulus specific
Alina Peter, Jarrod Dowdall, Liane Klein, Hanka Klon-Lipok, Kleopatra Kouroupaki, Joscha Schmiedt, Marieke Schoelvinck, Katharine Shapcott, Michael Schmid, Wolf Singer, Pascal Fries
- [S12-5](#) A theta rhythm in awake macaque V1 and V4 and its attentional modulation.
Georgios Spyropoulos, Conrado Arturo Bosman, Pascal Fries

Oscillation-based predictive mechanisms in speech processing.

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Predictive coding is a popular theory that accommodates numerous psycho-physiological phenomena, but which remains poorly supported at the biological level. That feed-forward and top-down propagation of sensory information use distinct frequency bands is an appealing assumption to explain a fundamental difference in the nature of the information that is passed forward and backward in sensory systems. However, whether distinct frequency bands carry predictions and prediction errors remains largely speculative. We will present EEG and MEG data and human depth electrode recordings that support the notion that top-down information could convey predictions on a lower frequency band than bottom-up signals. We will also discuss some ideas about how to explore the possible relation between predictive coding and frequency asymmetries in hierarchical information processing.

The Rhythms of Hierarchy

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Abstract body not available

The Spatially-Embedded Brain

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Using brain-wide retrograde tracing experiments in macaque, we are generating a consistent database of between area connections with projection densities, and distances (1). The network is neither a sparse small-world graph nor scale-free (2). Local connectivity accounts for 80% of labeled neurons (3), meaning that cortex is heavily involved in local function. Importantly link weights, are highly characteristic across animals, follow a heavy-tailed lognormal distribution over 6 orders of magnitude, and decay exponentially with distance.

The statistical properties of the cortex will give insight into the nature of the processing mode of the cortex (4). We have made a weighted network analysis that reveals a trade off between local and global efficiencies. An important finding is that a distance rule (EDR) predicts the binary features, the global and local communication efficiencies, clustered topography and the wire-minimization of the cortical graph (5, 6). These findings underline the importance of weight-based hierarchical layering in cortical architecture and hierarchical processing (7, 8). We have therefore evaluated the shapes and dimensions of cortical areas, which place different parts of the same area in different neighborhoods, with respect to EDR predictions of connectivity. We have shown that in the visual cortex central representations are preferentially linked to the ventral stream and peripheral representations to the dorsal stream.

Altogether, analysis of quantitative measures of connectivity suggest evolutionary optimization of areal shape, location and cortical folding and point to the need to consider the brain in space when considering the statistics of the inter-areal cortical network.

I will briefly mention on-going work on the mouse connectome that shows that the EDR model applies equally well across different species and different brain sizes suggesting general principals of organization. Interestingly however the core-periphery structure, indicative of a global work space cognitive architecture, includes primary areas in mouse and uniquely higher order areas in macaque.

References

1. N. T. Markov et al., A weighted and directed interareal connectivity matrix for macaque cerebral cortex. *Cereb Cortex* 24, 17 (2014).
2. N. T. Markov et al., Cortical high-density counter-stream architectures. *Science* 342, 1238406 (2013).
3. N. T. Markov et al., Weight Consistency Specifies Regularities of Macaque Cortical Networks. *Cereb Cortex* 21, 1254 (2011).
4. N. T. Markov, H. Kennedy, The importance of being hierarchical. *Curr Opin Neurobiol* 23, 187 (2013).
5. H. F. Song, H. Kennedy, X. J. Wang, Spatial embedding of structural similarity in the cerebral cortex. *Proc Natl Acad Sci U S A* 111, 16580 (2014).
6. M. Ercsey-Ravasz et al., A predictive network model of cerebral cortical connectivity based on a distance rule. *Neuron* 80, 184 (2013).
7. A. M. Bastos et al., Visual Areas Exert Feedforward and Feedback Influences through Distinct Frequency Channels. *Neuron* 85, 390 (2015).
8. G. Michalareas et al., Alpha-Beta and Gamma Rhythms Subserve Feedback and Feedforward Influences among Human Visual Cortical Areas. *Neuron* 89, 384 (2016).

Repetition-induced changes in gamma-band synchronization are stimulus specific

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Repeated encounters with visual stimuli often lead to reduced firing rate responses throughout the visual system. This phenomenon is known as adaptation or, especially in higher-order cortical areas, as 'repetition suppression'. However, behavioral performance typically remains stable or improves with repeated encounters despite reduced visual responses. To explain this apparent discrepancy, it has recently been proposed that stimulus repetition results in a more efficient stimulus representation by means of increased neuronal synchronization (Gotts et al., 2012).

Brunet et al. (2014) indeed showed that over the course of several hundred repetitions of grating stimuli, there was an increase in V1 and V4 LFP gamma-band power and V1-V4 gamma-band coherence. In V4, MUA and putative interneurons showed firing rate decreases, yet increases in gamma synchronization. Putative pyramidal cells showed no firing rate change, yet changes in synchronization that were positively correlated with stimulus drive. These results suggest that repetition leads to a sharpening of the stimulus representation by the gamma-synchronized ensemble.

However, the study repeated a small fixed set of gratings many times, and it therefore remained unclear whether changes were specific to the repeated stimuli, or whether a new set of stimuli would be affected by those changes. Further, the stimulus set precluded a generalization to more naturalistic or novel stimuli. Here, we investigate the stimulus specificity of repetition effects by presenting pseudo-randomly repeating, colored natural images initially novel to the animal. Monkeys were engaged in a change detection task on the presented objects (20 repetitions per image, ~2s presentation duration, with maximally 4 intervening stimuli between repetitions). Neural data were recorded from V1 and V4 in several rhesus monkeys. Visually induced MUA in both V1 and V4 showed repetition-related decreases. Natural stimuli induced gamma-band activity ranging from few ten to few thousand percent power increases with variability in peak frequency and spectral shape. Repetition could induce either decreases or increases in gamma-band activity depending on the particular stimulus, yet reliable across recording sessions. The interleaved and independent dynamics per stimulus demonstrate that repetition-related changes of gamma-band synchronization are specific to the respective stimulus.

Further, we provide preliminary evidence that repetition-related changes in V1 gamma are stimulus-location specific and therefore likely reflect a change in bottom-up processing. Collectively, these analyses demonstrate changes in stimulus representation on the timescale of seconds.

A theta rhythm in awake macaque V1 and V4 and its attentional modulation.

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Early and intermediate areas of the awake monkey visual cortex display slow rhythms during the execution of a visual attention task. These 3-5 Hz oscillations co-extend with the visual cortical stimulus representation, and predominate when covert attention is focused away from the stimulus driving the cortex. This slow rhythm synchronizes early and intermediate visual cortical areas. Local sensory processing, in the form of stimulus induced gamma oscillations, is influenced by the phase of the slow oscillation, and this influence is significantly weaker with attention. Microsaccadic eye movements do not account for the reported observations.

Symposium

S13: Neural circuits of pain

- [S13-1](#) Touch receptor-derived sensory information alleviates acute pain signalling and fine-tunes nociceptive reflex coordination
Stefan G. Lechner, Louise Gorham, Francisco Taberner, Vincenzo Prato, Alice Arcourt
- [S13-2](#) The organisation and functions of interneuron populations in the spinal dorsal horn
Andrew James Todd
- S13-3 Neurobiological principles of placebo and nocebo responses in pain
Ulrike Bingel - No abstract available
- [S13-4](#) Medial prefrontal cortex circuitry in chronic pain-related plasticity
Rohini Kuner, Linette Tan
- [S13-5](#) Pain related neural circuits in the medial prefrontal cortex
Oscar A. Retana, Manfred J. Oswald, Linette L. Tan, Rohini Kuner

Touch receptor-derived sensory information alleviates acute pain signalling and fine-tunes nociceptive reflex coordination

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Painful mechanical stimuli activate multiple peripheral sensory afferent subtypes simultaneously, including nociceptors and low-threshold mechanoreceptors (LTMRs). Using an optogenetic approach we demonstrate that LTMRs do not solely serve as touch receptors but also play an important role in acute pain signaling. We show that selective activation of neuropeptide Y receptor-2-expressing (Npy2r) myelinated A-fiber nociceptors evokes abnormally exacerbated pain, which is alleviated by concurrent activation of LTMRs in a frequency-dependent manner. We further show that spatial summation of single action potentials from multiple NPY2R-positive afferents is sufficient to trigger nocifensive paw withdrawal, but additional simultaneous sensory input from LTMRs is required for normal well-coordinated execution of this reflex. Thus our results show that combinatorial coding of noxious and tactile sensory input is required for normal acute mechanical pain signaling. Additionally we established a causal link between precisely defined neural activity in functionally identified sensory neuron subpopulations and nocifensive behavior and pain.

The organisation and functions of interneuron populations in the spinal dorsal horn

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Interneurons account for the vast majority of the neurons in laminae I-III of the dorsal horn, and they can be divided into two broad classes: excitatory (glutamatergic) neurons and inhibitory neurons that use GABA and/or glycine as their principal transmitter. The cells in each of these classes are morphologically and functionally heterogeneous, but recent studies have shown that several largely non-overlapping neurochemical populations can be identified within each class. The neurochemical approach allows selective manipulation of the function of these different populations by means of molecular genetic approaches. This has begun to reveal that specific types of interneuron play particular roles in somatosensory processing.

Medial prefrontal cortex circuitry in chronic pain-related plasticity

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A timely and fundamental question in the neurosciences revolves around how functional specificity is generated in highly redundant brain circuits. We identified a novel functional role of an important subdivision of the cingulate cortex, namely the mid-cingulate cortex (MCC), in central plasticity mediating the transition from acute to chronic pain. In functional mapping studies, the MCC emerged as a key nodal point in cortical and subcortical circuits activated in pain. This is one of the key regions that is activated in humans during a pain percept, yet it has not been interrogated functionally so far. With a view towards this goal, we employed in vivo optogenetic manipulations, viral-based circuit tracing and functional analyses in mice in this study. We report that the MCC exclusively gates sensory hypersensitivity to pain, but is dispensable for acute pain as well as pain affect. A most intriguing observation was that optogenetic activation of either the MCC or afferent pathways emerging from the MCC was sufficient by itself to induce a behavioral state of pain hypersensitivity in mice in the absence of any conditioning noxious afferent input from the periphery.

Taken together with previous seminal work on the rostral (pregenual) subdivision of the anterior cingulate (rACC), our work reveals a functional dichotomy between the rACC and the MCC in creating the multidimensional experience of pain. The ability of the MCC to trigger hypersensitivity independently of peripheral nociceptor input into the brain can explain changes in pain sensitivity reported in patients in the absence of (or persisting following healing of) obvious injuries or physical pathologies. Moreover, they provide a mechanistic basis for exacerbation of pain by psychosocial factors, such as stress and anxiety, that may deregulate basal activity in the MCC.

Pain related neural circuits in the medial prefrontal cortex

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Structures in the brain involved in nociception are also attributed various functions that are unrelated to pain. The medial prefrontal cortex (mPFC), for example, is not only engaged by painful stimuli, but is also active during memory tasks, decision making, and the processing of emotions like fear, among other contexts.

What is the morphological correlate that brings about these distinct functions within the same brain region? We would like to address the hypothesis that multifunctional structures like the mPFC contain dedicated pain circuits, and possibly pain specific neurons.

Our project employs methods with single cell resolution to analyze neuronal activity in different contexts, with the aim of answering the aforementioned questions. We use genetically modified mouse lines and adeno-associated viral vectors expressing marker proteins in an activity-dependent manner to visualize neuronal activity during painful and nonpainful conditions.

Furthermore, we are performing electrophysiological recordings to study the properties of functional ensembles with added temporal precision. We also seek to assess the functional contribution of these neural populations to live animal behavior.

Our experiments promise insights into the cortical circuitry underlying distinct cognitive processes on a cellular level. In addition, understanding the microscopic representation of acute nociceptive signaling will enable the investigation of the changes that take place during chronic and pathological pain conditions.

Symposium

S14: Tuning ion channels, myelin, and synapses for rapid axonal signaling

- [S14-1](#) Na⁺ channels in GABAergic interneuron axons: Speed versus energy efficiency
Peter Jonas, Hua Hu
- [S14-2](#) Activity-dependent facilitation of presynaptic potassium currents and short-term plasticity at a central synapse
LU-YANG WANG
- [S14-3](#) A biophysical foundation for rapid saltatory conduction in myelinated axons
Maarten Kole, Charles Cohen, Marko Popovic, Jan Klooster
- [S14-4](#) Mechanism of transmitter release at the calyx of Held synapse
Takeshi Sakaba
- [S14-5](#) Hyperpolarization-activated currents facilitate high-frequency action potential firing in cerebellar mossy fibers
Niklas Byczkowicz, Stefan Hallermann

Na⁺ channels in GABAergic interneuron axons: Speed versus energy efficiency

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Fast-spiking, parvalbumin-expressing GABAergic interneurons (PV+ interneurons) play a key role in several microcircuit functions, such as feedforward and feedback inhibition, high-frequency network oscillations, and temporal encoding of information in the brain (Hu et al., 2014, *Science* 345:1255-263). For all of these functions, the fast initiation, propagation, and termination of axonal action potentials (APs) is critically important. However, previous studies suggested that short APs are metabolically highly expensive (Carter and Bean, 2009, *Neuron* 64: 898–909). Together with the AP high frequency in PV+ interneurons in vivo, this suggests that a substantial part of the total energy budget of the brain may be used by processes associated with APs of PV+ interneurons. However, previous conclusions regarding AP energy efficiency in PV+ interneurons were made based on analysis of Na⁺ channels in interneuron somata. Whether these conclusions hold for PV+ interneuron axons, where the majority of voltage-gated Na⁺ channels is located, remains an important, but entirely open question. To estimate the energy efficiency of APs in interneuron axons, we performed confocally targeted subcellular patch-clamp recordings in interneuron axons. Na⁺ currents were recorded in outside-out patches using APs as voltage-clamp commands at near-physiological temperature (34–37°C). Our results indicated a Na⁺ inflow of 0.96 ± 0.27 pmole cm⁻² per AP in the interneuron axon. This value is lower than that comparable to that of other cell types, including axons of glutamatergic principal neurons (1.76 pmole cm⁻², Alle et al., 2009, *Science* 325:1405–1408). Thus, in terms of total Na⁺ inflow, PV+ interneurons appeared to be as energy efficient as other types of neurons. We next compared the total Na⁺ entry during the AP with the theoretical minimum (Na⁺ entry ratio) in the PV+ interneuron axons. Although the entry ratio in the interneuron axon (median value = 1.8, n= 17) appeared to be higher than in pyramidal neurons, it was substantially lower than the value of 4 previously obtained in the squid giant axon. Computer modeling of AP initiation and propagation in PV+ interneurons indicated an inflow of $\sim 500 \times 10^8$ Na⁺ ions during a single AP. Thus, the energy requirement per AP is significantly smaller in PV+ interneurons than in layer 5 neocortical pyramidal neurons (Hallermann et al., 2012, *Nat Neurosci* 15:1007–1014). In conclusion, AP signaling in GABAergic interneuron axons is more energy-efficient than previously thought.

Activity-dependent facilitation of presynaptic potassium currents and short-term plasticity at a central synapse

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Neurons convey information in bursts of spikes across chemical synapses where the fidelity of information transfer critically depends on synaptic input-output relationship. With a limited number of synaptic vesicles (SVs) in the readily releasable pool (RRP), how nerve terminals sustain transmitter release during intense activity remains poorly understood. Here we report that presynaptic K⁺ currents evoked by spikes facilitate in a Ca²⁺-independent but frequency- and voltage-dependent manner. Experimental evidence and computer simulations demonstrate that this facilitation originates from dynamic transition of intermediate gating states of voltage-gated K⁺ channels (Kvs), and specifically attenuates spike amplitude and inter-spike potential during high-frequency firing. Single or paired recordings from a mammalian central synapse further reveal that facilitation of Kvs constrains presynaptic Ca²⁺ influx, thereby efficiently allocating SVs in the RRP to drive postsynaptic spiking at high rates. We conclude that presynaptic Kv facilitation imparts neurons with a powerful control of transmitter release to dynamically support high-fidelity neurotransmission.

A biophysical foundation for rapid saltatory conduction in myelinated axons

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Saltatory conduction represents the acceleration of electrical signalling in myelinated nervous systems, restricting action potential generation to the nodes of Ranvier while rapidly propagating impulses along the intermediary myelinated internodes. Based on the seminal electrophysiological recordings from frog sciatic nerves by Huxley and Stämpfli (J. Physiol. 108:315–339, 1949) and Tasaki (Am. J. Physiol. 181:639–650, 1955) our current concept for saltatory conduction depicts glial myelin membranes as a near-perfect insulation of the axolemma. The equivalent circuit of the combined axo-myelin membranes is thought to reduce the net transverse capacitance and increase the net transverse resistance. However, given the submyelin spacing and the absence of direct characterization of biophysical properties of myelin its impact on axolemma and role to conduction velocity in mammalian axons remains controversial (Castelfranco and Hartline, Brain Res:1–23, 2016). Such biophysical insights can be obtained by patch-clamp recordings combined with morphology-constrained modelling of the electrical responses from the same cell. Here, we aimed to functionally identify the internodal membrane organization of large thick-tufted adult rat layer 5 pyramidal neurons in brain slices. Their axons are myelinated from the end of the axon initial segment onwards, along the primary axon internodes (~1.5 μm in diameter), generating an action potential conduction velocity of $\sim 4 \text{ m s}^{-1}$. We first examined the ultrastructure of internodes from recorded neurons filled with biocytin or HRP post hoc processed for electron microscopy (EM). The transmission EM indicated that layer 5 axons have on average ~ 14 myelin sheaths ($n = 7$ axons). Second, passive voltage responses from simultaneous soma-axon recordings were combined with detailed 3D morphological reconstructions, including dendrites, primary axons and collaterals and implemented in NEURON for detailed cable modelling ($n = 6$ high-quality models). Optimized cable models show that myelin has a rather low specific resistance (on average $8.59 \text{ k } \Omega \text{ cm}^2$ per myelin membrane), does not act as an insulator and neither contributes to a low capacitance of the axolemma. It will be discussed how alternative local circuits can fully account for action potential-evoked capacitive current flow, ensuring rapid and saltatory propagation from node to node.

Mechanism of transmitter release at the calyx of Held synapse

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The mechanism of synaptic transmission, particularly the mechanism of neurotransmitter release from the presynaptic terminal, remains unknown because direct access to the terminal is difficult. We used the calyx of Held in the auditory brainstem, which has a large presynaptic terminal, as a model system. By applying simultaneous recordings from pre- and postsynaptic compartments in acute slice preparation, we quantified the kinetics of exo- and endocytosis of synaptic vesicles. Particularly, we addressed which process during synaptic vesicle cycle could be rate-limiting during repetitive nerve activity. It turned out that synaptic vesicle replenishment after depletion of release-ready vesicles is rate-limiting during high-frequency stimulation at the calyx synapse under certain condition. Ca-CaM-Munc13 signaling pathways and possibly clearance of release sites by synaptic vesicle endocytosis may be important for the replenishment. In order to look at the process of synaptic vesicle replenishment more directly, we have recently dissociated the calyx terminal acutely and used total internal reflection microscopy for monitoring synaptic vesicle dynamics. Our results suggest that priming of tethered synaptic vesicles, rather than docking of synaptic vesicles to the release site, is the rate-limiting process after depletion of release-ready synaptic vesicles.

Hyperpolarization-activated currents facilitate high-frequency action potential firing in cerebellar mossy fibers

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Hyperpolarization-activated currents (I_h) have been described in axons and presynaptic terminals. However, the function of axonal I_h remains controversial. Cerebellar mossy fibers send sensory information to the cerebellum and can fire action potentials (APs) at frequencies of more than 1 kHz. Previous studies suggest that cerebellar mossy fiber boutons (cMFBs) have a strikingly large I_h , making them an ideal model to study the function of axonal I_h . In this study, whole-cell patch-clamp recordings from cMFBs in acute cerebellar brain slices showed a half-activation of I_h at -104 mV. Addition of cAMP to the intracellular solution shifted the half-activation of I_h in a dose-dependent manner to more depolarized potentials (-84 mV with 1 mM cAMP). Inhibition of I_h by application of 30 μ M of the selective blocker ZD-7288 significantly hyperpolarized cMFBs, increased the membrane time constant, and increased the input resistance. Furthermore, application of ZD-7288 reduced the maximum failure free frequency of APs and increased AP broadening and AP amplitude reduction during bursts of APs. ZD-7288 did not block sodium channels in cMFBs. Finally, inhibiting I_h with ZD-7288 slowed AP propagation, whereas enhancing I_h with 8-Br-cAMP accelerated AP propagation. These data reveal that axonal I_h secures reliable and fast propagation of APs.

Symposium

S15: Emerging complexity and functions of microRNAs-dependent regulation in neuroscience

- [S15-1](#) Long non-coding pseudogene transcripts compete with mRNAs that share microRNA recognition elements with them in human brain neurons
Hermona Soreq
- [S15-2](#) miRNA function in synapse development and plasticity
Gerhard Schratt
- [S15-3](#) Two is better than one. Cooperative gene regulation by microRNAs in Neural Stem Cells.
Carlos P. Fitzsimons
- [S15-4](#) Dissecting alternative pathways and functions of the microRNA biogenesis machinery in mammalian neurogenesis
Davide De Pietri Tonelli, Meritxell Pons-Espinal, Federica Marinaro

Long non-coding pseudogene transcripts compete with mRNAs that share microRNA recognition elements with them in human brain neurons

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MicroRNAs orchestrate brain functioning via interaction with microRNA recognition elements on target transcripts. However, the global impact of potential competition between coding and non-coding microRNA recognition elements sharing brain transcripts on the microRNA pool remains unexplored. Here, we report that non-coding pseudogenes that harbor microRNA recognition elements) are more tightly conserved and more efficiently expressed in human temporal lobe neurons than microRNA recognition elements-deficient pseudogenes. Non-coding pseudogenes that harbor microRNA recognition elements map to genomic regions with low histone marks associated with transcriptional suppression and participate in neuronal RNA-induced silencing complexes, indicating functional involvement. Furthermore, up- and down-regulation experiments validated mutual bidirectional co-regulation of non-coding pseudogenes that harbor microRNA recognition elements with microRNA recognition elements-sharing cholinergic transcripts; and global enrichment of non-coding pseudogenes that harbor microRNA recognition elements in single nucleotide polymorphisms associated with schizophrenia, bipolar disorder and autism suggests interaction with mental diseases. Our findings indicate functional roles of this subset of non-coding pseudogenes that harbor microRNA recognition elements in brain cognition, supporting physiological impact of the reciprocal co-regulation of non-coding pseudogenes that harbor microRNA recognition elements with microRNA recognition elements-sharing coding transcripts in human brain neurons.

This research is supported by the European Research Council (Advanced Award 321501).

miRNA function in synapse development and plasticity

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Our research group is interested in the role of microRNAs (miRNAs), a large class of small non-coding RNAs, in synapse development and plasticity in mammalian neurons, as well as the potential impact of miRNA regulation on higher cognitive functions and neurological disease. During the last years, we have identified key neuronal miRNAs and their targets that are involved in dendrite and spine morphogenesis in rat hippocampal neurons. One of these miRNAs is part of a large imprinted, mammalian-specific miRNA cluster. Induced expression of the miRNA cluster by neuronal activity is required for dendritic arborization and the downscaling of excitatory synapses, a form of homeostatic plasticity that is frequently disturbed in neurodevelopmental and psychiatric disorders. Accordingly, validated target genes of this miRNA cluster are frequently deregulated in neurological disease. Using miRNA cluster knockout mice, we obtained evidence for an involvement of this microRNA cluster in the control of anxiety-related and social behavior in mice. Behavioral phenotypes are accompanied by extensive changes in the hippocampal transcriptome, in particular related to proteins that function at both inhibitory and excitatory synapses. Mechanistically, cluster miRNAs are regulated at the level of transcription, dendritic transport and by a competing endogenous RNA encoded by a gene frequently mutated in autism-spectrum disorders (ASD). Our results point to a function of miRNAs in the control of synapse homeostasis and raise the possibility that impaired miRNA function could contribute to synaptic dysfunction in neurodevelopmental disorders, including ASD.

This work is supported by grants from the DFG (SPP1738, FOR2107) and the EU (FP7 “EpiMiRNA”)

Two is better than one. Cooperative gene regulation by microRNAs in Neural Stem Cells.

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MicroRNAs (miRNAs) are small RNA molecules involved in post-transcriptional regulation of gene expression and they act by binding to their mRNA targets at specific sites located at the mRNA 3'UTR. Although miRNAs are frequently studied individually, growing experimental evidence demonstrates that many mRNA targets are subjected to concerted regulation by multiple miRNAs. In particular, specific miRNA pairs inhibit target expression in a cooperative manner, leading to enhanced repression effectiveness and specificity. Importantly, distance constraints for miRNA binding sites at the target 3'UTR regulate optimal target repression. Using the regulation of specific targets as case study, we have shown that microRNA-124 and -137 cooperativity controls caspase-3 activity in hippocampal neural stem cells shortly after epileptic seizure induction. Our results suggest that microRNA cooperativity may contribute to the early neurogenic response to epileptic seizures in the dentate gyrus.

Dissecting alternative pathways and functions of the microRNA biogenesis machinery in mammalian neurogenesis

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The overarching goal of our research is to understand the role played by microRNAs (miRNAs) in neurogenesis in the mammalian brain. Our long-term goal is to develop and use miRNA-based technologies to develop novel RNA-based therapies for brain diseases.

Neurogenesis is the process of new neuron generation through the differentiation of neural stem/progenitors cells. Though the majority of neurons that comprise the mammalian brain are generated during embryonic development, some neurogenesis persists throughout life in specific niches of the mammalian brain. Adult neurogenesis may be considered as an intrinsic compensatory response to self-repair the adult nervous system, but it also influences brain functions, such as learning and memory. It therefore follows that understanding the mechanisms controlling neurogenesis may have potential implications for therapeutic development.

miRNAs are small non-coding RNAs with regulatory functions on the majority of messenger RNAs (i.e. mRNA target) and are rapidly emerging as a new layer of regulation of “virtually all” biological pathways, including neurogenesis.

Several studies have elucidated the crucial role(s) of miRNA-guided gene expression in embryonic neurogenesis (1). However, very little is known about the specific contribution alternative pathways of miRNA-biogenesis proteins in embryonic neurogenesis [2], and of miRNAs in adult hippocampal neurogenesis [3]. Ongoing experiments in our lab aim to dissect alternative pathways and functions of the miRNA biogenesis machinery in murine corticogenesis and in adult hippocampal neurogenesis.

References:

- 1) Barca-Mayo and De Pietri Tonelli. “Convergent microRNA actions coordinate neocortical development”. *Cell Mol Life Sci.* (2014) Feb 12.
- 2) Marinaro F., et al., “MicroRNA-independent functions of DGCR8 are critically required for neocortical development and TBR1 expression”. (2016), submitted.
- 3) Pons-Espinal, M., et al., “Synergic functions of miRNAs determines neuronal fate of adult neural stem cells”. (2016), submitted.

Symposium

S16: The evolutionary diversity of nervous system development - from worms to humans

- [S16-1](#) The genetic basis of natural variation in mushroom body size in *Drosophila melanogaster*
Patrick Callaerts, Liesbeth Zwarts, Lies Vanden Broeck, Elisa Cappuyens, Julien Ayroles, Michael Magwire, Veerle Vulsteke, Jason Clements, Trudy Mackay
- [S16-2](#) Evolution of neurogenesis in arthropods – conserved features and flexible tools
Angelika Stollewerk
- [S16-3](#) Comparison of the circadian clock of social and solitary bees
Katharina Beer
- [S16-4](#) Developmental conservation and diversity of the insect brain
Gregor Bucher
- [S16-5](#) Neural Stem and Progenitor Cells and Neocortex Expansion in Development and Evolution
Wieland B. Huttner

The genetic basis of natural variation in mushroom body size in *Drosophila melanogaster*

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The brain integrates a variety of sensory stimuli to control context-appropriate behavioral responses. In insects, mushroom bodies are higher order brain structures that integrate and process sensory information. The size of the mushroom bodies has been regarded as a proxy for cognitive ability and behavioral plasticity and mushroom bodies across insects reveal prominent size differences. Thus understanding the genetic basis of differences in mushroom body size could provide insights into the evolution of insect mushroom bodies and by extension the evolution of the brain and complex behaviors. However, the genetic substrate and the selective pressures acting upon brain size as a quantitative trait are poorly defined. We have used the *Drosophila* Genetic Reference Panel to identify polymorphic variants affecting natural variation in mushroom body size allowing us to discover gene networks controlling mushroom body development and plasticity. We further revealed correlations of mushroom body morphology with behavior and lifespan suggesting that changes in mushroom body structure and function impact these traits. We propose that the identified gene networks that control development and plasticity serve as the genetic substrates that control natural variation in and evolution of brain size.

Evolution of neurogenesis in arthropods – conserved features and flexible tools

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Arthropods show considerable variations in early neurogenesis of the central and peripheral nervous system. This includes the pattern of specification, division and movement of neural precursors and progenitors. In all metazoans with nervous systems, including arthropods, conserved genes regulate neurogenesis, which raises the question of how the various morphological mechanisms have emerged and how the same genetic toolkit might generate different morphological outcomes. Here I address this question by comparing central and peripheral neurogenesis across arthropods and show how variations in the regulation and function of the neural genes might explain this phenomenon and how they might have facilitated the evolution of the diverse morphological mechanisms of neurogenesis.

Comparison of the circadian clock of social and solitary bees

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The seasonal and daily changes in floral resources are a great challenge for bees. To predict the regular oscillations in the environment bees have evolved a circadian clock, which is involved in timing daily activities, the bees' time memory and their sun-compass orientation.

In a comparative study we investigate the development of the circadian clock in the eusocial honey bee (*Apis mellifera*) and the solitary bee (*Osmia bicornis*). Both are generalist pollinators native in Europe, but to cope with daily and seasonal changes they developed different mechanisms in their communal and reproductive life style. The social life style of honey bees has an impact on the behavior governed by the endogenous circadian clock: whereas younger nurse bees exhibit no circadian locomotor activity and older forager bees show strong circadian activity rhythms. The solitary bee on the other hand does not have the social context of a hive community, but faces the same oscillating changes in environment.

We examined possible differences in the development of circadian rhythms in locomotor activity of honey bees and solitary bees after emergence. Therefore we designed a set-up to monitor individual bees either solitary or in social context of a mini colony. Unlike the honey bee, solitary bees show strong circadian rhythmicity during the first few days after emergence. We hypothesized that the young honey bees do not have a completely developed circadian system, when emerging from the brood cell.

Immunofluorescent staining of Neuropeptide PDF (Pigment Dispersing Factor), which has been identified as important part of the circadian clock in different insects, provided further evidence to this assumption. Studying the neuro-architecture of the PDF network in whole mount brains of honey bees sampled at different developmental stages, we could show that it gains complexity throughout honey bee development, even during their adulthood. By further investigating the circadian system of both bee types we aim to achieve a better understanding of effects of life style on the endogenous clock and the functional consequences.

Developmental conservation and diversity of the insect brain

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The insect brain is built by a set of neuropils that can be found in most taxa. However, relative size, shape and timing of development differ quite a lot between insects, probably reflecting evolutionary adaptations. One example is the central complex (CX), which fully develops during embryogenesis in hemimetabolous insects while it develops postembryonically in Dipterans. The red flour beetle *Tribolium castaneum* takes an intermediate position in that the central CX develops partially during embryogenesis.

Using a candidate gene approach we found-not unexpectedly-a high degree of conservation in early neuroectodermal patterning between holometabolous insects and vertebrates and we identified several genes required for embryonic central complex development. As this approach might be biased towards conserved gene functions, we have been performing a genome wide RNAi screen, which revealed FoxQ2 as a novel top component of insect neuroectoderm patterning.

Given this high conservation of early patterning – how can we explain the diversity of neuropils between taxa? I will present an approach to tackle this question using the heterochronic development of the CX as model and making use of genome editing in the genetic model systems *Drosophila melanogaster* and *Tribolium castaneum*.

Neural Stem and Progenitor Cells and Neocortex Expansion in Development and Evolution

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Our group studies the molecular and cellular mechanisms of neurogenesis in the developing neocortex in the context of mammalian brain evolution, specifically the various types of cortical stem and progenitor cells (CSPCs), their modes of division, their lineages, and the neuron production resulting therefrom. With regard to (i) the site of mitosis and (ii) the absence or presence of ventricular contact at mitosis, three principal classes of CSPCs can be distinguished. First, CSPCs that reside in the ventricular zone (VZ) and that contact the ventricle where they undergo mitosis, i.e. the neuroepithelial cells, apical radial glial cells and apical intermediate progenitor cells, collectively referred to as apical progenitors (APs). Second, CSPCs that reside in the subventricular zone (SVZ) where they typically undergo mitosis and that have delaminated from the ventricle, i.e. the basal (or outer) radial glial cells and basal intermediate progenitor cells, collectively referred to as basal progenitors (BPs). Third, CSPCs that undergo mitosis in the basal VZ or in the SVZ and that retain ventricular contact at mitosis, called subapical progenitors.

Our group has been studying the following issues related to these CSPCs in the developing mouse, ferret, marmoset, macaque and human neocortex: (1) the various lineages from APs to BPs; (2) the machinery underlying BP delamination; (3) symmetric versus asymmetric cell divisions; (4) the microcephaly gene *Aspm*; (5) the AP marker prominin-1/CD133; (6) membrane particles released into the ventricle; (7) extracellular matrix, integrins, and progenitor self-renewal; (8) cell cycle length; (9) transcriptomes of embryonic mouse and fetal human neocortical germinal layers and specific progenitor subpopulations.

Recent insights into the cell biology of CSPCs, molecular pathways and factors, and their role in neocortex expansion in development and evolution will be presented.

Symposium

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Hanna Chole, Pierre Junca, Jean-Christophe Sandoz, Gérard Arnold

Developmental and adult neuronal plasticity of olfactory synaptic microcircuits in the mushroom-body calyx of social Hymenoptera

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Mushroom bodies (MBs) are prominent neuropils in the insect brain that undertake high-level sensory integration and the organization of complex behaviors that involve learning, the formation of associative memories, and spatial orientation. Hymenopteran insects possess particularly large multimodal MBs with doubled calyces (major sensory input structures of the MBs) containing a large number of neurons and are divided into modality-specific subregions. Neuronal circuits within all calyx subregions are organized into an array of synaptic complexes termed microglomeruli (MG). Each MG comprises a single central presynaptic bouton from an axonal terminal of an olfactory or visual projection neuron, which is embedded in many f-actin rich postsynaptic profiles, most of them originating from MB intrinsic neurons called Kenyon cells, and a few processes from GABAergic, octopamine, or dopamine positive extrinsic neurons.

The talk will first highlight a remarkable structural plasticity of MG associated with postembryonic brood care during pupal development in nectar-feeding ants and honeybees. We investigated that thermoregulatory rhythmic nursing behavior mediated by *Camponotus mus* workers on the developing pupae leads to an increase in MG density and total MG numbers in the olfactory lip region of the young adult MB calyx. Similar results were found in honeybee workers where the synaptic organization in the young adult olfactory lip is affected by slight deviations within and beyond the natural range of a constant pupal rearing temperature experienced in the brood area, and these effects persist after the first week of adult life. Interestingly, the favorable temperature range and the optimal temperature regime are species-specific in Hymenoptera. These temperature-mediated differences in initial MG numbers in the MB calyx may have important consequences for later stages of adulthood. The second part of the talk will highlight aspects of cellular and subcellular processes that shape olfactory MG in the calyx during adult maturation in *Camponotus rufipes* ants and honeybees. During the natural transition from nursing to foraging an overall reduction of MG is accompanied by a significant increase in postsynaptic contacts of Kenyon cell dendritic spines. As a result, we infer that dendritic spines are key candidates to the reorganization of MG that occur during the age-related transition between nurse and forager. Our results suggest that the different levels of structural neuronal plasticity in olfactory microcircuits play important roles in the regulation of social organization in Hymenopteran societies.

Supported by the DFG, SFB 1047 "Insect timing" (Projects B5) to CG.

Social contact as a reinforcement in olfactory learning in honeybees

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Honeybees' foraging behavior critically depends on associative learning, in which foragers associate floral cues, prominently odors, with appetitive reinforcement from flower nectar. In the Lab, this process is usually studied using the Pavlovian conditioning of the proboscis extension response (PER), in which an odor (CS) is associated with sucrose solution (US). Interestingly, olfactory learning about floral resources is not limited to the foraging situation, and honeybee workers can learn chemosensory information directly from successful foragers within the hive. Previous work attributed this learning to a simple classical association between the floral scent adsorbed on the returning foragers' body and a sugar reward given by this forager via trophallaxis. Remarkably, however, nectar transfer is not performed in all dual interactions with returning foragers, suggesting that other mechanisms may be involved. Here, we asked whether social cues may play a role in this transmission, i.e. if interaction with another forager can represent an appetitive reinforcement for bees. We found that simple antennal contact with a fed nestmate, in absence of any sugar stimulation, can induce PER in harnessed worker bees. In addition, bees can learn to associate an odor CS with this antennal contact with a nestmate. After such association, the odor alone triggers the PER. This suggests that simple social contact can act as an appetitive US in honeybees. We currently study the mechanisms implied in this new conditioning focusing on the physical nature of this social US. Our current data suggest the implication of antennal movements produced by the US nestmate.

Re-evaluation of learned information in *Drosophila*

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Animals constantly reassess the reliability of learned information to optimize their behavior. On retrieval, consolidated long-term memory can be neutralized by extinction if the learned prediction was inaccurate. Alternatively, retrieved memory can be maintained, following a period of lability in which it is reconsolidated. Although extinction and reconsolidation provide opportunities to alleviate problematic human memories, we lack a detailed mechanistic understanding of memory updating processes. Here we identify neural operations underpinning re-evaluation of memory in *Drosophila*. Accuracy of prediction during reactivation of sugar-reinforced olfactory memory can lead to either extinction or reconsolidation. Each process recruits activity in specific parts of the mushroom body output network and different subsets of reinforcing dopaminergic neurons. Memory extinction requires output neurons with dendrites in the α and β lobes of the mushroom body, which drive negatively reinforcing dopaminergic neurons that innervate the same zones. The aversive valence of new extinction memories neutralizes previously learned odor preference. Memory reconsolidation requires the α β mushroom body output neurons. This pathway recruits negatively reinforcing dopaminergic neurons innervating the same compartment and re-engages positively reinforcing dopaminergic neurons to reconsolidate the original reward memory. These data establish that recurrent and hierarchical connectivity between mushroom body output neurons and dopaminergic neurons supports memory re-evaluation driven by reward prediction error.

A temporal code for sugar concentration from the gustatory neurons of bumblebees

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Bees and other insect pollinators have mouthparts are specialized consuming sugary solutions such as floral nectar. Bees prefer to drink solutions high in sugar concentrations but the composition of nectar varies in concentration over a range from 8-70% (wt/vol). We predict, therefore, that the gustatory neurons in the sensilla of bees should be sensitive to changes in sugar concentration and capable of detecting sugars over a large range. Here, we report electrophysiological recordings of the population of neurons housed in the galeal sensilla of the buff-tailed bumblebee, *Bombus terrestris*. The gustatory neurons of *B. terrestris* begin responding to 2.5-5 mM solutions of sucrose. The detection threshold for fructose was between 5-10mM and for glucose was between 10-25 mM. Surprisingly, stimulation with sucrose solutions of greater than 50mM produced a distinctive bursting pattern of firing in two neurons, in which a single action potential fired by one neuron is followed by a ~20 ms period of inhibition in a cycles of 40 Hz. This pattern of firing was also observed when galeal sensilla were stimulated with > 50 mM fructose solutions, maltose and melezitose, but only rarely with glucose or other sugars. We show that the concentration of sugars is represented by a temporal pattern of firing of the gustatory neurons from the mouthparts and propose that the brain uses this information to guide feeding behaviour.

Antennal response to odorants with innate or acquired hedonic values in honey bees (*Apis mellifera*)

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In order to survive, animals must produce adaptive behaviors when facing potentially positive (food) or negative (danger) situations. In honey bees, olfactory cues are highly relevant for communication among nestmates, foraging and nest defense. We studied the effect of the valence of odors on the movements of crucial sensory organs for bees: their antennae. We developed a tracking system based on a motion capture principle for monitoring harnessed bees' antennal movements at a high frequency rate. As bees' antennae are highly mobile, we asked whether odor-evoked antennal movements may contain information about their acquired hedonic value. The hedonic value of an odor can be innate or acquired through learning. We here analyze (i) the spontaneous antennal response toward odors, whose hedonic value was measured based on the spontaneous orientation of bees, and (ii) the change in antennal movements to odorants as a result of appetitive or aversive conditioning, inducing opposite acquired hedonic values. In the appetitive conditioning of the proboscis extension response (PER), an odor (CS) was associated with sucrose solution (US), while in the aversive conditioning of the sting extension response (SER), an odor CS was associated with a thermal shock US. Spontaneous antennal responses to natural odorants seem to be separated in fast-forward or slow backward movements. While queen and brood pheromones, as well as royal jelly odor, induced fast forward movements, alarm pheromones induced backward movements and a decrease in velocity. Differential appetitive conditioning had a strong effect on antennal movements, bees responding to the odorant associated with sugar with a marked forward motion of the antennae and a strong velocity increase. By contrast, differential aversive conditioning had little effect on antennal movements. These experiments unravel the existence of both innate and acquired, hedonic-based, antennal responses in bees.

Symposium

S18: Computations - from sensations to decisions

- [S18-1](#) Absolute versus relative perception of auditory processing - how spatial acoustic context determines sound localization
Benedikt Grothe, Andrea Lingner, Michael Pecka
- [S18-2](#) Adaptive active sampling behaviour underlies contextual modulation in an early sensory system
Andreas Schaefer
- [S18-3](#) Cortical top-down control of early olfactory processing
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- [S18-3](#) Cortical top-down control of early olfactory processing
Wolfgang Kelsch, Markus Rothermel
- [S18-4](#) Multiple fly visuo-motor behaviors predicted by a single biologically plausible circuit
Andrew D Straw, Andreas Poehlmann
- [S18-5](#) Top-down inputs onto lateral hypothalamus determine signaling of feeding-related cells
Suzanne van der Veldt, Marta Carus-Cadavieco, Maria Gorbati, Franziska Bender, Tatiana Korotkova, Alexey Ponomarenko

Absolute versus relative perception of auditory processing - how spatial acoustic context determines sound localization

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It is common knowledge that our perception of the worlds is not reflecting the actual physical environment. We see, hear, and smell only fractions and features of the physical world most relevant for our behavior as shaped by evolution. Nevertheless, if it comes to neuronal foundations of sensory processing we tend to forget this fact and, for instance, treat the binaural auditory system as an absolute sound localization system. Therefore, computations performed by the lower auditory brainstem have traditionally been investigated and data interpreted in the conceptual framework of stable computational space maps based on interaural time and level difference processing (ITD and ILD, respectively). Although the idea of space maps has been challenged in the last decade (compare Grothe et al. 2011), the idea of absolute sound localization persisted.

Recently, we discovered unexpected feedback-systems that modulate the population output of the binaural comparators, the medial and lateral superior olives (MSO, LSO, respectively) in the mammalian auditory brainstem (Magnusson et al. 2008, Stange et al. 2013). This feedback is mediated by slow, pre-synaptic GABA-B-receptors that decrease the synaptic strength of the direct MSO and LSO inputs. Model-predictions based on these findings let us to expect systematic errors in absolute sound localizations. Psychophysical experiments with human subjects confirmed these assumptions (Stange et al. 2013). Our current psychophysical tests show that auditory space is computed in a highly dynamic way depending on the acute acoustic context causing to compressions and dilations for enhanced stimulus segregation of moving sounds. Coherent with current population coding scenarios and suggests that the mammalian auditory system evolved for relative, not absolute, localization of dynamic stimuli.

Magnusson AK, Park TJ, Pecka M, Grothe B, Koch U (2008) Retrograde GABA signaling adjusts sound localization by balancing excitation and inhibition in the brainstem. *Neuron* 59:125–137

Stange A, Myoga MH, Lingner A, Ford MC, Alexandrowa O, Felmy F, Pecka M, Siveke I, Grothe B (2013) Adaptation in sound localization: from GABAB receptor-mediated synaptic modulation to perception. *Nature Neurosci* 16:1840-1847, DOI:10.1038/NN.3548

Grothe B, Pecka M, McAlpine D (2010) Mechanisms of sound localization in mammals. *Physiol Rev* 90: 983-1012

Adaptive active sampling behaviour underlies contextual modulation in an early sensory system

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Sensory circuit activity has often revealed correlates of information about behavioral context. Such representations could arise either from physiological changes to the network, or from changes in stimulus sampling behaviour. The olfactory bulb (OB) is ideally suited to studying this, with both inputs from higher centers and clear coupling of activity to the sniff cycle. Using whole cell recordings in the OB of passive and behaving mice, we identified learning-related changes in mitral and tufted cell odor responses. Sniffing behavior also underwent parallel changes, which correlated with the changes in odor response. In absence of learning, sniffing could alter both baseline activity and odor responses. Learning-related changes occurred prior to both the typical reaction time and earliest estimates of decision time (a single sniff cycle), while sniff changes correlated with the motivational state of the animal. Therefore, highly motivated mice may modulate their sampling strategy, altering odor representations to facilitate decision. Thus, contextual representations in sensory circuits can be explained by alteration in stimulus sampling behavior.

Cortical top-down control of early olfactory processing

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The bottom-up flow of sensory information is complemented by cortical top-down projections that are thought to modify sensory representations according to the animals' current state. We will present here recent data on the prominent top-down projection from the anterior olfactory nucleus (AON) back to the main olfactory bulb (MOB) in rodents. This top-down model system may help to understand their role in generating neuronal coding states for state dependent perception of environmental cues and consequent initiation of behaviors.

In our recent work, we found that optogenetically induced oxytocin release enhanced olfactory exploration and same-sex recognition of adult rats. Consistent with oxytocin's function in the AOC particularly in social cue processing, region-selective receptor deletion impaired social recognition, but left odor discrimination and recognition intact outside a social context. Oxytocin transiently increased the drive of the AON projecting to MOB interneurons. In vivo, cortical top-down recruitment of MOB interneurons dynamically enhanced the inhibitory input to MOB projection neurons and increased the signal-to-noise of their output. Based on these findings, we now explored dynamical interactions of AON and MOB coding during conspecific exploration and specific contribution of the AON through highly parallel single unit recordings.

Additionally we expressed GCaMP selectively in AON projection neurons and imaged fluorescence signals from their axon terminals in the MOB. Surprisingly, odorants evoked large signals that were transient and coupled to odorant inhalation both in the anesthetized and awake mouse, suggesting that feedback from AON to the MOB is rapid and robust across different brain states. The strength of AON feedback signals increased during wakefulness, suggesting a state-dependent modulation of cortical feedback. Finally, we demonstrated that AON feedback projections were also activated when stimulating other neuromodulatory centers – for example, the HDB. Our results point to the AON as a multifunctional cortical area that provides ongoing feedback to the MOB and also serves as a descending relay for other neuromodulatory systems.

Multiple fly visuo-motor behaviors predicted by a single biologically plausible circuit

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In the fruit fly, two prominent behaviors requiring sophisticated neuronal computation are visual object fixation and figure ground discrimination, yet the neural substrates remain unknown. Experiments in motion blind, walking flies revealed object responses due to a position system distinct from the T4-T5 cell dependent motion pathway (Bahl et al., 2013). We performed behavioral experiments in which we blocked synaptic output from T4-T5 cells and observed a lack of stripe fixation at fast dynamics and loss of figure ground discrimination ability. Thus, for mediating these behaviors, the T4-T5 motion dependent system is necessary and other systems cannot compensate for disruption of T4-T5 cell output (Fenk, Poehlmann and Straw, 2014).

To address the question of whether the T4-T5 motion system is sufficient to mediate such behaviors, we implemented two dynamical models based on measured properties of well-studied HS cells (Schnell et al., 2010), immediate post-synaptic targets of T4-T5. The key component of both models is motion asymmetry in the responses of model neurons, whereby front-to-back motion elicits stronger responses than back-to-front motion. The first model is a simplified phenomenological model and the second is more physiologically realistic. Both couple a bilateral pair of neurons to turning behavior. We showed that these models are sufficient to mediate object tracking and figure ground discrimination in the presence of noise and predict fixation in front of moving backgrounds (Fenk, Poehlmann and Straw, 2014). Thus, we propose that neurons that respond asymmetrically to motion, including but not limited to HS, are sufficient to allow flies to track small field objects. It will be interesting to extend these modeling efforts with further anatomical, physiological and behavioral results to work towards a deeper understanding of fly visuo-motor behaviors. Some visuo-motor behaviors will require additional components to model faithfully, but given the biological plausibility of the present minimalistic models, they are important to consider as a starting hypothesis for the neuronal computations underlying a range of visuo-motor behaviors.

Top-down inputs onto lateral hypothalamus determine signaling of feeding-related cells

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Lateral hypothalamus (LH) controls feeding behaviors, yet little is known about the role of top-down inputs in regulation of LH. One of major inputs to LH is provided by the lateral septum. We investigated role of this input, combining optogenetics with multisite electrophysiological recordings in behaving mice in the free-access feeding paradigm. Gamma-rhythmic activation of this pathway elicited food approach. To investigate neuronal mechanisms underlying behavioral effects of gamma-rhythmic entrainment of the LH, we analyzed the timing of LH neuronal activity during gamma oscillations. We found two groups of LH cells, fired preferentially at distinct phases of gamma oscillations. Further, using excitatory (ChETA) and inhibitory (eNPAC2.0) opsins, we showed that input from the LS inhibit a subset of LH neurons, thus enabling their phase-shifted, i.e. temporally separated, signaling during gamma oscillations. Next we studied whether different timing of LH neuronal firing during gamma relates to feeding-related activity of LH cells. Firing of a subset of LH neurons in the free-access feeding paradigm matched location of the food zone (FZ-match cells), while other cells were preferentially active distantly from the food zone (FZ-mismatch cells). FZ-match cells prominently reduced their firing during the gamma oscillation trough, when they are mostly influenced by the LS inhibition, and fired with an increased probability during the subsequent rising phase of the gamma cycle. Further, FZ-match index predicted firing of the cell in particular phases of gamma oscillation. Thus, LS gamma oscillatory input during food seeking enables fine-time scale separation of LH cells according to their feeding-related activity.

We gratefully acknowledge support by the DFG (Exc 257 NeuroCure, TK and AP, SPP1665, AP), The Human Frontier Science Program (HFSP; RGY0076/2012, TK, DB), and GIF (I-1326-421.13/2015, TK).

Symposium

S19: Epigenetic mechanisms of behavior and physiological regulation

- [S19-1](#) Epigenetic mechanisms of behavior and physiological regulation
Andre Fischer
- [S19-2](#) Epigenetic regulation of the oxytocin system within the lateral septum in social fear conditioning
Inga Neumann, Rohit Menon
- [S19-3](#) The balance between heat stress resilience and vulnerability is mediated by dynamic DNA methylation and de-methylation along the Corticotropin-Releasing-Hormone gene
Noam Meiri, Tomer Cramer, Tania Kisliouk
- [S19-4](#) Epigenetic mechanisms underlying parental high-fat diet induced obesity in the offspring
Aron Weller, Asaf Marco, Tatiana Kisliouk, Tzlil Tabachnik, Noam Meiri
- [S19-5](#) Gene Regulation and Epigenetics of a Lifetime Body-Size Memory in *Drosophila*
Laura Spindler, Tammo Krause, Burkhard Poeck, Roland Strauss

Epigenetic mechanisms of behavior and physiological regulation

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Abstract body not available

Epigenetic regulation of the oxytocin system within the lateral septum in social fear conditioning

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Anxiety and fear are adaptive emotional responses to threatening situations. However, when becoming maladaptive, they can lead to anxiety disorders including social anxiety disorder (SAD), which is defined as persistent fear and avoidance of social situations. Treatment of SAD is rather unspecific and combines psychotherapy and pharmacotherapy and is only affective in 38 % of patients and associated with a high rate of relapse (1). Therefore, in order to reveal the molecular and neuronal underpinnings of SAD, we have established a mouse model of social fear using a social fear conditioning (SFC) paradigm. Social fear acquisition is induced by punishment of social contacts with conspecifics during fear conditioning (2).

The neuropeptide OXT has been proposed as a potential therapeutic agent for SAD due to its pro-social, anxiolytic and anti-stress effects. We could previously reveal that infusions of OXT into the lateral ventricle or bilaterally into the septum abolished fear expression in socially fear conditioned (SFC+) mice via activation of central OXT receptors (OXTR). Detailed investigation of the oxytocinergic system in SFC+ mice revealed an increase in OXTR binding in the lateral septum, which normalized after social fear extinction, while local OXT release in response to social stimuli was found to be blunted in the paraventricular nucleus (PVN) of SFC+ mice (3).

These findings could be confirmed at mRNA level, as SFC+ mice showed elevated *oxtr* mRNA expression specifically in the lateral septum, but neither in the dorsal hippocampus nor amygdala, compared with non-conditioned SFC- mice, as measured using quantitative real-time PCR 2h after fear acquisition. Similarly, the attenuated OXT release in SFC+ mice was reflected by reduced *oxtr* mRNA levels in the hypothalamic PVN 2h after social fear extinction. These results point towards the regulation of *oxtr* and *oxtr* at DNA level possibly via epigenetic modifications.

Histone deacetylases (HDACs) are key enzymes, which modify chromatin and, thus, contribute to the regulation of gene expression. Therefore, we studied the local expression of various transcription regulators including HDAC as epigenetic markers in SFC+ versus SFC- mice. Indeed, an increased expression of *hdac1* was found in the lateral septum of SFC+ mice 2h after fear acquisition, and this increase returned to basal levels following fear extinction. Indicating specific epigenetic regulation of the *oxtr*, stimulation of Neuro 2A cells with MS275 (2 μ M), a potent HDAC1 inhibitor, for 24 h increased *oxtr* mRNA expression by 150%. Conversely, treatment of Neuro 2A cells with C646, a specific CBP/p300 inhibitor, decreased *oxtr* mRNA expression. In line with this, chromatin immunoprecipitation revealed an enrichment of H3K27ac and depletion of H3K9me3 at the *oxtr* promoter following MS275 treatment, whereas treatment with C646 increased H3K9me3 in Neuro 2A cells. Finally, we could demonstrate that bilateral infusion of MS275 in the lateral septum 90 min before social fear extinction training significantly facilitated fear extinction.

Taken together, we have proven that SFC-induced changes in *oxtr* expression in SFC+ mice are, at least in part, mediated via epigenetic mechanisms. The increased *oxtr* expression may be mediated via histone acetylation, which have previously been shown to augment gene expression, or DNA methylation (4). Importantly, infusion of an HDAC1 inhibitor facilitated extinction learning in SFC+ mice supporting these findings at a behavioural level. On-going studies are determining the link between the oxytocinergic system and histone modifications in the context of SFC. These findings extend our understanding of the role of brain OXT in social fear and suggest that epigenetic mechanisms may be a contributing factor to the psychopathology of SAD as recently suggested (4).

Supported by Bayerische Forschungsstiftung and Deutsche Forschungsgemeinschaft.

1. Gelernter CS, Uhde TW, Cimbolic P, Arnkoff DB, Vittone BJ, Tancer ME, et al. (1991): Cognitive-behavioral and pharmacological treatments of social phobia. A controlled study. *Archives of General Psychiatry*. 48:938-945.
2. Toth I, Neumann ID, Slattery DA. Social fear conditioning - a novel and specific animal model to study social anxiety disorder. *Neuropsychopharmacology* 37(6):1433-43.
3. Zoicas I, Slattery DA, Neumann ID (2014): Brain oxytocin in social fear conditioning and its extinction: involvement of the lateral septum. *Neuropsychopharmacology* 39:3027-3035.
4. Ziegler C, Dannlowski U, Brauer D, Stevens S, Laeger I, Wittmann H, et al. (2015): Oxytocin receptor gene methylation: converging multilevel evidence for a role in social anxiety. *Neuropsychopharmacology* 40:1528-1538.

The balance between heat stress resilience and vulnerability is mediated by dynamic DNA methylation and de-methylation along the Corticotropin-Releasing-Hormone gene

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Determining whether stress will lead to future stress-resilience or vulnerability depends on a delicate balance of a probably adjustable stress response set-point, which is regulated during postnatal sensory development, involving the HPA axis. Here we demonstrate that heat stress during the critical period of thermal control establishment in chicks, “programs” a habituated or sensitized response, depending on the ambient temperature. This is mediated by epigenetic modifications, specifically DNA-methylation and -demethylation.

The mRNA of CRH in the hypothalamic PVN and plasma corticosterone levels were elevated a week after heat conditioning in chicks which were trained to be vulnerable to heat, while they declined in chicks that were trained to be resilient, demonstrating correlative changes in the HPA axis. Interestingly, global 5mc% and 5hmc% changed significantly between the two different heat conditioned groups, a week after their conditioning. Resilient chicks displayed low 5mc% alongside high 5hmc%, while vulnerable chicks displayed an opposite pattern of high 5mc% and low 5hmc%. This pattern was repeated when specific CpG methylation sites along the CRH gene were evaluated, indicating that these dynamic changes contribute to the differences in CRH expression levels, and therefore might take part in the formation of the habituation and resilience phenomena. Since the habituated animals displayed a form of demethylation, we aimed to attenuate this via intracranial injection of Parp inhibitor, disrupting the activity of the dynamic DNA-demethylation TET-enzyme family. Indeed, 5hmc% declined. This in turn resulted in obliteration of the ambient temperature resilience showed previously by non-injected chicks.

It is highly plausible to conclude that the mechanism underlying the balance between heat stress resilience and vulnerability is regulated by epigenetic adaptations.

Epigenetic mechanisms underlying parental high-fat diet induced obesity in the offspring

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Children's likelihood to overeat and develop obesity is affected by parental obesity. Changes in epigenetic programming have been implicated as potential mechanisms underlying this phenomenon. Using a rat model, we exposed only the first generation to chronic high fat diet (HFD) and followed the effect on two consecutive generations of standard fed offspring. We focused on the promoter of the hypothalamic neuropeptide *Pomc*, which is crucially involved in control of food intake. HFD consumption by non-mated female rats (F0) significantly increased body weight and plasma leptin levels and attenuated *Pomc* mRNA expression. This was associated with hypermethylation of the *Pomc* promoter. As expected, high leptin levels in the HFD group, increased expression of the transcription factor Sp1. Nevertheless, Binding of Sp1 to the hypermethylated *Pomc* promoter was significantly reduced. Furthermore, perinatal exposure to maternal HFD lead to long term acquired alteration in DNA methylation patterns and posttranslational modifications of histone H3 lysine 9 (H3K9) that affect *Pomc* transcription in the F1 and F2 offspring. As a potential tagging of the nucleosome at the *Pomc* promoter for histone post-translation modification, we describe the binding of methyl binding domain 1 (MBD1) to the methylated *Pomc* promoter, interacting with SETDB1 methyltransferase to promote the formation of methylation of H3K9. This combined DNA and histone methylation produces a repressor complex potentially attenuating the expression of *Pomc*. These findings contribute to our understanding of the mechanisms through which environmental cues are translated into stable changes in the *Pomc* gene, leading to obesity.

Gene Regulation and Epigenetics of a Lifetime Body-Size Memory in *Drosophila*

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One of the last steps in the neurodevelopment of a *Drosophila* fly is to learn and consolidate the own body size. We have shown that naïve (dark-reared flies) do not know their body reach and try to surmount gaps that clearly exceed their reach by far. We found that naïve flies learn their body size from the parallax motion they create while walking in visual patterns; they keep this body-size memory for the rest of their life. We have identified sets of neurons innervating the protocerebral bridge (pb) in the central brain of the fly that are required to establish this memory. Employing GRASP (GFP reconstitution across synaptic partners) revealed that intrinsic lateral pb neurons are presynaptic to columnar neurons that connect the pb with the fan-shaped body and the noduli of the central complex. Moreover, these different types of neurons require opposing cAMP/PKA signalling levels and activation of the transcriptional regulator CREB to establish the individual body reach. It is hypothesised that opposing activity of CREB in the two populations may lead to permanent differential changes in the expression levels of CREB target genes or genes downstream of those. We speculate that the lifetime body-size memory might be consolidated through epigenetic mechanisms to ensure a fixed change in opposing gene regulation. Although DNA methylation has not been observed in developing or adult *Drosophila*, most genes involved in epigenetics of vertebrates have their counterpart in flies and some of them have been shown to be involved in memory formation. A genetic screen, based on RNAi knock-down strategy, was initiated to identify possible candidate genes executing epigenetic regulation of long-term memory consolidation. RNA interference was limited to the lateral pb neurons that require CREB activity for memory consolidation. By using behavioural and histological analysis, out of ca. 100 candidates, more than 20 genes have been identified that evoked a defect in the body-size memory upon RNAi in the relevant neurons. The promising factors comprise chromatin-associated proteins with counteracting function regulating gene silencing and activation. This approach will elucidate which epigenetic modifications are required to consolidate this unusually long-lasting memory in flies.

Symposium

S20: Common ground plan of the insect brain architecture

- [S20-1](#) High-throughput systematic identification of novel neurons in the *Drosophila* brain as a reference for comparative analysis
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High-throughput systematic identification of novel neurons in the *Drosophila* brain as a reference for comparative analysis

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Among insect model organisms *Drosophila* has a unique advantage that systematic identification of neurons is much more straightforward thanks to its advanced molecular genetics. During the past two centuries neuroscientists have tended to focus on a few selected brain regions to analyse neurons in regions extensively, while other neurons in neighbouring regions remain hardly investigated. However, such neurons also deserve systematic analyses if we want to obtain a global overview of the entire neuronal network in the brain.

A potential problem in this respect was the fact that, other than a few well-investigated brain regions, there were even no commonly accepted terms and clearly defined boundaries for the rest of the brain. This made it very difficult to describe the locations of neurons and their projection targets. To address this we formed a consortium of 15 laboratories across the world and established a system of nomenclature and boundary definition of the entire insect brain. Although the current version is based on the *Drosophila* brain, we accommodated available knowledge of the brains of other species as much as possible so that the system should be applicable and expandable across Insecta.

Neurons are made by distinct numbers of neural stem cells, or neuroblasts. By genetically labelling such stem cells early during neurogenesis and visualising their progeny in the adult, we found that the progeny of each of the about 100 stem cells in the fly brain arborizes in distinct brain regions to form a clonally defined unit architecture. Interestingly, distinct functional modules of the brain - such as the lower and higher sensory centres as well as the mushroom body and central complex - are each formed by a group of several clonal units that we call a "clan". Such hierarchical organization of clonal units and clans should have evolutionary implication; identification of clonal unit structures in other insects should provide important insights on the insect brain evolution.

More than ten thousand *Drosophila* transgenic strains have been generated to drive cell-specific gene expression using GAL4 and LexA transcription factors. Using this system we have made systematic identification of neurons that have not been investigated in the olfactory, visual, auditory, gustatory as well as learning and memory systems. Currently we are analysing the somatosensory system to establish an overview of information flow from the peripheral sensory neurons to putative somatosensory centres in the brain. The resulting view showed clear similarity between mammals and *Drosophila*, again suggesting conserved building principle of the neuronal network since the Cambrian Period.

The expression patterns of those expression driver strains are not very specific, in part because a single gene is active in various cell types. This made it especially difficult to identify specific neurons in the lesser known brain regions, or *Terra Incognita*. Using the new-generation split-GAL4 system it is possible to combine two driver strains to induce expression only in the cells where both are active. Using this system we are now performing systematic identification of neurons in the superior and posterior parts of the brain, where higher-order sensory integration and motor control should occur. Knowledge to be obtained should serve as a reference for analysing comparable regions in other insects.

Single cell morphology of the lateral clock neurons in *Drosophila melanogaster*

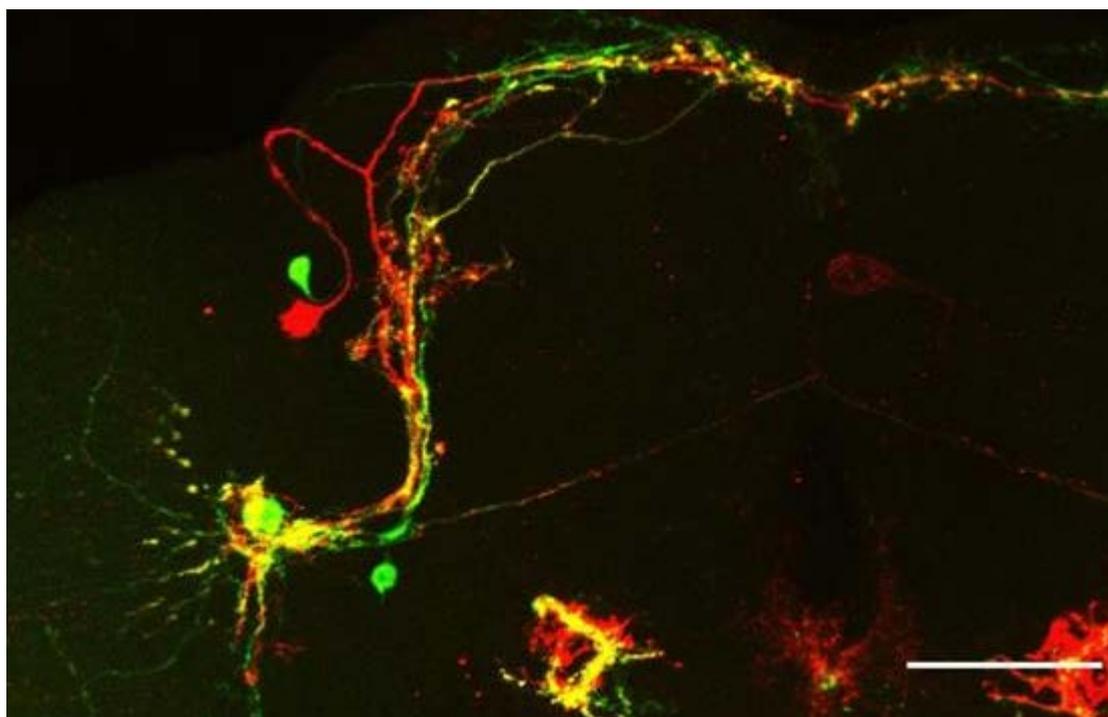
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The endogenous clock of *Drosophila melanogaster* consists of about 150 neurons, which share the molecular machinery to produce self-sustained circadian rhythms in the absence of so-called *Zeitgebers* (e.g. light/dark or temperature cycles). The mechanisms and the involved key genes and proteins, crucial for circadian clock function (e.g. *period*, *timeless*, *clock*, *cycle*) have been extensively studied and are well known (reviewed by Helfrich-Förster, 2004). The neuronal anatomy and projection patterns composed by the different clock neuron subgroups are also sufficiently described (Helfrich-Förster *et al.*, 2007), but due to the limitations of antibody-stainings and Gal4 driver lines, we still lack detailed anatomical information on a single-cell level.

To circumvent these problems, we used the Flybow system (Hadjieconomou *et al.*, 2011 and Shimosako *et al.*, 2014), which has the advantage of multicolor single-cell labeling in Gal4 target tissues. Relying on a modified heat-shock Flippase to drive alterations in fluorescence protein expression, Flybow eludes the poor inducibility and toxicity of other brainbow adaptations to *Drosophila*, that rely on the Cre recombinase (reviewed by Rodriguez *et al.*, 2012). With this approach we stochastically expressed one out of four fluorescence proteins in the Gal4 expressing cells to describe the anatomy of the lateral clock neurons that comprise the so-called Evening oscillator (E-cells) in *Drosophila*. Our results point out the heterogeneity of these clock neurons, not only in function (Yao and Shafer, 2014), but also displayed in their morphology.

We finalized our anatomical study of the E-cells by revealing the post- and presynaptic sites with *UAS-DenMark-mCherry* (Nicolai *et al.*, 2010) and *UAS-nSyb-EGFP* (Zhang and Broadie, 2003), respectively.



Comparison of the sky-compass pathway in different insect species

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Visual cues play an important role in spatial orientation of many insect species. As a global reference, the sun's horizontal direction (solar azimuth) is of particular interest. Through scattering of sunlight in the atmosphere, several additional cues arise that are all tightly coupled to the solar azimuth: a pattern of polarized light, a color gradient and an intensity gradient. All of these skylight cues can be used to maintain a constant orientation over short distances (dung beetles), migrate over long distances (locusts and monarch butterflies) or for vector navigation (bees and desert ants).

The neuronal network that underlies these behaviors has been investigated in a number of insect species and is conserved to a degree, that the same types of neuron can be identified across species borders. The sky-compass system is therefore an excellent example to illustrate both the common ground-plan underlying insect brains as well as its adaptations for different tasks.

In my talk I will compare the sky-compass networks of a long-distance migrant, the desert locust, and a path-integrating insect, the honeybee. In both species, the sky-compass pathway originates in the dorsal rim area of the medulla, where transmedulla neurons receive polarization input from specialized photoreceptors in the dorsal rim area of the compound eye. These neurons then run through a narrow layer in the medulla where they presumably pick up unpolarized skylight information and project to the anterior optic tubercle in the central brain. From there, projection neurons transmit sky-compass signals to the bulbs of the lateral complex where they form conspicuously large synaptic complexes with tangential neurons of the lower division of the central body (CBL). Columnar neurons connect the CBL to the individual slices of the protocerebral bridge, where tangential neurons establish a polarotopic map, i.e. neighboring slices of the protocerebral bridge house neurons with systematically varying tuning for polarized light. At the output stage of the network, neurons with synaptic input in connecting the protocerebral bridge and the upper division of the central body to the lateral accessory lobe.

Species specific differences concern the branching pattern of the transmedulla neurons, which in honeybees are restricted to the dorsal half of the medulla, and the ultrastructure of the synaptic connection between the neurons of the anterior optic tubercle and the central complex. While in locusts the synaptic connections are exclusively established via divergent dyads, honeybees additionally have divergent tetrads.

Other groups have shown, that in *Drosophila* the homologs of the central-complex neurons described above are involved in visual tasks such as stripe fixation or place learning. Moreover, the azimuth position of a vertical stripe is represented in the *Drosophila* central complex in a similar way as the polarization angles in the locust's central complex.

Taken together this shows that a basic layout of a neuronal network in the insect brain can be employed and modified for different behavioral tasks.

Evolution of a social insect brain – insights from comparative studies

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Social insects (ants, bees, wasps, termites) are among the most socially advanced organisms in the animal kingdom. The enormous evolutionary success and ecological importance of social-insect colonies are largely based on efficient communication systems, cooperative division of labor, and powerful navigational skills. The multitude of different tasks within a social insect colony requires a rich diversity of behavioral repertoires across individuals and, at the same time, high levels of behavioral flexibility within individuals. What are the structural and functional neuronal adaptations that have promoted the evolution of a social insect brain?

The recent efforts of a consortium of 15 laboratories around the world to define neuroanatomical boundaries and connections of brain neuropils and to introduce a unified nomenclature system has greatly facilitated comparative studies on commonalities and variations in a common ground plan of the insect brain architecture. In the search for adaptations of the insect brain to specific requirements for a social life style we focus on comparative structural and functional analyses of brains in selected species of Hymenoptera (ants, bees, wasps). I will highlight the anatomical organization and function of multiple antennal-lobe output tracts promoting sophisticated parallel olfactory processing, underline the importance of an expansion in parallel synaptic microcircuits within olfactory and visual compartments of the mushroom bodies for long-term memory formation, and point out the role of plasticity in conserved visual pathways to the central complex for navigation and central place foraging.

Comparison of social with nonsocial Hymenoptera and other insect groups suggest that evolutionary adaptations promoting insect sociality are based on combined variations in a common ground plan of the insect nervous system rather than the emergence of novel brain structures or a simple expansion of individual brain compartments. In addition, social insect brains, in particular the mushroom bodies, express high levels of developmental and adult neuroplasticity. In conclusion, comparative analyses in Hymenoptera support the hypothesis that preadaptations in multimodal sensory supply and parallel processing together with enhanced capacities for long-term plasticity in high-order integration centers represent important steps in the evolution of insect sociality.

Supported by the German Research Foundation (DFG), SFB 1047 “Insect Timing” and SPP 1392 “Integrative Analysis of Olfaction”.

Genealogical Correspondence of Brain Centers across Pancrustacea

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Molecular phylogenetics identifies the closest relatives of insects as blind simple crustaceans called remipedes that live in subterranean aquifers [1,2]. This relationship conflicts with neurocladistics and neurophylogenetics that resolve brain organization of insects closest to that of eumalacostracan crustaceans, such as shrimps and crabs. A prevailing view promoted by molecular phylogeneticists [3] and supported by recent neurophylogenetic studies [4,5], is that correspondences of brain organization are convergent: the evolution of functionally comparable centers originating from different ancestors. The implication is that certain centers and pathways are apomorphies specific to insects [4] while centers providing the same functions in crustaceans are apomorphies specific to that group. If a vocabulary describing commonalities of pancrustacean brain organization is to aid in understanding brain function [6] then it is crucial that conflicts such as these are either resolved or vindicated.

Two prominent forebrain centers have been the subject of intense research, using mainly insects to interpret their possible functions. Cardinal properties ascribed to the iconic mushroom bodies, second order olfactory centers in insects, are that they are crucial for learning and memory [7]. Cardinal properties suggested for the insect central complex are its roles in action selection [8]. Rarely, however, have such centers and their properties been considered in the light of evolution. Second-order olfactory centers and midline centers are ubiquitous to most crustaceans, including remipedes [4]. If insects originated from the crustacean stem lineage and if those centers in insects and crustaceans are homologous then they would have originated over half a billion years ago, evolving in a benthic ecology quite different from terrestrial habitats. What would have been their ancestral roles in behavior? If those centers common to insects and crustaceans evolved independently in the two groups can any inferences be made about crustacean brain function from what we know of insect neurobiology, or vice versa?

In this symposium I will describe three examples of recent work that reject the view of convergent evolution. Second order olfactory centers in insects are not apomorphic. The central complex is an ancient neuropil whose original role is, and was, that of a decision-making center integrating opportunistically whatever sensory information is available to the organism [9]. And, fossil brains of stem euarthropods provide enough information to support homology of brain organization across Pancrustacea [10,11].

1. von Reumont, B. M. et al. *Mol. Biol. Evol.* 29, 1031-1045; 2. Oakley, T. H. et al. *Mol. Biol. Evol.* 30, 215-233; 3. Lozano-zFernandez, J. et al. *Phil. Trans. R. Soc. B* 371, 20150133; 4. Fanenbruck, M., et al. *Proc. Nat. Acad. Sci. USA* 101, 3868–3873; 5. Stemme, T., et al. *Cell Tissue Res.* 363, 635–648; 6. Ito, K., et al. *Neuron* 81, 755–765; 7. Vogt, K., et al. *eLife* 3, e02395; 8. Martin, J. P., et al., *Curr. Biol.* 25, 1–9; 9. Strausfeld and Hirth, *Science* 340, 157–161; 10. Ma, X., et al. *Nature* 490, 258–261; 11. Strausfeld, N. J., et al. *Curr. Biol.* 26. In press.

Symposium

S21: System memory consolidation during sleep

[S21-1](#) Cueing memory reactivation during sleep
Susanne Diekelmann

[S21-2](#) Neuronal oscillations mediating sleep-dependent memory consolidation
Til Ole Bergmann

[S21-3](#) The neurochemical mechanisms of sleep-dependent memory consolidation
Gordon B. Feld

[S21-4](#) Effects of sleep on immunological memory processes
Tanja Lange, Jürgen Westermann, Johannes Textor, Jan Born

[S21-5](#) The Effect of Sleep on Operant Conditioning in *Aplysia Californica*
Albrecht Vorster, Jan Born

[S21-6](#) No effects of increased acetylcholine on odor-induced memory reactivation during slow wave sleep
Jens Gerrit Klinzing, Jan Born, Bjoern Rasch, Susanne Diekelmann

Cueing memory reactivation during sleep

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Sleep strengthens and stabilizes newly acquired memories in an active process of system consolidation. This process is assumed to rely on covert reactivations of new memories that occur spontaneously after learning, mainly during slow-wave sleep (SWS), but can also be externally triggered by learning-associated memory cues. In a series of experiments, we show that the application of associated odor cues during SWS leads to an immediate stabilization of new memories, whereas similar odor reactivations during the wake state destabilize memories. Functional magnetic resonance imaging showed that odor reactivation during SWS mainly activated hippocampal regions and the retrosplenial cortex, whereas during wakefulness reactivation was primarily associated with activations in prefrontal areas. Applying different types of reminders further revealed different effects of reactivation during sleep and wakefulness. In the wake state, only an incomplete reminder but not a complete reminder labilized the memory traces, whereas both the incomplete reminder and the complete reminder stabilized memory representations during sleep. Moreover, odor reactivations during a short sleep episode can accelerate sleep-dependent consolidation processes, leading to memory improvements that are normally seen only after longer sleep periods. Such odor-induced reactivations do not only stabilize memory representations but can also restructure memories thereby fostering the extraction of explicit knowledge. This evidence collectively suggests that reactivation has different effects on memory traces during wakefulness and sleep, with reactivation during sleep enhancing the consolidation and reorganization of new memory representations.

Neuronal oscillations mediating sleep-dependent memory consolidation

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Newly acquired memories are initially hippocampus-dependent and need to undergo a process of active system consolidation, during which they are redistributed to neocortical sites for long-term storage. This process is thought to take place during phases of quiet wakefulness and non-rapid-eye movement (NREM) sleep and is presumably based on the repeated reactivation of memory engrams (patterns of hippocampo-neocortical connections) which gradually drives the establishment of respective direct cortico-cortical connections. During NREM sleep, control via brainstem neuromodulatory systems (in particular the cholinergic one) enables a specific kind of oscillatory activity in the thalamo-neocortico-hippocampal system that facilitates memory reactivation. NREM oscillatory activity is characterized by the neocortical slow oscillation (SO; < 1 Hz), the thalamic sleep spindle (~12-15 Hz), and the hippocampal ripple (> 80 Hz). The intricate interaction of SOs, spindles and ripples constitutes a set of hierarchically nested oscillations, which provides the fine-tuned temporal and spatial structure that is required to orchestrate the reactivation of memory traces and the information flow between hippocampus and neocortex. I will present studies investigating the function of these oscillations in humans both non-invasively by combining surface electroencephalography (EEG) with transcranial magnetic stimulation (TMS) or functional magnetic resonance imaging (fMRI), as well as invasively by means of direct intracranial electroencephalography (iEEG) recordings from epilepsy patients. Together, these studies demonstrate that the intricate coupling of slow oscillations, spindles and ripples can indeed provide the required mechanism that mediates the hippocampo-neocortical dialogue and information transfer during sleep.

The neurochemical mechanisms of sleep-dependent memory consolidation

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Sleep benefits memory by strengthening and transforming initially labile memory traces through repeated reactivations that are thought to initiate neurochemically mediated plastic processes. This consolidation process has been described in most detail for the hippocampus dependent declarative memory domain, where the hippocampus acts as a hub that initially and only temporarily binds the representation, which is transferred to the cortex during consolidation. While more and more is known about the boundary conditions of this effect, i.e., about which memory is improved by sleep under what conditions, and about the electrophysiological mechanisms, the relevant neurochemical underpinnings are currently understudied. Initial landmark studies indicate that the neuromodulatory milieu of early slow wave rich sleep is essential for the consolidation of memory. Essentially, in contrast to encoding (i.e., the learning of new information), consolidation relies on the low tones of acetylcholine and cortisol present during early sleep. Correspondingly, blocking the action of the major excitatory neurotransmitter glutamate at AMPA and NMDA receptors is ineffective in blocking consolidation, whereas this intervention has well documented effects on encoding. Intriguingly, enhancing NMDA receptor activity improves sleep-dependent memory consolidation. In a recent study we could rule out that this paradoxical effect is due to signalling shifting to the metabotropic glutamate receptor 5, which is functionally coupled to the NMDA receptor, as its blockade had no influence on memory. Moreover, we recently discovered that direct electrical coupling via gap junctions may play a major role for consolidation during sleep. In fact, such an essential contribution of direct electrical coupling of neurons nicely fits the importance of fine-tuned electrical oscillatory brain activity orchestrating reactivations that has emerged as the driving force of sleep-dependent memory consolidation.

Effects of sleep on immunological memory processes

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Immunological memory is the formation of antigen-specific T and B cells that store antigenic information for the long-term. Like in the central nervous system, also immunological memory can be subdivided into the processes of encoding, consolidation and recall. During encoding, antigen-presenting cells of the innate immune system serve as an initial store. They take up the antigen, process it and present parts thereof on their surface. During consolidation, the antigenic information is then transferred to T and B cells of the adaptive immune system that represent the long-term store. Consolidation takes place in secondary lymphatic tissues at the so called immunological synapse, where antigen-presenting cells activate T cells with the cognate T cell receptor that structurally fits to the presented antigen. Activated T cells proliferate and in turn support the generation of antibody-producing B cells. Some of these T and B cells survive for the long-term and during recall, these persisting antigen-specific T and B cells can recognize the antigen upon re-encounter and thus allow more efficient immune defense. There is first evidence from studies in animals and humans that sleep promotes the consolidation process at the immunological synapse. In particular slow wave sleep and its unique endocrine constellation with high levels of pro-inflammatory hormones and low levels of anti-inflammatory hormones may support the interaction between antigen-presenting cells and T cells by facilitating their traffic to secondary lymphatic tissues and their cell-to-cell signaling. Presumably these mechanisms mediate enhancing effects of sleep on the formation of antigen-specific T cells and antibodies following vaccination that were observed in healthy subjects. In analogy to its effects on gist abstraction during neurobehavioral declarative memory consolidation, slow wave sleep may additionally serve to select and store the most relevant antigenic information in the T and B cell system.

The Effect of Sleep on Operant Conditioning in *Aplysia Californica*

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Sleep is known to support memory consolidation. This has been shown for mammals and, more recently, also for invertebrates such as bees and *Drosophila*. Here, we investigated whether sleep affects memory consolidation in *Aplysia californica* which has an even simpler nervous system and is a well-studied model of synaptic memory formation. The animals were tested on an operant avoidance learning task ('learning that a food is inedible') three times (Learning, Retrieval 1, Retrieval 2), with a 17 h interval between tests. One group of animals had undisturbed sleep after Learning (Sleep group), the other stayed awake, and recovered sleep after Retrieval 1 (Wake group). Compared with the Wake animals, the Sleep group displayed significantly better performance at Retrieval 1. Moreover, performance was correlated with sleep during the retention interval in the Sleep group at Retrieval 1.

No effects of increased acetylcholine on odor-induced memory reactivation during slow wave sleep

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Sleep-associated memory consolidation is assumed to rely on the reactivation of newly acquired memory representations in the hippocampus, particularly during slow wave sleep (SWS). Mnemonic cues such as odors can be associated with a learning task and then presented again during subsequent sleep to facilitate consolidation of the learned material. Such reminder cues presumably bias hippocampal memory reactivation in favor of the associated content. Reactivated memory traces are communicated to distributed neocortical networks eventually leading to the formation of stable parametric memory representations. This information flow has been suggested to depend on a low cholinergic tone which is characteristic for SWS and results in the disinhibition of connections between hippocampus and neocortex.

In the present study, we pharmacologically increased acetylcholine levels by administering the acetylcholine-esterase inhibitor physostigmine during a 40-minute sleep period. Before sleep, subjects learned card locations in a 2D object location task in the presence of an odor. This odor was presented again during SWS of a subsequent 40-minute sleep period. We expected that increased cholinergic tone would suppress performance improvements known to be triggered by odor-induced memory reactivation.

Contrary to our hypothesis, physostigmine did not suppress the memory-enhancing effect of odor-induced memory reactivation. Odor stimulation during SWS significantly improved memory retention, independently of whether subjects received placebo or physostigmine. We speculate that odor stimulation may trigger neocortical consolidation mechanisms, complementary to previously demonstrated hippocampal targets.

Symposium

S22: From monocytes to microglia - conditions influencing the fate of myeloid cells in the brain

[S22-1](#) Microglial aging
Ingo Bechmann

[S22-2](#) Live analysis of T-cell interactions with myeloid cells within nascent autoimmune CNS lesions
Alexander Flügel

[S22-3](#) Therapeutic potential of myeloid cells in neurodegenerative diseases
Josef Priller

[S22-4](#) Myeloid cells in the CNS
Marco Rudolf Prinz

[S22-5](#) Astrocytes-restricted NF- κ B activation enhances microglial response and induces a transient neuroprotection on Motor Neurons during ALS disease progression
Najwa Ouali Alami, Christine Schurr, Tobias Boeckers, Thomas Wirth, Albert Ludolph, Bernd Baumann, Francesco Roselli

Microglial aging

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In addition to "resting" and "activated" microglia, a third form has been described by W. Streit (2006) in human brains from aged individuals, i.e. dystrophic ("senescent") microglia. We have shown that degenerating neuronal structures positive for tau (neuropil threads, neurofibrillary tangles, neuritic plaques) are invariably colocalized with severely dystrophic rather than with activated microglial cells. Ultrastructural analysis provided evidence for a damaged cytoskeleton in dystrophic microglia. Given the crucial function of microglia for synaptic plasticity, we are currently exploring factors driving microglial aging.

Live analysis of T-cell interactions with myeloid cells within nascent autoimmune CNS lesions

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The initiators of autoimmune CNS processes are T cells carrying receptors on their membrane that are directed against distinct brain-derived proteins. In order to recognize these targets within the CNS, the pathogenic T cells require the help of brain-resident antigen-presenting cells that are derivatives of the myeloid lineage and are distributed as meningeal-, perivascular-, or choroid plexus macrophages, or as parenchymal microglia throughout the CNS tissues. All these cells constitutively express MHC class I and II molecules and thus they have the capacity of displaying these neural antigens to the CNS invading T cells. However, currently it is not clear which cells function as T cell activators in vivo during the course of the autoimmune process. By combining two-photon imaging and functional characterization in a model for MS, namely experimental autoimmune encephalomyelitis (EAE), we track effector T cells and their interactions with myeloid cells in the CNS in detail. We report that contacts with CNS resident myeloid cells critically regulate the migration pattern and functionality of the pathogenic T cells.

Therapeutic potential of myeloid cells in neurodegenerative diseases

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While microgliosis has long been recognized as a pathological feature of neurodegenerative diseases, only recently have innate immune cells been implicated in the etiology of these diseases. Microglia, the resident immune effector cells of the central nervous system (CNS), are dysfunctional in amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD). Therefore, we have been interested in strategies to support microglia. We found that bone marrow-derived cells can attenuate disease progression in animal models of ALS and AD. In particular, we discovered a subset of myeloid progenitors that efficiently target the damaged CNS. A single intravenous injection of these myeloid progenitors attenuated the disease course in the mutant SOD1G93A mouse model of ALS even when administered after disease onset. Moreover, the transplantation of these cells reduced cerebral amyloid load in animal models of AD. The myeloid progenitors exerted long-lasting anti-inflammatory effects, suggesting that they may be promising candidates for cell therapy in neurodegenerative diseases.

Myeloid cells in the CNS

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The diseased brain hosts a heterogeneous population of myeloid cells, including parenchymal microglia, perivascular cells, meningeal macrophages and blood-borne monocytes. To date, the different types of brain myeloid cells have been discriminated solely on the basis of their localization, morphology and surface epitope expression. However, recent data suggest that resident microglia may be functionally distinct from bone marrow- or blood-derived phagocytes, which invade the CNS under pathological conditions. During the last few years, research on brain myeloid cells has been markedly changed by the advent of new tools in imaging, genetics and immunology. These methodologies have yielded unexpected results, which challenge the traditional view of brain macrophages. On the basis of these new studies brain myeloid subtypes can be differentiated with regard to their origin, function and fate in the brain.

Astrocytes-restricted NF- κ B activation enhances microglial response and induces a transient neuroprotection on Motor Neurons during ALS disease progression

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive paralysis due to upper and lower Motor Neurons loss.

SOD1 G93A transgenic mice recapitulate the human disease and have revealed non-cell-autonomous mechanisms involved in MNs degeneration. However, the role of astrocytes and microglia in different phases of ALS progression is still unclear: there is no consensus whether and when astrocytes-driven inflammation may be protective or detrimental.

Our thesis put Astrocytes as an important actor that drives neuro-inflammation contributing to the neurodegenerative process and consequently playing a critical role in the disease progression. We used a genetic strategy based on key transcriptional regulator of inflammation NF- κ B to drive astrocytic inflammatory response at different time points during disease progression.

We used a genetic strategy based on key transcriptional regulator of inflammation NF- κ B to drive astrocytic inflammatory response at different time points during disease progression.

We generated a triple transgenic mouse in which astrocyte-specific NF- κ B activation is achieved by doxycycline-inducible expression of a constitutively active allele of IKK2 in the SOD1 G93A mouse model; the activation of NF- κ B was restricted to astrocytes using a GFAP-mini-promoter-driven tTA gene.

Compared to mSOD1 littermates, the GFAP.tTA/tetO.IKK2-Ca/mSOD1 G93A triple transgenic mice (IKK2 induced from P20) displayed a prolonged onset phase but a reduced progression phase, leading to an unchanged lifespan, despite significant but biphasic effects on motor performance during the disease course.

In the early stages, when beneficial effects on motor performance are detected, we observed a significant decrease in MN disease markers (such as misfolded SOD1 burden, LC3A and p62 build-up) in correspondence of a massive rise in microglial density, amoeboid morphology and of a CD45+ microglial subpopulation appearance together with a significant CD4+ cells infiltration.

However, starting from P70 in the triple-tg mouse microglia assumed a CD68+ phenotype with a senescent morphology, whereas MN markers levels were equal to the mSOD1 mice, suggesting a loss of the beneficial effect driven by astrocytic IKK2/NF- κ B activation. This effect keep going on at p90 as well, accompanied with a degenerating microglia.

Taken together our data demonstrated a bi-phasic role of inflammation in ALS pathogenic cascade, where astrocyte-orchestrated amplification of the inflammatory response is beneficial at early stages, but it turns to detrimental at later time points.

The elucidation of the mediators involved, like the phosphorylation of STAT-6, that include IL-4 pathway, involved in the proliferation processes and lymphocytic function and clearly expressed in astrocytes, may offer new entry points for translational therapeutic interventions, together with Wnt signaling.

Acknowledgments:

We would like to thank Prof. B. Baumann and the institute of physiological chemistry for creating the triple transgenic mouse model and the departments of Neurology and Anatomy and cell biology.

Symposium

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Anton Miroshnikov, Philipp Schlegel, Andreas Schoofs, Hückesfeld Sebastian, Albert Cardona, Michael Pankratz

Neuronal connectome of the *Platynereis* larva

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Precise neuronal synaptic connectivity and its modulation by chemical signaling are ultimately responsible for the circuit dynamics controlling effector activity during behavior. We currently have little information on the complete synaptic connectivity (connectome) of entire neuronal circuits and how these are modulated. The establishment of new, small, relatively simple model organisms could greatly benefit neuroscience by allowing whole-body connectomics and by expanding the range of nervous system phenomena that can be studied. We work on the larval stages of the marine annelid *Platynereis dumerilii* that has recently emerged as a powerful experimental system for the study of neural circuits and neuromodulation in a whole-body context. We use connectomics, neurogenetics, activity imaging, and behavioral experiments to understand how circuits influence behavior and physiology in the planktonic larvae of *Platynereis*. By studying different larval stages, we can also gain insights into how circuit maturation influences larval behavior during development.

Neural circuits for multisensory integration and memory-based behavioral choice

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Animals sense the local environment, learn and remember past events, predict future ones, and combine current and past information to choose and coordinate appropriate motor responses. Underlying these capabilities is the nervous system, which continuously integrates multiple sources of information and chooses one response in exclusion to all others. These operational patterns depend on the synaptic-level structure of the wiring diagram of the central nervous system (CNS). Knowing the complete wiring diagram of a CNS would facilitate the interpretation of functional and behavioral data, and enable the formulation of circuit-level hypotheses of neural function. Together with collaborators around the world we are currently mapping the synaptic-level wiring diagram of the entire nervous system of the larva of *Drosophila*, an established model system for developmental biology that is now emerging as a powerful model system for studying the structure-function relationship in neural circuits. I will present the technology behind the collaborative reconstruction of neural circuits and our current understanding of brain-wide circuit patterns underlying sensory processing and memory-based behavioral choice.

The calycal microglomerulus: a small circuit in the spotlight

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The insect mushroom body (MB) is essential for the formation and retrieval of multiple types of memory, including olfactory associative memories. Within its input region, the MB calyx, olfactory information is delivered from second order projection neurons (PN) to the Kenyon cells (KCs). Here, large PN boutons contact multiple claw-like dendrite termini of KCs, forming characteristic Microglomeruli (MGs).

With the availability of a whole brain electron microscopy (EM) volume of an adult female fly (Bock et al., unpublished), we reconstructed the complete circuitry of a MG and identified all the neurons that compose it and their local connections. Starting off from a PN bouton, we identified all cell types pre- and postsynaptic within the MG synaptic complex by tracing to identification and annotating each synaptic connection. This allowed us to describe the connectome of a single MG. Our data suggest that MGs are autonomous computational relays.

Deconstruction and reconstruction of olfactory computations in zebrafish

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The olfactory system has emerged as a major model to study high-dimensional and non-topographical neuronal codes and computations. Many such computations are thought to depend on the connectivity between specific ensembles of neurons (the “wiring diagram”). In principle, dense reconstructions of wiring diagrams can be achieved by volumetric electron microscopy (EM) based on automated sectioning techniques and subsequent anatomical reconstructions of neurons and synapses. We have advanced techniques for serial block face scanning EM (SBEM), established an efficient workflow for manual skeleton tracing of neurons in 3D EM datasets, and developed efficient procedures for error correction. These methods allowed us to reconstruct all 1047 neurons in the olfactory bulb (OB) of a zebrafish larva. Prior to circuit reconstruction we measured odor responses of OB neurons by multiphoton calcium imaging. Activity measurements were mapped onto reconstructed neurons to analyze direct relationships between the connectivity of a neural circuit and its function (“functional connectomics”).

The OB receives input from sensory neurons that terminate in distinct neuropil units, the glomeruli. In adults, the olfactory bulb consists of principal neurons, the mitral cells, and two layers of interneurons: a superficial interneuron network containing periglomerular and short-axon cells, and a deep interneuron network containing numerous granule cells. We classified neurons of the larval OB based on their morphology and identified two new rare cell types. Surprisingly, most interneurons were classified as superficial interneurons while typical granule cells were almost completely absent. These and additional results show that, at early developmental stages, the OB contains a “core circuitry” corresponding to the superficial interneuron network while the deep network develops later. This “core circuitry” is similar to the neuronal circuitry of the insect antennal lobe.

Neuronal computations in the OB depend on interactions between glomeruli that are mediated by interneurons. We found that interneurons often had widespread projections, and that subsets of interneurons preferentially innervated common subsets of glomeruli. Hence, inter-glomerular interactions are neither random nor organized by an obvious topographical principle but governed by glomerular identity. The annotation of synapses between OB neurons is still ongoing. However, results already provide strong evidence that the specific interactions between glomeruli mediate transformations of odor-evoked activity patterns that support the classification of natural odorants. Hence, these results indicate that “higher-order” computations can be determined by specific wiring diagrams, and that mechanistic insights into such computations can be obtained by functional connectomics approaches.

This work was supported by the Novartis Research Foundation, the Swiss Nationalfonds (SNF), and the Human Frontiers Science Program (HFSP).

Complete connectome of a learning circuit

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Mushroom bodies (MB) are central brain structures that enable insects to learn and remember different sensory stimuli. Kenyon cells (KC), the intrinsic cells of the MB, are known to link rewarding and punishing experiences with olfactory stimuli in bees, fruit flies and other insects. Activity dependent presynaptic plasticity has been implicated in learning, based on the joined activation of KCs by olfactory projection neurons and dopaminergic MB input neurons. Hence, it was suggested that a memory trace is located at the KC synapses to the MB output neurons. Yet, the synaptic architecture of the entire MB circuit was never shown.

To address this issue we reconstructed the complete wiring diagram of the MB from a first instar *Drosophila* larval brain electron microscopy (EM) dataset. We reconstructed the anatomy and connectivity of all KCs (223 cells) in both brain hemispheres and all pre- and postsynaptically connected neurons (166 cells).

Analyzing the patterns of olfactory input into KC dendrites revealed two different types of KCs. In the first group of KCs, each cell receives specific input from a single olfactory projection neuron. In the second group, the KCs receive input from two to six olfactory projection neurons in a manner indistinguishable from random. This olfactory input to KC connectivity reduces redundancy and maximizes dimensionality of the odor representation. MB input neurons and MB output neurons tile the entire MB in eleven non-overlapping compartments. In each compartment, we found a canonical circuit motif of connections between KCs, MB input neurons, and MB output neurons. While it was known that MB input neurons connect to KCs and KCs synapse onto MB output neurons we found additional connections. Surprisingly KCs synapse back onto MB input neurons and MB input neurons directly connect to MB output neurons within the compartment. Furthermore, input and output neurons participate in feedback and feed-across connections creating a neuronal basis that could support long-term memory and inhibition between the appetitive and aversive centers of the MB.

Moreover we found that MB output neurons connect to one another, creating a hierarchical feed-forward circuit and inhibitory pathways between the appetitive and aversive MB centers.

In summary, our EM reconstruction describes all cells and connections in an insect MB for the first time. The discovery of a repeated canonical motif, combined with the power of *Drosophila* genetics, will allow the careful dissection of the role of each interaction between the cells involved in learning and memory. Knowing the connectivity and circuit motifs supports the formation of functional models, which can now be tested in behavioral assays and allows us to answer questions in a targeted way.

The Sensory-Motor-Architecture of Feeding Networks in Flies

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Due to constant development of new tools, the fruit fly *Drosophila melanogaster* has become one of the major model organisms for studying the neuronal basis of behavior. Despite its superficial simplicity and an approx. 5x10³-fold reduction of neurons compared to mouse, the *Drosophila* larva is still capable of complex tasks, such as evaluation of food sources and subsequent adaptation of its foraging strategy.

In both flies and mammals, central olfactory pathways were shown to have similar organization. In comparison, much less is known about the organization of neural circuits that integrate other types of external and internal sensory information such as gustatory stimuli.

Based on ssTEM scans of an entire CNS we investigate the organization of afferent and efferent neurons which control feeding behavior in the subesophageal zone (SEZ). By combining EM-reconstruction and confocal microscopy for visualizing gustatory afferents, we provide a compartmentalization of the primary taste center in the SEZ depending on gustatory receptor neurons and their sensory organ origin. Based on this, we define areas that are specifically targeted by either external, pharyngeal or enteric sensory neurons.

Next, we investigate the second order connectivity of those non-olfactory sensory neurons by focusing on the connectivity to four potential downstream systems. Therefore, we first identified and characterized the feeding motor neurons, efferent serotonergic neurons (Se0), neuroendocrine neurons of the pars intercerebralis (IPCs, DH44, DMS) [1] and a cluster of 20 bitter gustatory interneurons which produce the neuropeptide Hugin [1,2].

Analysis shows that external and pharyngeal gustatory neurons are unlikely to make direct synaptic connections onto either the motor- or the neuroendocrine system, suggesting that integration of gustatory stimuli occurs deeper within the circuitry. However, we find highly selective, monosynaptic connections between enteric sensory neurons and the neurosecretory cells (NSC) and between presumed non-gustatory sensory neurons (e.g. mechano) and neurons of the motor/serotonergic system.

This data provides a framework for studying the integration of afferent signals from different body regions to induce appropriate changes in behavior.

[1] Hückesfeld et al., 2016, Nature Com

[2] Schlegel et al., 2016, eLife ;10.7554/eLife.16799

Symposium

S24: Breaking News

- [S24-1](#) PARKINSONIAN RATS RESPOND TO ULTRASONIC VOCALIZATIONS: A NEW ANIMAL MODEL OF PARADOXICAL KINESIA
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- [S24-2](#) Repetitive magnetic stimulation restores alterations in synaptic excitation/inhibition-balance of hippocampal slice cultures in the Poly(I:C) gestational immune activation model of schizophrenia
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Ankita Ravi Vaswani, Jan-Hendrik Spille, Martin Schwarz, Wolfgang Hübner, Ulrich Kubitscheck, Sandra Blaess
- [S24-6](#) 5-HT_{2A} agonist TCB-2 reduces neuropathic pain through up-regulation of KCC2
Irene Sanchez-Brualla, Pascale Boulenguez, Cécile Brocard, Sylvie Liabeuf, Annelise Viallat-Lieutaud, Xavier Navarro, Esther Udina, Laurent Vinay, Frédéric Brocard
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- [S24-10](#) Role of serotonergic signaling in regulation of astrocytes morphology.
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PARKINSONIAN RATS RESPOND TO ULTRASONIC VOCALIZATIONS: A NEW ANIMAL MODEL OF PARADOXICAL KINESIA

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Parkinson's disease is a neurodegenerative basal ganglia disease which leads to a global deterioration in motor function, such as bradykinesia (slowness of movement) as one of the most characteristic clinical features. Immobile patients who become excited by external stimuli may be able to make quick movements, such as catching a ball or running. There are several reports of this phenomenon called paradoxical kinesia which refers to a sudden transient ability of akinetic Parkinsonian patients to perform motor tasks they are otherwise unable to perform. The mechanisms underlying this phenomenon are unknown due to a paucity of valid animal models that faithfully reproduce paradoxical kinesia. Our aim was to develop a new method to evaluate paradoxical kinesia in cataleptic rats by presenting species-relevant signals, namely rat ultrasonic vocalizations. To test the effects of ultrasounds in cataleptic animals, male rats received haloperidol (0.5mg/kg, IP); 60 min after injection, the bar test was performed during which a given rat was exposed to different playback presentations of appetitive 50-kHz USV, aversive 22-kHz USV, or relevant acoustic controls. The time until a rat steps down from the bar with both forepaws was considered the catalepsy time. Every animal was exposed to all acoustic stimuli in random order. Cataleptic rats rapidly stepped down from the bar when exposed to playback of appetitive 50-kHz USV, but not in response to either aversive 22-kHz USV or acoustic controls. In addition, rats exposed to 50-kHz USV showed clear approach behavior towards the sound source. Our animal model fulfills the criterion of face validity and provides a completely new approach to studying paradoxical kinesia which might be useful for uncovering the mechanisms behind this phenomenon and improving behavioral therapies for Parkinson's disease.

Repetitive magnetic stimulation restores alterations in synaptic excitation/inhibition-balance of hippocampal slice cultures in the Poly(I:C) gestational immune activation model of schizophrenia

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Activation of the maternal immune system during gestation has been identified as a major risk factor for psychiatric disorders in the offspring. To study the effects of gestational infection on neural and behavioral dysfunction in the offspring, preclinical models of maternal immune activation (MIA) have been developed. Here we prepared entorhino-hippocampal slice cultures from the offspring of the well-established polyinosinic–polycytidylic acid [Poly(I:C)] MIA model of schizophrenia to test for MIA-associated alterations in hippocampal circuits. Whole cell patch-clamp recordings disclose alterations in synaptic excitation/inhibition-balance in CA1 pyramidal neurons in slice cultures prepared from Poly(I:C) offspring: an increase in inhibitory synaptic strength is observed, while excitatory synapses are not affected. Using a conventional figure-of-eight coil, that is also used in clinical practice, we employed repetitive magnetic stimulation (rMS) to test whether we can influence the synaptic phenotype in MIA-slice cultures. Indeed, 10 Hz rMS reversed the increased inhibitory synaptic strength in MIA-slice cultures without affecting excitatory synaptic strength. These results demonstrate that rMS can restore MIA-associated alterations in synaptic excitation/inhibition-balance. Thus, ‘organotypic in vitro MIA models’ may serve as preclinical drug and intervention screening assays to evaluate new therapeutic strategies of early stages in psychiatric diseases associated with gestational infection. [supported by Federal Ministry of Education and Research, Germany; GCBS-WP1: 01EE1403B]

The choreography of learning walks is crucial for the navigational performance of *Cataglyphis* desert ants

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Cataglyphis desert ants are famous model organisms for navigation. When they begin their foraging career outside the nest, they must start to navigate visually using celestial cues (e.g. the polarization pattern of the sky, or the sun's azimuthal position) or visual landmarks. For that, the ant and its brain have to accomplish new tasks! This transition from indoor worker to forager takes between one and three days. In this time the ants perform so-called learning walks and their brains undergo neuronal changes. We studied the learning walks of different *Cataglyphis* species in their habitats offering two dissimilar environments: Saltpans in Tunisia are barren and offer almost no natural landmarks. In contrast, glades in a pine forest in Greece are much more cluttered and the trees and bushes there serve as a diverse panorama. Using high-speed video analysis techniques, we show that the choreography of learning walks of *Cataglyphis* ants is very robust and includes different rotational movements with stopping phases. The naïve newcomers exhibit a characteristic sequence of learning walks. In the beginning, the ants perform meandering learning walks only within a few centimeters distance from the nest entrance and in all directions of the compass. But then the ants move further away and start to forage after three to seven learning walks. Our experiments show that the ants must perform learning walks at the beginning of their foraging career to acquire landmark information about their nest's surroundings and enable successful navigation later on as foragers. Remarkably, the ants perform their learning walks even when the visual cues at the nest entrance have been altered. In addition, we studied which neuronal changes accompany the behavioral transition of *Cataglyphis* desert ants at the beginning of their foraging career.

This study was supported by the German Research Foundation (DFG), Collaborative Research Center SFB1047 "Insect Timing" [Project B6 to W.R. and R.W.].

Representation of the auditory space in the barn owl's midbrain: Does every spike matter?

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The barn owl is a nocturnal bird of prey and as such a specialist in sound localization. It is known to catch prey in complete darkness, like a mouse rustling on the floor. For localizing sound sources in the horizontal plane (azimuth) it relies primarily on the interaural time difference (ITD). An implementation of the Jeffress Model with axonal delay lines and coincident detectors allows the detection of ITDs as small as 10 microseconds. Detection of ITD occurs in narrow frequency channels. This leads to so called phase ambiguities whenever the period of a sound wave is smaller than the physiological ITD range (approx. +/-270 microseconds in the barn owl). The ambiguity is resolved by across-frequency integration. In the external nucleus of the inferior colliculus (ICX), so-called space-specific neurons respond to a broad frequency range. The responses of the neurons show reduced ambiguities when acoustically stimulated with suitable frequency contents. These neurons also incorporate information about the interaural level difference (ILD) that changes with elevation and thus many neurons tuned to different ITDs and ILDs form a neural map of auditory space.

Stimulation with broadband noise at best binaural cues (100 ms duration, medium stimulus level) evokes response rates that are often below 60 spikes per second. Considering the stimulus duration, only six action potentials are produced under these nearly optimal conditions for a single stimulus. Even though across frequency integration is reducing ambiguities, neurons may fire at half that rate at phase-equivalent ITDs, separated from the best ITD by the period of the neuron's center frequency. The absolute difference of spike counts is very small. This may cause as problem in separating the true ITD from its phase-equivalents.

Therefore, we took a closer look on the time course of spiking and spike-frequency adaptation (SFA). SFA describes the decrease of a neuron's response during an ongoing stimulus. In several series of experiments, we investigated SFA as a function of the ITD and the frequency contents of a stimulus. Here we try to address the question, how SFA influences the reliable representation of auditory space.

Extracellular recordings were obtained from neurons in ICX of anesthetized American barn owls (*Tyto furcata*). The stimuli were presented via earphones with different ITDs and frequency bandwidth to study the influences of those parameters for SFA. Peri-stimulus time histograms (PSTH) were assembled from responses to 20 or 200 repetitions of the stimulus.

At optimal conditions (broadband noise and best ITD), the great majority of neurons responded in a "phasic-tonic" (or primary like) manner, i.e. with a strong onset peak followed by a sustained response over the whole stimulus duration. The tonic component was strongest for responses to the unit's best ITD and became weaker for less favorable ITDs. Stimulation with smaller bandwidths also usually led to stronger SFA. However, neurons tended to fire at the stimulus onset even at suboptimal conditions. In other words, the difference of the response rate a neuron exhibits for different ITDs arises continually during the ongoing stimulus.

Facing the task of reliably detecting and localizing prey, the neural circuits of the owl seem to be optimized to signaling the presence of a sound (onset response) and refining the representation of its direction if and while the information becomes available during ongoing sounds.

Understanding the mechanisms that regulate the migration of midbrain dopaminergic neurons in the developing brain.

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Midbrain dopaminergic neurons (MbDNs) are involved in the regulation of voluntary movement, reward behavior and cognitive processes. MbDNs are born in the floor plate of the ventral midbrain from where they migrate to form three anatomically distinct structures: the substantia nigra (SN), the ventral tegmental area (VTA) and the retro rubral field (RRF). We showed previously that MbDNs that give rise to the VTA migrate radially from the floor plate, while those that form the SN migrate first radially and then tangentially to take up a more lateral position. As they migrate, MbDNs simultaneously extend their axonal projections towards their forebrain targets. The molecular mechanisms that coordinate MbDN migratory behavior and axonal outgrowth are not understood. We are investigating the role of the Reelin signaling pathway in these processes. Reelin is an extracellular matrix protein that is known to regulate neuronal migration in the cortex and other brain areas. Disabled 1 (Dab1) is a key regulator of Reelin signaling. When we inactivate Dab1 in differentiated MbDNs, we find that MbDNs are medially clustered and fail to migrate to the SN, indicating that Dab1 is directly required in MbDNs for their correct localization. We are currently investigating how the loss of Dab1 affects the cell dynamics of migrating MbDNs. To monitor the morphology, process orientation and axonal outgrowth of MbDNs, we inactivate Dab1 in a small subpopulation of fluorescently marked MbDNs. This mosaic labeling of MbDNs allows for easier visualization of MbDNs that are densely clustered during development. We study these fluorescently labeled MbDNs with a combination of time-lapse imaging in organotypic slice cultures and light sheet microscopy of the dopaminergic system in cleared whole-mount embryonic brains.

5-HT_{2A} agonist TCB-2 reduces neuropathic pain through up-regulation of KCC2

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After a spinal cord injury, the expression of the potassium chloride cotransporter KCC2 is reduced in motoneurons and in dorsal horn interneurons from laminae I and II. This causes the loss of chloride homeostasis, which impairs GABA/glycine inhibition of these cell types, causing spasticity and neuropathic pain, respectively.

TCB-2, an agonist of 5-HT_{2A} receptors, has shown to increase GABA/glycine inhibition on motoneurons after a complete spinal cord transection, reducing spasticity through a mechanism that involves KCC2 upregulation. Here, we show that in a model of neuropathic pain -spinal cord hemisection-, TCB-2 reduces mechanical and thermal hyperalgesia. Moreover, immunohistochemical analysis shows that TCB-2 injection increased KCC2 expression in the dorsal horn of the segments caudal to the injury site. TCB-2 effects on the algesimetry tests were reduced by the intrathecal injection of DIOA ((dihydro-indenyl)oxy]alkanoic acid), an inhibitor of KCC2, showing that the analgesic effect of TCB-2 was dependent upon KCC2 activity. Finally, TCB-2 proved to reduce mechanical allodynia after a peripheral nerve injury, also increasing KCC2 expression on the dorsal horn of the injured side, quantified by immunohistochemistry. These findings show the interest of TCB-2 as a drug that enhances KCC2 expression and is able to reduce neuropathic pain after an injury to the nervous system.

Oscillatory entrainment and firing patterns encode visual-tactile information in first-order thalamic nuclei

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Behavioral performance relies on the integration of information from different sensory modalities. This process takes place both at cortical and subcortical level. Phase reset of network oscillations and modulation of neuronal firing along direct axonal projections between primary somatosensory (S1) and primary visual (V1) cortices have been identified as underlying mechanisms of cross-modal processing. However, their disruption led to persistence of multisensory interactions, suggesting that integration of different senses occurs already earlier on the sensory tract. To decide whether first-order thalamic nuclei contribute to cross-modal processing, we assess the impact of uni- (light flash or whisker deflection) and bimodal stimulation (i.e. simultaneous light flash and whisker deflection) on oscillatory activity and neuronal firing within thalamocortical networks by performing simultaneous extracellular recordings from S1, ventral posteromedial nucleus (VPM), V1 and lateral geniculate nucleus (LGN) in pigmented rats *in vivo*. Bimodal stimulation modulates VPM and LGN firing 40 - 80 ms, but not 0 - 40 ms post-stimulus. In addition, it times the neuronal firing by shortening the spiking onset in VPM and by augmenting cross-sensory thalamo-cortical and thalamo-thalamic spike-LFP coupling. At network level, bimodal stimulation enhances evoked responses in VPM, but not LGN. Our results indicate that visual-tactile stimulation modulates network oscillations as well as spiking patterns in first-order thalamic nuclei.

Inhibitory synaptic adhesion proteins regulate anxiety processing

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Anxiety is a debilitating condition that affects 3% - 10% of adult population in the world. Mapping of the brain activity in patients and in mouse models showed that anxiety- related behavior is triggered by abnormally high firing of basal amygdala and its output nucleus, centromedial amygdala. A plausible mechanism for this increased excitatory drive is dysfunction of inhibitory synapses, but proteins that contribute to such dysfunction, and may serve as novel targets for pharmaceutical intervention, are largely unknown. Previously, we showed that deletion of inhibitory synaptic protein Neuroligin 2 causes robust anxiety phenotype and upregulates excitatory projection neurons in basal amygdala, that in turn robustly enhance the firing of centromedial amygdala under anxiogenic conditions. Importantly, mutations in Neuroligin 2 were identified in patients with schizophrenia, that shows prominent comorbidity with anxiety. These findings indicate that Neuroligin 2 plays an important role in maintaining the function of anxiety processing brain regions. To identify additional inhibitory synaptic proteins involved in anxiety regulation, we currently investigate the function of novel protein IgSF9b that interacts with Neuroligin 2 in vitro and hence potentially modulates the anxiety phenotype of Neuroligin 2 knock out mice. We found that in sharp contrast to Neuroligin 2 knock outs, deletion of IgSF9b decreases anxiety behavior and enhances the activation of inhibitory interneurons in basal amygdala under anxiogenic conditions. Moreover, deletion of each protein affects distinct subsets of inhibitory synapses in basal and centromedial amygdala. Strikingly, those differential effects are combined in mice lacking both proteins in a way that completely restores anxiety expression and firing of centromedial amygdala to wild type level. These data raise an intriguing possibility that Neuroligin 2 and IgSF9b bi directionally regulate anxiety processing. Finally, we sought to determine whether in addition to regulating the number of active neurons, Neuroligin 2 and IgSF9b may coordinate the neural activity in basal and centromedial amygdala during exposure to anxiogenic conditions. For that we established simultaneous local field potential recordings in basal and centromedial amygdala of behaving mice. Our preliminary data shows that deletion of Neuroligin 2 enhances the theta (4-8 Hz) power in both regions in mice exposed to anxiogenic conditions, suggesting that Neuroligin 2 shapes the dynamics of anxiety processing circuit. Taken together, our findings provide an insight into molecular mechanisms of anxiety and potential drug targets for its treatment.

Differential regulation of synaptic proteomes after appetitive and aversive auditory learning in mice

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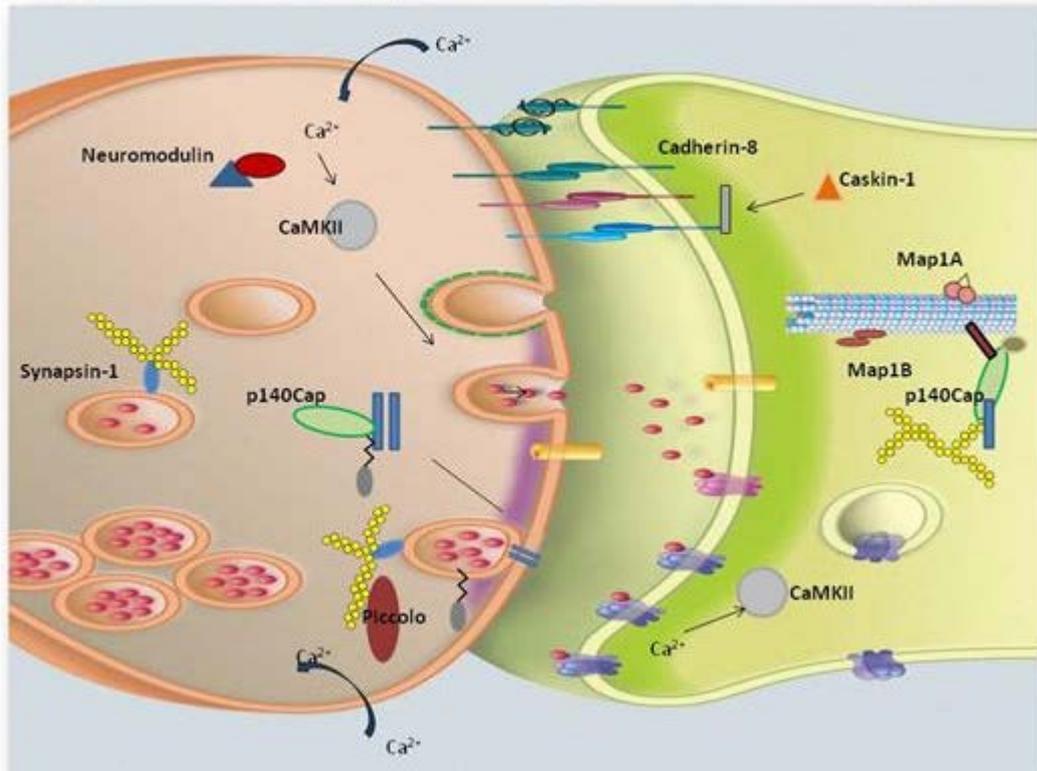
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Learning and memory formation can be modulated by positive or negative reinforcement. However, only sparse knowledge exists how the polarity of reinforcement affects synaptic plasticity required for learning and memory consolidation. Therefore, we designed an auditory detection and discrimination paradigm in mice that allows the application of appetitive and aversive motivation in order to perform a comparative proteomic characterization of synaptic protein and phosphorylation profiles after reward vs. punishment. Electrical brain stimulation in the medial forebrain bundle was delivered as appetitive reinforcement when mice correctly responded to a CS+ tone by crossing the hurdle in a shuttle box. Mild footshock punishment was applied as aversive reinforcement upon missed hurdle crossing or crossing in response to CS- tones. For each reinforcement polarity, separate groups of mice were trained for one training session and sacrificed 6h later for biochemical enrichment of postsynaptic densities (PSD) from four brain areas, i.e. the auditory cortex, frontal cortex, hippocampus, and striatum. A label-free proteomics approach based on TiO₂-enrichment and high resolution mass spectrometry on an Orbitrap Velos-Pro mass spectrometer was used to identify and quantify synaptic protein expression and phosphorylation signatures in comparison of the two reinforcement polarities.

The proteomic screen revealed 102 proteins and 345 phosphopeptides significantly changing their relative synaptic abundance after learning as compared to naive controls. Moreover, the relative abundance of 32 proteins and 137 phosphopeptides was differentially regulated between aversive and appetitive reinforcement (see figure 1). One of the most promising candidate proteins is p140Cap, showing polarity-dependent learning-induced changes in synaptic protein abundance and phosphorylation. This protein, also known as SRC kinase signaling inhibitor 1, is a key element involved in dendritic spine morphology by promoting actin stability and nucleation as well as functionally linking actin cytoskeleton to microtubule dynamics. Furthermore, p140Cap is also located within the presynapse where it can interact with several proteins involved in synaptic vesicle secretion. We have addressed the particular phosphorylation sites of p140Cap with respect to its known multiple adapter functions. Mutations of selected serine residues have been studied in vitro using the BioID approach to characterize altered interactomics patterns.

Figure 1: Identification of polarity-dependent learning-induced changes in protein phosphorylation



Role of serotonergic signaling in regulation of astrocytes morphology.

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Serotonin is an important neurotransmitter regulating various brain functions via activation of specific serotonin receptors. However, the contribution of serotonergic signaling in regulation of the numerous astrocytic functions is only poorly elucidated. Astrocytes possess a unique morphology which enables single astrocytes to sense and modulate signaling and plasticity at adjacent neuronal synapses. It is known that astrocytes' Ca²⁺ signaling is implicated in these functions. Properties and propagation of Ca²⁺ signals depend on diffusion and therefore on astrocyte morphology, which is dynamic itself. However, it remains poorly understood which signaling cascades are critical in controlling astrocyte morphology. We therefore propose to uncover the mechanisms by which small GTPases of the Rho family, which can be regulated by serotonin receptors, control astrocyte morphology and astrocyte Ca²⁺ signaling.

We showed that knockdown of a defined serotonin receptor lead to a more ramified morphology in cultured mouse hippocampal astrocytes (figure 1A). Furthermore, transient overexpression of the small GTPase RhoA resulted in drastic morphological changes displayed by decreased perimeter, compactness and size (figure 1B). Sholl analysis also revealed impact of RhoA on the arborization of mouse hippocampal astrocytes. Moreover, our recent experiments suggest that astrocytes Ca²⁺ dynamics correlate with their morphology. Together, these data indicate that serotonin receptors are critically involved in regulation of astrocyte morphology and Ca²⁺ signaling.

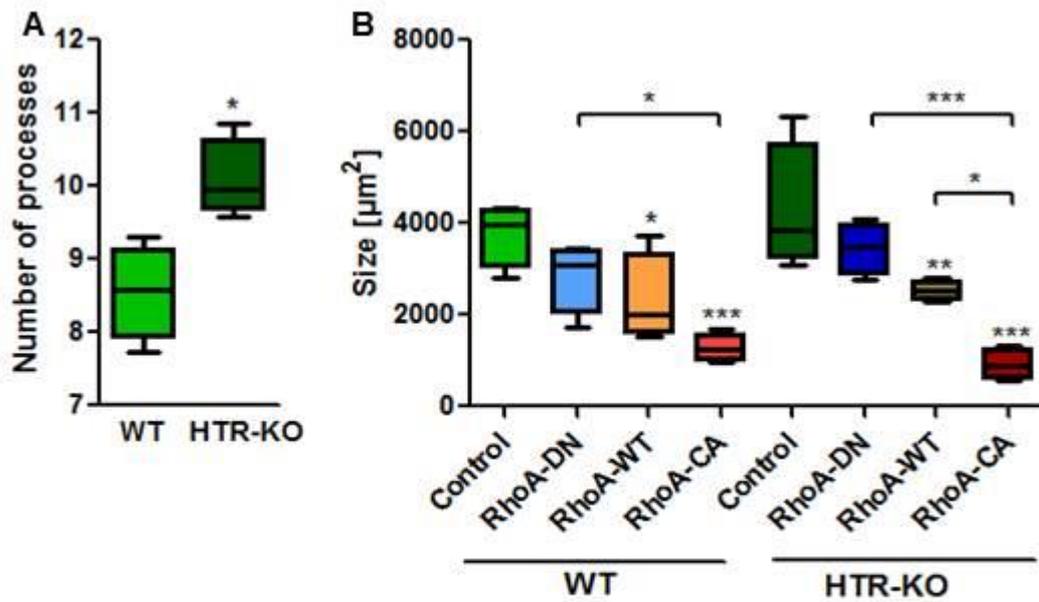


Figure 1: **A)** Sholl Analysis reveals differences in the mean number of processes between wildtype (WT) and serotonin receptor knockout (HTR-KO) astrocytes from the mouse hippocampus. **B)** AAV-induced overexpression of different activity altered mutants of the small GTPase RhoA leads to reduction in cell size in both, WT and HTR-KO astrocytes in hippocampal cultures. DN= dominantly negative, WT= wildtype, CA= constitutive active.

Symposium

S25: Spike timing-dependent plasticity: from functions in circuits towards possible treatment of humans

[S25-1](#) STDP and its function in neural circuits
Jochen Triesch

[S25-2](#) How spike patterns shape spike timing-dependent plasticity rules and underlying signaling mechanisms
Elke Edelmann, Efrain Cepeda-Prado, Volkmar Leßmann

[S25-3](#) Non-invasive assessment of timing-dependent plasticity in the human motor system
Patrick Ragert

[S25-4](#) Repetitive transcranial magnetic stimulation: Are we exploiting spike-timing dependent plasticity for the treatment of patients?
Andreas Vlachos

[S25-5](#) Synaptic input and output of hilar mossy cells during sharp wave ripples.
Aarti Swaminathan, Ines Wichert, Nikolaus Maier, Dietmar Schmitz

STDP and its function in neural circuits

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Since its theoretical prediction and experimental confirmation in the 1990s, spike timing-dependent plasticity (STDP) has been a topic of intense interest for theoreticians and experimentalists alike. Since its discovery, STDP has been observed experimentally in various preparations ranging from cultured neurons to intact circuits. Furthermore, plasticity phenomena consistent with an STDP interpretation have been reported in human subjects. Classic STDP rules have a causal nature. They increase the efficacy of a synaptic connection if the presynaptic spike helped to trigger a post-synaptic spike and decrease it if the order is reversed. This suggests a fundamental role of STDP in the brain's ability to learn causal models allowing the prediction of future events. However, the unstable nature of STDP dictates that it must be combined with homeostatic mechanisms.

A family of computational models developed in our lab, self-organizing recurrent neural network models (SORNs), have revealed how the combination of STDP and homeostatic plasticity mechanisms can solve the instability problem and endow recurrent spiking networks with predictive learning abilities (Lazar et al., 2009). More recently, an extended version of this model has demonstrated how the combination of STDP with homeostasis can account for the major properties of spontaneous brain activity and its relationship to stimulus-evoked activity (Hartmann et al., 2015). Furthermore, models from the SORN family have recently explained various structural and dynamic features of the cortical connectome including the statistical distribution of excitatory synaptic efficacies, the long-term dynamics and lifetime distributions of synapses, as well as the over-abundance of bidirectional connections and certain graph motifs (Zheng et al., 2013; Miner & Triesch, 2016). Ongoing work is extending these findings to inhibitory synaptic connections onto excitatory neurons.

Taken together, these results suggest that STDP mechanisms are playing a central role in shaping both the structure and function of cortical circuits. More computational and experimental work is needed, however, to fully appreciate the diversity of STDP rules among different pairs of cell types and their functional meaning.

References:

Hartmann, C., Lazar, A., Nessler, B., & Triesch, J. (2015). Where's the Noise? Key Features of Spontaneous Activity and Neural Variability Arise through Learning in a Deterministic Network. *PLoS Comput Biol*, 11(12), e1004640.

Lazar, A., Pipa, G., & Triesch, J. (2009). SORN: a self-organizing recurrent neural network. *Frontiers in computational neuroscience*, 3, 23.

Miner, D., & Triesch, J. (2016). Plasticity-driven self-organization under topological constraints accounts for non-random features of cortical synaptic wiring. *PLoS Comput Biol*, 12(2), e1004759.

Zheng, P., Dimitrakakis, C., & Triesch, J. (2013). Network self-organization explains the statistics and dynamics of synaptic connection strengths in cortex. *PLoS Comput Biol*, 9(1), e1002848.

How spike patterns shape spike timing-dependent plasticity rules and underlying signaling mechanisms

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Cellular mechanisms that mediate synaptic plasticity are believed to underlie learning and memory related processes in the brain. In this respect, investigating the signaling cascades that enable induction and expression of long-term potentiation (LTP) and long-term depression (LTD) are prerequisites to correlate cellular synaptic changes with memory formation. To induce synaptic plasticity events that resemble the respective cellular processes related to learning and memory *in vivo*, the use of physiologically relevant protocols is of utmost interest. One suitable model to investigate such *in vivo* like types of synaptic changes is spike timing-dependent plasticity (STDP). STDP consists of exactly timed coincident activation of pre- and postsynaptic neurons in forward or backward sequence that induce bidirectional and timing-dependent changes in synaptic efficacy, termed t-LTP and t-LTD, respectively. Besides accurate timing of pre- and postsynaptic activity, timely availability of neuromodulators (e.g., dopamine) and mediators of synaptic plasticity during LTP/LTD (e.g., brain-derived neurotrophic factor, BDNF) are essential. Here we focus mainly on actions of BDNF to mediate STDP.

Using whole cell patch clamp techniques, we asked whether BDNF is necessary to induce STDP in CA1 pyramidal neurons of acute hippocampal brain slices of rats and mice. Furthermore, we addressed the questions, whether BDNF is a common mediator that is equally involved in synaptic plasticity induced by different STDP paradigms. To address these questions we applied variants of two distinct STDP paradigms. Besides a canonical paradigm consisting of one presynaptic stimulation paired with also just one postsynaptic action potential (1:1 paradigm), we also employed a pairing of one presynaptic and four postsynaptic stimulations (1:4 paradigm) as our second STDP paradigm (termed theta burst like t-LTP). Pairing frequency was set to 0.5Hz, while the repeat number of paired stimulation was varied. Our results show that BDNF release for induction of t-LTP is recruited specifically by inclusion of the postsynaptic high frequency theta burst and that a certain minimal number of repeats are mandatory for efficient BDNF secretion. Inducing t-LTP with a low repeat number led to a shift from BDNF-dependent towards BDNF-independent mechanism of expression of t-LTP. For t-LTP induced by the canonical 1:1 STDP paradigm, we did not observe any BDNF effect at high or low number of repeats. However, the 1:1 induced t-LTP types were strongly dependent on modulation by dopamine, as was evident from experiments making use of D1-like and D2-like dopamine receptor antagonists (for more details, see Poster by Cepeda-Prado et al., this meeting). According to our data, BDNF-dependent and independent t-LTP types can be induced subsequently and exist simultaneously and independently at the same synaptic connections.

In summary, our data indicate that LTP can be induced robustly with different STDP paradigms that might mimic physiologically relevant changes in synaptic efficacy. However, depending on the applied STDP paradigms different signaling mechanisms for establishing t-LTP can be activated. Altogether, this suggests that multiple and parallel ways of synaptic plasticity can co-exist at selected Schaffer collateral CA1 synapses in the hippocampal circuit.

The work is supported by the DFG (SFB779/B06 and ED 280-1/1).

Non-invasive assessment of timing-dependent plasticity in the human motor system

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Over the past decades, a huge variety of stimulation protocols have been introduced to investigate functional neuroplasticity non-invasively in the living human brain. Paired associative stimulation (PAS) is a specific form of non-invasive brain stimulation which consists of a median nerve stimulation followed by transcranial magnetic stimulation (TMS) applied to the contralateral primary motor cortex (M1). Several studies indicated that the outcome of PAS is based on timing-dependent rules, comparable to those identified in animal studies. More specifically, synchronous arrival of inputs (here median nerve stimulation and TMS) to M1 usually results in increased excitation whereas asynchronous arrival decreases it. Since those effects are NMDA receptor- dependent and can outlast the stimulation period for several minutes, such alterations have been considered as long-term potentiation (LTP)- and long-term depression (LTD)-like effects in humans. Here, I will give a detailed overview about PAS and its potential to assess and induce timing dependent plasticity in healthy individuals as well as in neurological diseases. Finally, I will also discuss potential determinants influencing the outcome of PAS in humans.

Repetitive transcranial magnetic stimulation: Are we exploiting spike-timing dependent plasticity for the treatment of patients?

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Repetitive transcranial magnetic stimulation (rTMS) of the human brain can lead to long-lasting changes in cortical excitability. This observation has led to the therapeutic use of rTMS in brain diseases associated with alterations in excitation/inhibition balance. However, the cellular and molecular mechanisms of rTMS-based therapies remain not well understood. To learn more about the effects of rTMS we established an in vitro model of repetitive magnetic stimulation using organotypic brain slice cultures. Our work discloses that rTMS is capable of inducing calcium-dependent changes of both excitatory and inhibitory synapses. Strikingly, these changes are not observed in all synapses of a neuron. Since rTMS induces a considerably large electromagnetic field, it is conceivable that many neurons will be depolarized simultaneously in the stimulated tissue. This situation makes it likely that spike-timing dependent plasticity (STDP) is employed during stimulation and may thus account for some of the input-specific effects observed after rTMS. Indeed, using compartmental modelling of back- and anterograde propagating action potentials we are able to demonstrate that rTMS-induced STDP can account for our results. It is therefore interesting to consider that rTMS-based therapies may already exploit STDP in clinical settings. Thus, rTMS represents an interesting tool for future translational synaptic plasticity studies.

Synaptic input and output of hilar mossy cells during sharp wave ripples.

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Hippocampal sharp waves and associated ripple oscillations have been implicated in the consolidation of episodic memories and future planning. In this study, we focused on the dentate gyrus, which serves as the first stage of information processing coming from the entorhinal cortex to the hippocampus. The function of the dentate gyrus and the hilar region in particular, during these network events has not been explored in detail.

We investigated the role of hilar mossy cells during SWRs by combining field recordings in the CA3 region with whole cell recordings from verified mossy cells during spontaneous SWRs in mouse hippocampal slices. We found that mossy cells receive SWR-associated excitatory and inhibitory synaptic inputs. Moreover, a large fraction of mossy cells discharged during SWRs. Together, our data suggest an unexpected involvement of mossy cells in this network event.

Symposium

S26: New insights into functional and molecular dynamics of presynaptic calcium channels

- [S26-1](#) N-type voltage-gated calcium channels: role of α_2 subunits in trafficking and function
Annette Dolphin
- [S26-2](#) Assembly and dynamics of macro-molecular complexes in CNS synapses
Bernd Fakler
- [S26-3](#) Presynaptic calcium influx and buffering at a fast central synapse
Stefan Hallermann
- [S26-4](#) Local Protein Degradation Controls Presynaptic Calcium Influx and Homeostatic Synaptic Plasticity
Martin Müller
- [S26-5](#) Calcium channel surface dynamic influences synaptic transmission
Jennifer Heck, Pierre Parutto, Romy Freund, Anna Ciuraszkiewicz, Arthur Bikbaev, Maria Andres-Alonso, Ivan Bykov, David Holcman, Martin Heine

N-type voltage-gated calcium channels: role of $\alpha 2\delta$ subunits in trafficking and function

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Voltage-gated calcium channels (VGCCs) permit depolarisation-dependent Ca²⁺ entry into excitable cells to control many functions, including neurotransmitter release in neurons, and muscle contraction. For the CaV1 and CaV2 sub-families, the channels are known to be heteromeric, consisting of an $\alpha 1$ pore-forming subunit, associated with auxiliary subunits $\alpha 2$ and β . I will describe our studies on the role of the $\alpha 2$ subunits in calcium channel trafficking and function [1-3]. Most recently we have used protein engineering to probe the role of proteolytic processing of $\alpha 2$ in calcium channel function. Related to this, I will describe evidence for a key role of $\alpha 2^{-1}$ in the development of neuropathic mechanical hypersensitivity in rodent models of neuropathic pain [4, 5].

References

- [1] Canti, C., Nieto-Rostro, M., Foucault, I., Heblich, F., Wratten, J., Richards, M.W., Hendrich, J., Douglas, L., Page, K.M., Davies, A., et al. 2005 The metal-ion-dependent adhesion site in the Von Willebrand factor-A domain of alpha2delta subunits is key to trafficking voltage-gated Ca²⁺ channels. *Proc. Natl. Acad. Sci. USA* 102, 11230-11235.
- [2] Hoppa, M.B., Lana, B., Margas, W., Dolphin, A.C. & Ryan, T.A. 2012 alpha2delta expression sets presynaptic calcium channel abundance and release probability. *Nature* 486, 122-125.
- [3] Cassidy, J.S., Ferron, L., Kadurin, I., Pratt, W.S. & Dolphin, A.C. 2014 Functional exofacially tagged N-type calcium channels elucidate the interaction with auxiliary alpha2delta-1 subunits. *Proc. Natl. Acad. Sci. U. S. A* 111, 8979-8984.
- [4] Bauer, C.S., Nieto-Rostro, M., Rahman, W., Tran-Van-Minh, A., Ferron, L., Douglas, L., Kadurin, I., Sri Ranjan, Y., Fernandez-Alacid, L., Millar, N.S., et al. 2009 The increased trafficking of the calcium channel subunit $\alpha 2\delta$ -1 to presynaptic terminals in neuropathic pain is inhibited by the $\alpha 2\delta$ ligand pregabalin. *Journal of Neuroscience* 29, 4076-4088.
- [5] Patel, R., Bauer, C.S., Nieto-Rostro, M., Margas, W., Ferron, L., Chaggar, K., Crews, K., Ramirez, J.D., Bennett, D.L., Schwartz, A., et al. 2013 alpha2delta-1 gene deletion affects somatosensory neuron function and delays mechanical hypersensitivity in response to peripheral nerve damage. *J Neurosci* 33, 16412-16426.

Assembly and dynamics of macro-molecular complexes in CNS synapses

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Voltage-gated calcium channels (VGCC) convert membrane potential changes into intracellular signaling by rapidly changing the intracellular calcium ion concentration. This property has been well studied at the presynapse where VGCC act as a part of multi-protein complexes with highly variable subunit composition. These complexes determine their functional properties, subcellular localization and molecular dynamics. The characteristics of action potential-induced calcium influx through calcium channels dictate in turn the probability and short-term plasticity of synaptic neurotransmitter release. In this symposia we want to spot light on new insights on the traffic (Annette Dolphin), and macromolecular organization of presynaptic calcium channels (Bernd Fakler), their role in the regulation of fast neurotransmission (Stefan Hallermann) and in synaptic homeostatic plasticity (Martin Müller). Investigators have used broad spectra of methodological approaches ranging from live-cell imaging, quantitative proteomics, genetics in mouse and in fly, super-resolution microscopy, and mathematic modeling. With this symposium, we like to discuss the function of calcium channels beyond the ion conduction and highlight their role in the integration of presynaptic release machinery and as a target for manifold regulations.

Presynaptic calcium influx and buffering at a fast central synapse

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The rate at which signals can be transmitted between single neurons limits the speed of information processing in the central nervous system. Cerebellar mossy fiber boutons display synchronous release of neurotransmitter up to about 1 kHz. We combined high-resolution electrophysiological techniques, quantitative two-photon calcium imaging, and finite element modeling of calcium diffusion and buffering to analyze the mechanisms enabling reliable high-frequency transmission. At cerebellar mossy fiber boutons the gating of calcium channels is extremely fast, enabling efficient calcium channel opening during rapid presynaptic action potentials. Furthermore, calcium influx is tightly coupled to the release sensor. Finally, immobile calcium buffers have a low affinity and a low binding ratio, enabling rapid clearance of calcium at the active zone. Thus, our data help to explain the calcium dynamics at the active zone of fast central synapse.

Local Protein Degradation Controls Presynaptic Calcium Influx and Homeostatic Synaptic Plasticity

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The first genes have been linked to homeostatic modulation of neurotransmitter release, but it is largely unknown how their protein products are regulated during homeostatic plasticity. Here we explore the role of local protein degradation in the homeostatic control of neurotransmitter release at the *Drosophila* neuromuscular junction. We demonstrate that the acute induction and the sustained expression of homeostatic potentiation of release induced by postsynaptic glutamate perturbation are blocked after pharmacological or genetic proteasome perturbation. We further show that presynaptic proteasome inhibition increases the peak amplitude of presynaptic spatially-averaged calcium transients evoked by single action potentials without affecting calcium-transient decay kinetics. Presynaptic proteasome interference also slows EPSC decay kinetics, and leads to an increased EGTA-sensitivity of release without causing apparent changes in synapse morphology. Interestingly, proteasome perturbation does not potentiate release after loss of *dysbindin*, a schizophrenia-susceptibility gene that has been linked to homeostatic plasticity. Finally, we provide genetic evidence that dysbindin ubiquitination opposes neurotransmitter release. Together, our data suggest that local protein degradation controls specific presynaptic mechanisms and molecules during homeostatic plasticity and baseline synaptic transmission.

Calcium channel surface dynamic influences synaptic transmission

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The localization of voltage gated calcium channels (VGCCs) and vesicles at the presynaptic active zone is critical for the release probability of neurotransmitters. Using single particle tracking photoactivation localization microscopy (sptPALM) we showed that the majority of synaptic VGCC is confined but mobile within the presynaptic membrane. Several molecular interactions between VGCCs and presynaptic scaffold proteins in mammalian synapses as RIM, RIM-binding protein and Bassoon have been reported to influence the localization of VGCCs as well as the recruitment of synaptic vesicles and have a major impact on synaptic transmission. Described binding motives for channel scaffold interactions are located at the distal C-terminus of VGCCs. In order to probe whether interactions between VGCCs and scaffold proteins manipulate channel localization and mobility, we used two C-terminal splice variants of VGCCs. Here, alternative splicing of exon 47 results in the expression of a shorter C-terminus ($\Delta 47$) lacking a variety of protein-protein interactions.

Both splice variants, $Ca_v2.1_{\Delta 47}$ and $Ca_v2.1_{+47}$ accumulated into the presynaptic terminals and co-localized with presynaptic proteins as Bassoon, RIM and Munc13 and the vesicular protein synapsin. Within ~50 % of synapses, the endogenous $Ca_v2.1$ population was similarly replaced by both tagged $Ca_v2.1$ C-terminal splice variants. Despite the differences in the C-terminus, the $Ca_v2.1_{\Delta 47}$ splice variant promotes a stronger accumulation of scaffold proteins. However, the shorter $Ca_v2.1_{\Delta 47}$ was significantly more mobile compared to $Ca_v2.1_{+47}$ but had similar confinement and dwell time within small energy domains. Synaptic calcium signals in $Ca_v2.1_{+47}$ or $Ca_v2.1_{\Delta 47}$ dominated synapses were similar. Finally, we performed a light-inducible immobilization of $Ca_v2.1$ by fusing *cryptochrome2olig* to the N-terminus of the channel. The artificial clustering of calcium channels lead to altered kinetics and strength of the vesicular release.

Our data suggest that recruitment of calcium channels is independent of the C-terminal domains interacting with scaffold proteins. Whereas an effect on the channel surface mobility is likely. Further, we hypothesize that aggregation and immobilization of VGCCs at the presynapse favors multi-vesicular release and thus modulates the variability of presynaptic release properties.

Symposium

S27: The neuroscience of good and evil: translational insights into pro- and antisocial decision-making.

- [S27-1](#) The role of attention in third-party punishment
Bernd Weber
- [S27-2](#) Neurobiological contributions to a better understanding of human aggression: what can we learn from recent studies?
Katja Bertsch
- [S27-3](#) The neural basis of social choice in rodents
Marijn van Wingerden, Julien Hernandez-Lallement, Tobias Kalenscher
- [S27-4](#) Animal models of anti-social behaviour: role of oxytocin and vasopressin
Trynke de Jong, Vinicius E.M. Oliveira, Inga D. Neumann
- [S27-5](#) Hornets have it: a conserved olfactory subsystem for social recognition in Hymenoptera
Antoine Couto, Aniruddha Mitra, Denis Thiéry, Frédéric Marion-Poll, Jean-Christophe Sandoz
- [S27-6](#) Seeing faces in random noise: A brain network for illusory face perception
Ina Hübener, Andreas Jansen

The role of attention in third-party punishment

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Social norms are important for human societies. Norm violations (e.g. unfair transgression) are often met with punishment even by people that are not directly affected. However, punishing the offender is not the only possible option for a bystander. Driven by empathic concerns, they may also give a helping hand to the victim. In a pre-registered and fully incentivized eye-tracking study (N = 47) we present evidence that the influence of empathic concern on behavior is conveyed via attentional processes. In a within-subject design we first analyzed the relationship between choices, decision processing measures and empathic concern. The results show that the bystander's decision to intervene and the respective attention distribution leading up to the choice systematically varied with the person's level of empathic concern. In order to test whether these differences in processing can also be externally induced, in a second step we instructed participants to focus on specific components of the norm violation, namely the (un)fair conduct of the offender or the feelings of the victim. The data shows that the observed (intrinsic) attentional bias in the baseline condition could not be successfully influenced by the experimental manipulation. We discuss the theoretical and practical implications of our results.

Neurobiological contributions to a better understanding of human aggression: what can we learn from recent studies?

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Aggression can be defined as any behavior that is intended to harm another individual against his will. Although it may help an individual to defend himself in critical situations, aggression and violence are a global problem ranging amongst the most prevalent causes of injury and death worldwide. A better understanding of the underlying psychological and neurobiological mechanisms of aggression and violence has therefore been one of the most intriguing subjects for psychologists and neurobiologists. Both disciplines have found evidence for several different forms of aggression: a cold-blooded, instrumental and a hot, impulsive form, which are supposed to be associated with activations in different brain regions as well as diverse psychological impairments, developmental trajectories, and psychiatric disorders. In my talk, I will present results from recent studies on antisocial personality disorder/psychopathy (“cold-blooded aggression”) and on borderline personality disorder (“hot aggression”) and, try to integrate them with those of animal studies. I will also discuss possible implications of these results for the treatment of extremely aggressive individuals with a particular focus on the neuropeptide oxytocin and its effects on patients with borderline or antisocial personality disorder.

The neural basis of social choice in rodents

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Pro-social choice is ubiquitous in human behavior, even in anonymous, one-shot interactions. The adaptive advantage of pro-social behavior between individuals or within a group of cooperating individuals has been studied extensively, but a proximate mechanism for pro-social choice has been more elusive. In order to study the mechanisms and neural implementation of pro-social choice in more detail, we developed a rodent version of the Pro-Social Choice Task (PCT). We found that rats, in general, prefer a mutual Both Reward (BR), generating reward for both actor and a partner rat over an Own Reward (OR) alternative that would only reward the actor but not the partner. Importantly, the social nature of this effect was supported by indifference between BR/OR options when the partner was a puppet instead of a real rat. Substantial between-individual variation in pro-social choice was found, though most rats showed a within-session increment in pro-social choice, highlighting the importance of social reinforcement learning mechanisms. The neural basis of pro-social choice in rats was investigated through bilateral lesions of the BLA. Leaving magnitude discrimination intact, these lesions selectively abolished pro-social choice relative to sham-operated controls.

A putative mechanism for social reinforcement learning in rats lies in ultrasonic vocalizations serving as feedback on the emotional state of the partner animal. We have found that rats prefer vocalizations associated with a positive affective state over those coupled with negative events. The role of USVs in driving pro-social choice in the PCT will be discussed in the framework of a social reinforcement learning mechanism.

Animal models of anti-social behaviour: role of oxytocin and vasopressin

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Social decision-making is of incredible importance to all social animals, including humans and rats. To approach or avoid, to attack or befriend, to help at a cost or harm for a profit – the wrong decision may waste valuable energy at best or cause social exclusion or even severe physical damage at worst.

Social neuroscientists are currently making rapid progress to understand how the brain controls social decision-making. Established and innovative rodent models and paradigms are crucial to delineate neuronal networks and neurotransmitters underlying pro- and antisocial interactions.

Oxytocin (OXT) and vasopressin (AVP) are neuropeptides that play a strong modulatory role in social interactions in rodents. In general, OXT is considered to be a pro-social, anti-aggressive neuropeptide whereas AVP tends to facilitate aggression.

We addressed two gaps in the current knowledge. First, we tested the hypothesis that OXT and AVP affect aggressive interactions in a sex-specific manner. Thus, we studied the effects of OXT and AVP in our novel rat model of virgin female aggression. Surprisingly, the data point to a dominant anti-aggressive effect of AVP, executed via the dorsolateral septum, with OXT playing a less robust role. These data

Second, we set out to develop a novel animal model for callous and unemotional traits and instrumental aggression. I will present here the current state of our innovative sexual aggression test in male rats, including preliminary data on the effects of OXT and AVP treatments.

Hornets have it: a conserved olfactory subsystem for social recognition in Hymenoptera

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Cuticular hydrocarbons (CHCs) are critical cues for intra- and interspecific interactions as they provide information about species membership, fertility status and colony membership. Although the chemical and behavioral aspects of nestmate discrimination are well documented in social Hymenoptera, much less is known about the detection and processing of these olfactory signals. In carpenter ants, previous work identified a female-specific olfactory subsystem potentially in charge of processing CHC information, through antennal detection by basiconic sensilla. It is still unclear however whether this specialized olfactory subsystem exists in other eusocial hymenopteran families which also actively apply nestmate discrimination. We thus assessed the existence of a possible homologous subsystem in Vespidae, a family in which eusociality appeared independently from ants. We analyzed the distribution of basiconic sensilla on the antenna of the hornet *Vespa velutina* and explored the projection pattern of its sensory neurons into the first olfactory processing center, the antennal lobe. The neurons from these sensilla project to a conspicuous cluster of small glomeruli with anatomical and immunoreactive features reminiscent of the ant CHC subsystem. Extracellular electrophysiological recordings further show that neurons within hornet basiconic sensilla respond preferentially to CHCs. These observations suggest that a CHC subsystem is conserved across distinct eusocial Hymenoptera families, and may have represented an ancestral preadaptation for elaborate intraspecific communication, potentially facilitating the multiple emergence of eusociality among Hymenoptera.

Seeing faces in random noise: A brain network for illusory face perception

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The perception and processing of faces is an elementary human property. A whole network of brain areas - spanning from the occipital to the frontal lobe - evolved to detect and evaluate a face with its anatomical features and its corresponding emotions. Faces convey such primal information for our social life and the survival of our species, that our brain occasionally even perceives a face, when there only exist face-like structures in non-face objects like the milk froth of our morning coffee, clouds or the bark of a tree. Such illusory perception is called face pareidolia.

While the majority of research on face processing has focused on a bottom-up analysis, where increasingly complex facial information is processed in a cascade of specialised brain areas known as the "core and extended network" of face perception, far less is known on neural correlates of illusory face perception, which heavily relies on top-down mechanisms. Only recently, some studies set out to explore the underlying brain mechanisms involved in this fascinating phenomenon. These first results indicate a participation of the "core network" (fusiform face area = FFA; occipital face area = OFA) together with prefrontal brain areas. However, the precise role of the prefrontal cortex and its interaction with the "core network" are still poorly understood.

Therefore, we used functional Magnetic Resonance Imaging (fMRI) and Psychophysiological Interaction (PPI) analysis to investigate which prefrontal brain area could mediate the top-down process of illusory face perception and how it might be functionally interconnected with the "core network" of face perception. To trigger illusory face perception, subjects were presented with pure noise images, but were led to believe, that 50 % of them contained a face. On average faces were detected in 37.66 % of trials.

fMRI whole brain activation pattern elicited by face relative to no-face responses in concert with the PPI analysis revealed a distributed network specialised in illusory face perception. The network spans from occipitotemporal (e.g. FFA and OFA) over parietal (e.g. precuneus and inferior parietal lobule) to sub-lobar (e.g. insula) and prefrontal (e.g. inferior frontal gyrus (IFG) and orbitofrontal cortex) brain regions. Interestingly, and contrary to normal face processing, the "core network" of face perception (including FFA and OFA) was found to be lateralised to the left hemisphere. Besides, the results imply a crucial contribution of the left OFA and the right IFG.

Furthermore, a reverse correlation analysis with behavioural face and no-face ratings was conducted to reveal individual internal representations. The resulting classification images revealed face like structures, which proves that subjects actually experienced illusory face perception.

Finally, building on previous studies and the results of this study, a network model for illusory face perception was proposed, which puts the OFA in the centre between bottom-up inputs from the primary visual cortex and top-down influences from the IFG.

Symposium

S28: Glia - all the same? Increasing evidence for glial heterogeneity

- [S28-1](#) Mechanistic insights of oligodendroglial cell generation from neural stem cells
Felix Beyer, Janusz J. Jadasz, Patrick Küry
- [S28-2](#) The dual-specificity phosphatase Dusp15 is a downstream effector of Sox10 and Myrf in myelinating oligodendrocytes
Melanie Küspert, Katharina Muth, Sandra Piefke, Matthias Weider, Elisabeth Sock, Irm Hermans-Borgmeyer, Michael Wegner
- [S28-3](#) Glutamate Receptor targeting in Glial Cells
Stephanie Griemsmann, Andrea Mölders, Nadine Erlenhardt, Barbara Biermann, Akitoshi Miyamoto, Hiroko Bannai, Nikolaj Klöcker
- [S28-4](#) Study of brain metabolism by single cell imaging
Rodrigo Lerchundi
- [S28-5](#) Analysis of purinergic P2Y1 receptor function in cortical astrocytes and cerebellar Bergmann glia
Carmen V. Bohn, Hannah M. Jahn, Xianshu Bai, Andreas Helfer, Julian Michely, Hans H. Maurer, Anja Scheller, Frank Kirchhoff
- [S28-6](#) Study of astrocyte-specific and inducible GABA_B receptor deletion in the mouse brain
Laura Schlosser, Hannah M. Jahn, Xianshu Bai, Laura C. Caudal, Gebhard Stopper, Anja Scheller, Frank Kirchhoff

Mechanistic insights of oligodendroglial cell generation from neural stem cells

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Glial heterogeneity attracted increasing attention in the past years and led to the surprising finding of several cellular sub-populations among the four major glial cell types in the central nervous system (CNS), namely astrocytes, microglia, NG2 cells, and oligodendrocytes. In order to understand this heterogeneity it is important to elucidate whether the differences of these sub-populations depend on region specific signals within the CNS or whether they are intrinsically encoded. Moreover, it will be of interest to reveal to what extent these mechanisms are conserved among different species and to describe fate determination and differentiation processes of neural stem cells (NSCs) giving rise to different glial cell types during both, development as well as in the adult. We found that the cyclin-dependent kinase inhibitor protein CDKN1C (p57kip2) acts as negative regulator of Schwann cell- as well as of oligodendroglial precursor cell (OPC) differentiation without being associated with cell cycle control. In addition, it is also in charge of oligodendroglial fate determination of adult NSCs. Upon suppression of p57kip2 in adult NSCs of both, rat and mouse origin, these cells initiate an OPC-like expression profile concomitant with a downregulation of astrocytic markers. Our current investigations aim to reveal whether the modulation of p57kip2 expression in stem cells can lead to fully matured, myelinating oligodendrocytes independent of extrinsic signalling and to what degree these oligodendroglial cells contribute to glial heterogeneity in *in vivo* environments.

The dual-specificity phosphatase Dusp15 is a downstream effector of Sox10 and Myrf in myelinating oligodendrocytes

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The transcription factors Sox10 and Myrf play essential roles in myelinating oligodendrocytes and their loss causes severe myelination defects in mice. Downstream targets and interaction partners of both factors in the central nervous system are only partly known until now. ChIP-Seq and expression analyses identified the dual specificity phosphatase Dusp15 as a novel target of Sox10 and Myrf and a promising candidate regulator of oligodendrocyte differentiation. Sox10 and Myrf directly bind to the *Dusp15* promoter and synergistically activate *Dusp15* expression. In mice deletion of Sox10 in oligodendrocyte precursors leads to loss of *Dusp15* expression. In the developing spinal cord *Dusp15* is induced in early Myrf-positive oligodendrocytes and expression ceases again in myelinated oligodendrocytes. *Dusp15* expression therefore marks a distinct subpopulation of myelinating oligodendrocytes during development, and a role in the regulation of early differentiation events is implicated. In the adult central nervous system, only few oligodendroglial cells express *Dusp15* arguing that it may be involved in glial heterogeneity. Recently, *Dusp15* was also shown to be induced in lesioned white matter of MS patients, pointing to functions during adult remyelination. Functional assays in oligodendrocyte-like cells showed a reduced expression of early and late myelin markers after knockdown of *Dusp15*. Interestingly, knockdown and overexpression experiments in primary oligodendrocytes revealed a positive function of *Dusp15* in early differentiation events but a negative effect on late differentiation. These functional data together with the sharp peak of *Dusp15* expression in early myelinating oligodendrocytes suggest a role in exact timing of myelination. As a membrane-located phosphatase *Dusp15* may mediate intrinsic cues originating from Sox10 and Myrf to modulate the phosphorylation state and activity of ligand receptors and their signal transducers. This may change cellular responses to extrinsic factors and as a result modulate timing and magnitude of myelin gene expression, which are also important in adult remyelination events.

Glutamate Receptor targeting in Glial Cells

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Glial cells are active partners of neurones in normal brain function and sense neuronal activity. Increasing evidence highlights the communication between neurones and glial cells via neurotransmitters like glutamate. For this purpose glial cells express a variety of ligand-gated ion channels and transporters. In the present study, we focus on glutamate receptors that are involved in fast synaptic transmission between neurones, i.e. the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) which consists of four pore-forming subunits GluA1-4 and a number of auxiliary subunits.

In addition to the well-characterized neuronal expression patterns, AMPA receptors are also expressed in glial cells. Thus, NG2 cells receive direct synaptic input from neurons via AMPA receptors which might be a critical determinant of NG2 lineage progression. Here, we show that NG2 cells in a purified culture model express functional AMPA receptors on their surface.

Also astrocytes make close contacts to neurones, representing the third compartment of the tripartite synapse model. It is well known that they express glutamate transporters to remove the neurotransmitter from the synaptic cleft. The expression of AMPA receptors, however, is subject of controversial discussion. Here, we show that purified and cultured astrocytes do express functional AMPA receptors. Glutamate induced currents in astrocytic outside-out patches, which were blocked by GYKI. Taking advantage of a transgenic GFAP-GFP mouse line we were able to isolate astrocytes from various brain regions and confirmed the expression of all four pore-forming AMPA receptor subunits in astrocytes. Additionally, the astrocyte-specific expression of several auxiliary subunits was analyzed by quantitative real time PCR.

Together, these data indicate the functional expression of AMPA receptors in glial cells which possibly contribute to communication between neurones and glia. It remains to be elucidated whether AMPA receptors are targeted to glial plasma membrane subdomains.

This work was funded by SPP 1757 Glial Heterogeneity and YoungGlia.

Study of brain metabolism by single cell imaging

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Astrocytes have a close relationship with neurons in the brain. For a long time, these cells have only been considered as a structural support for neurons. However, experimental evidence obtained in the last decades suggests that astrocytes play a central role in the coupling between neuronal activity and energy metabolism.

During brain activity, glutamate released by excitatory synapses is taken by astrocytes through sodium-coupled transport systems, evoking increases of sodium that should be recovered in term of seconds. Considering that the concentration of sodium in the extracellular space is ten times bigger than in the cytosol, the dissipation against the gradient should require a high expend of ATP. At the same time, other molecules like potassium and ammonium are also released during activity, modulating the energetic status of the glial cell through the increase of glycolysis and inhibition of mitochondrial activity.

The development of genetically encoded fluorescent sensors sensitive to metabolites has also opened the door to study the relation between sodium transient and the modulation of a cell metabolism. By using fluorescent ATP sensors and a sodium fluorescent probes we observed that levels of ATP change dynamically in response to intracellular changes of physiological sodium produced by different stimulus in astrocytes (culture and tissue). This data suggests a bigger role of sodium in the modulation of astrocytic metabolism.

Analysis of purinergic P2Y1 receptor function in cortical astrocytes and cerebellar Bergmann glia

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Astrocytes express neurotransmitter receptors that form microdomains for signaling pathways along their perisynaptic and perivascular processes. The purinergic metabotropic P2Y1 receptor is involved in long-range intercellular signaling of astrocytes including gliotransmitter release.

To investigate and temporally control the expression of the P2Y1 receptors more specifically in cerebellar Bergmann glia as well as in cortical astrocytes, we took advantage of GLAST-CreERT2 x floxed P2Y1 receptor mice. For precise temporal control of gene recombination, we explored the pharmacokinetic properties of tamoxifen when injected intraperitoneally using HPLC-MS. For analysis we used adolescent mice of 8 to 12 weeks. Our HPLC-MS analysis showed a fast uptake of tam and its most active metabolite 4-hydroxytamoxifen (4-OHT) in the brain peaking already at 8 h post injection. Similarly fast was the clearance of tam and 4-OHT: both were undetectable already 48 h after injection. The efficiency of tam-induced recombination was determined by qRT-PCR of genomic DNA purified from brain homogenates of cKO mice and by quantifying reporter-positive cells in GLAST-CreERT2 x floxedR26-tdTomato mice, revealing the percentage of astrocytes among all cells: 20 % (brainstem), 8 % (cerebellum), 22 % (cortex), 30 % (hippocampus) and 31 % (optic nerve). The astroglial *p2ry1* gene deletion resulted then in significant reductions of P2Y1 receptor mRNA of 61 % in the cerebellum and 43 % in the cortex.

We are now in a position to study the impact of astroglial P2Y1 receptors, e.g. in motor behavior or brain trauma.

Study of astrocyte-specific and inducible GABA_B receptor deletion in the mouse brain

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Astrocytes are decisively involved in synaptic transmission. For this purpose, astrocytes are equipped with transmitter receptors that sense neuronal activity. One of these receptors is the metabotropic GABA_B receptor, a sensor of the main inhibitory neurotransmitter -aminobutyric acid (GABA). In neurons, the activation of GABA_B receptors (formed by dimerization of GABA_{B1} and GABA_{B2} subunits) leads to a signaling pathway involving inhibitory G proteins. In astrocytes, however, the downstream signaling of GABA_B receptors and its function in the neuron-glia network is still unclear. Recent reports suggest a link to intracellular Ca²⁺ signals.

Therefore, we generated a genetically modified mouse model in which we could induce an astroglia-specific deletion of the essential GABA_B receptor subunit GB1, and simultaneously record astroglial Ca²⁺ signals (GLAST-CreERT2 x floxed GABA_BR1 mice (GB1-cKO) x floxed R26-GCaMP3).

Genomic DNA recombination, i.e. gene deletion, was induced by intraperitoneal tamoxifen injection and quantified by real-time PCR. Analysis of genomic DNA purified from cerebellum, cortex and hippocampus revealed a significant reduction of floxed *gabbr1* alleles after three weeks. In addition, also fluorescence *in situ* hybridization (FISH) revealed a reduction of the mRNA in cortical astrocytes while leaving GABA_B mRNA still present in neurons. Confocal microscopy analysis of immunostained brain sections, showed a significant reduction of GABA_BR1 protein from astrocyte membranes in cortex, hippocampus and cerebellum. Our current *in vivo* two-photon recordings reveal a complex spatial and temporal pattern of Ca²⁺ signals in wildtype astrocytes.

Expanding these observations to mutant astrocytes will reveal the impact of astroglial GABA_B receptors for neuron-glia communication, and behavior.

Symposium

S29: To eat? To sleep? To run? Coordination of innate behaviors by hypothalamic circuits

- [S29-1](#) CNS-Dependent Regulation of Glucose Homeostasis
Jens Claus Bruening
- [S29-2](#) Thalamic integration of LH circuits in sleep-wake states
Antoine Adamantidis
- [S29-3](#) Inhibitory interplay between orexin/hypocretin neurons and eating
Denis Burdakov
- [S29-4](#) Gamma oscillations organize top-down signaling to hypothalamus and enable food seeking
Tatiana Korotkova, Marta Carus-Cadavieco, Maria Gorbaty, Li Ye, Franziska Bender, Suzanne van der Veldt, Yubin Hu, Natalia Denisova, Franziska Ramm, Karl Deisseroth, Alexey Ponomarenko
- [S29-5](#) The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia
Gretel Betiana Kamm, Kun Song, Hong Wang, Jörg Pohle, Fernanda de Castro Reis, Paul Heppenstall, Hagen Wende, Jan Siemens
- [S29-6](#) Retrograde chemogenetic dissection of the central noradrenergic system: Implications for analgesic and aversive neuronal circuits
Stefan Hirschberg, Yong Li, Eric J Kremer, Andrew D Randall, Anthony E Pickering

CNS-Dependent Regulation of Glucose Homeostasis

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Melanocortin neurons in the arcuate nucleus of the hypothalamus integrate different hormonal signals from the periphery of the organism, which communicate fuel availability of the organism, such as leptin, insulin and glucose. In turn, these neurons coordinate behavioral and autonomic responses to adapt food intake and energy expenditure. From an evolutionary point of view it is reasonable that these neurocircuits not only adapt food intake and energy expenditure according to fuel ability, but that they also coordinate the fluxes of fuels across different organs. The presentation will focus on the identification and functional characterization of melanocortin-dependent neurocircuits, which also control peripheral insulin sensitivity and glucose homeostasis.

Thalamic integration of LH circuits in sleep-wake states

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Lecture will describe a role of GABA cells in lateral hypothalamus (LH) and their projections to TRN (reticular thalamic nucleus) in sleep-wake states. Optogenetic activation of this circuit recapitulates state-dependent changes of TRN neuron activity in behaving mice and induces rapid arousal during NREM, but not REM sleep. Further, activation of this circuit induces sustained cortical arousal during deep anesthesia.

Inhibitory interplay between orexin/hypocretin neurons and eating

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In humans and rodents, loss of brain orexin/hypocretin (OH) neurons causes pathological sleepiness, whereas OH hyperactivity is associated with stress and anxiety. OH cell control is thus of considerable interest. OH cells are activated by fasting and proposed to stimulate eating. However, OH cells are also activated by diverse feeding-unrelated stressors [14-17] and stimulate locomotion and "fight-or-flight" responses. It would be difficult to combine the latter responses and behaviours with eating, raising the question of whether OH neurons directly stimulate eating at all. Indeed, loss of OH cells produces obesity, suggesting that OH cells do not facilitate net energy intake. Here we revisited and re-examined the relation of OH cells to eating at the level of natural physiological activity of OH cells. First, we monitored eating-associated dynamics of OH cells using fiber photometry in free-feeding mice. OH cell activity decreased within milliseconds after eating onset, and remained in a down state during eating. This OH inactivation occurred with foods of diverse tastes and textures, as well as with calorie-free "food," in both fed and fasted mice, suggesting that it is driven by the act of eating itself. Second, we probed the implications of natural OH cell signals for eating and weight in a new conditional OH cell-knockout model. Complete OH cell inactivation in adult brain induced a hitherto unrecognized overeating phenotype and caused overweight that was preventable by mild dieting. These results support an inhibitory interplay between OH signals and eating, and demonstrate that OH cell activity is rapidly controllable, across nutritional states, by voluntary action.

Gamma oscillations organize top-down signaling to hypothalamus and enable food seeking

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How the brain initiates, maintains and coordinates innate behaviors is largely unknown. Lateral hypothalamus (LH) is crucial for the regulation of feeding behaviors. Yet little is known about the regulation of LH by top-down inputs from cognitive control regions. Combining optogenetics with multisite electrophysiological recordings in behaving mice, we report that gamma oscillations coordinate signaling between medial prefrontal cortex (mPFC), lateral septum (LS) and LH. We show that gamma-rhythmic signaling within this circuit selectively drives food-seeking, and identify cell types involved in generation of LS-LH gamma oscillations: LS somatostatin cells and LH GABA cells. We further show a microcircuit mechanism through which gamma-rhythmic entrainment in the LS-LH circuit enables a function-selective reorganization of LH neurons' activity. Upstream, gamma-rhythmic activation of mPFC-LS pathway directs goal-oriented behavior and improves performance in a food-rewarded learning task. Overall, our work identifies a novel top-down pathway, which utilizes gamma synchronization to guide activity of subcortical networks and to regulate feeding behavior by dynamic reorganization of functional cell groups in hypothalamus.

We gratefully acknowledge support by the DFG (Exc 257 NeuroCure, TK and AP, SPP1665, AP), The Human Frontier Science Program (HFSP; RGY0076/2012, TK, DB), and GIF (I-1326-421.13/2015, TK).

The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia

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Body temperature homeostasis is critical for survival and requires precise regulation by the nervous system. The hypothalamus serves as principal thermostat that detects and regulates internal temperature.

We demonstrate that the ion channel TRPM2 is a temperature sensor in a subpopulation of hypothalamic neurons. TRPM2 limits the fever response, and may detect increased temperatures to prevent overheating.

Furthermore, chemogenetic activation or inhibition of hypothalamic TRPM2-expressing neurons in vivo decreased and increased body temperature, respectively. Such manipulation may allow analysis of the beneficial effects of altered body temperature on diverse disease states.

Identification of a functional role for TRP channels in monitoring internal body temperature should promote further analysis of molecular mechanisms governing thermoregulation and foster the genetic dissection of hypothalamic circuits concerned with temperature homeostasis.

Retrograde chemogenetic dissection of the central noradrenergic system: Implications for analgesic and aversive neuronal circuits

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The Locus coeruleus (LC) is the principle noradrenergic nucleus in the CNS. Anatomical studies suggest that the LC has distinct output modules, providing selective innervation of some target areas. For example, there are non-overlapping populations that innervate the prefrontal cortex (PFC) and the spinal cord (SC).

Such modular design could have great impact on the way the noradrenergic system is implicated in pathologies. For instance, a functional deficit of pontospinal noradrenergic control is associated with the development of neuropathic pain. Frontline treatments, like noradrenaline reuptake inhibitors, are believed to act by restoring spinal noradrenergic tone. However, their use is commonly associated with CNS side-effects, which are caused in other brain areas (such as the PFC) limiting their therapeutic utility.

Here we adopt an “engineered” excitatory ionophore (PSAM) that is inert to endogenous ligands but retains sensitivity to synthetic compounds. Lentiviral and canine adenovirus type 2 based vectors with catecholaminergic neuron specific promoter for efficient expression of PSAM and a reporter fluorophore (EGFP) were developed. These vectors facilitate direct and retrograde targeting and activation of subpopulations of noradrenergic neurons *in vivo*.

In vitro and *in vivo* electrophysiological recordings from transduced LC neurons demonstrate reversible and dose-dependent control of LC discharge over the whole physiological range (1-15Hz). Extracellular recordings from SC neurons that respond to a range of noxious and innocuous stimuli demonstrate that engagement of the descending noradrenergic system specifically attenuates nociceptive processing in the SC.

Furthermore, we show that some of the adverse effects caused by tonically increased noradrenergic activity are due to the PFC projecting LC neurons. Of particular interest is that increased tonic activity of the descending noradrenergic neurons does not cause conditioned place aversion. These findings suggest that by using a selective therapeutic strategy to activate pontospinal noradrenergic neurons it may be possible to dissociate the analgesic benefits from the confounding CNS side-effects.

Symposium

S30: Illuminating normal and diseased brain function with in vivo fluorescence imaging

- [S30-1](#) Mouse auditory cortex is required for anticipatory motor response
Xiaowei Chen, Jingcheng Li, Xiang Liao, Israel Nelken
- [S30-2](#) In vivo imaging studies of striatal ensemble neural dynamics in normal and parkinsonian states
Mark J. Schnitzer, Jones Parker, Biafra Ahanonu, Benjamin Grewe, Jin Zhong Li, Michael Ehlers
- [S30-3](#) Exploring the complexity of dementia neuropathology with in vivo optical imaging
Jaime Grutzendler
- [S30-4](#) Restoring brain function in Alzheimer's mouse model by BACE inhibition
Marc Aurel Busche, Aylin Keskin, Maja Kekus, Helmuth Adelsberger, Ulf Neumann, Derya Shimshek, Beomjong Song, Tingying Peng, Hans Förstl, Matthias Staufenbiel, Israel Nelken, Arthur Konnerth
- [S30-5](#) Deep two-photon calcium imaging in vivo
Antje Birkner, Carsten H. Tischbirek, Arthur Konnerth

Mouse auditory cortex is required for anticipatory motor response

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Animals often anticipate and react to upcoming behaviorally-relevant sensory stimuli from the environment. The neural correlates of the anticipation of repetitive sensory stimulation have been previously observed in auditory cortex. However, it remains unclear whether this cortical area is a key node relevant for transforming the expected neural responses into behavioral consequences. We therefore used in vivo cellular imaging and fiber photometry to record mouse primary auditory cortex to elucidate its role in processing anticipated stimulation. We found neuronal ensembles in layers 2/3, 4 and 5 that were activated in relationship to anticipated sound events following rhythmic stimulation. These neuronal activities correlated with the occurrence of anticipatory motor responses in an auditory learning task. Optogenetic gain- and loss-of-function manipulation experiments revealed an essential role of such neuronal activities in producing the anticipatory behavior. These results suggest that primary auditory cortex is critical for coding predictive information and transforming it into anticipatory motor behavior. Our study establishes a causal link between neuronal activity in primary sensory cortex and anticipatory behavior.

In vivo imaging studies of striatal ensemble neural dynamics in normal and parkinsonian states

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Imbalanced neural activity across the striatum's direct and indirect pathways is hypothesized to underlie multiple psychiatric and movement disorders. However, there has been no definitive proof of pathological imbalanced activity in any of these conditions, due to the lack of cell-type specific recordings of striatal neural activity. In Parkinson's disease, dopamine depletion is thought to disrupt motor control by unbalancing the dynamics of striatal D1- and D2-dopamine-receptor-expressing medium spiny neurons (MSNs), which are respectively purported to promote and suppress movement. To test these ideas, we imaged the dynamics of thousands of individual D1- and D2-MSNs in freely behaving mice, before and after dopamine depletion. We found that both MSN types normally co-activate prior to locomotion, contradicting the notion that the two pathways have purely opponent roles in motor control. A parkinsonian state causes a pathologic disruption of how local ensembles of MSNs normally encode movement and unbalances activity across the two striatal pathways. We have also examined the differential effects of D1- or D2-receptor agonism, or the dopamine precursor L-DOPA on these pathologies. Overall, impaired ensemble neural coding and imbalanced pathway dynamics may jointly cause the characteristic motor impairments of Parkinson's disease. While present treatment approaches generally aim to re-balance striatal neural activity, our results recommend that next-generation therapeutics for basal ganglia disorders should also target impaired neural ensemble coding.

Exploring the complexity of dementia neuropathology with in vivo optical imaging

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Age related dementia is associated with a complex set of neuro-pathological features that include abnormal amyloid deposition, glial activation, loss of myelin and microvascular pathology. I will present methodologies that we have implemented and developed to explore these pathological processes using high-resolution in vivo and fixed tissue optical microscopy. I will also discuss novel insights that we have gained related to the role of glial cells in the evolution of amyloid deposition and the development of axonal dystrophy around amyloid plaques

Restoring brain function in Alzheimer's mouse model by BACE inhibition

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Amyloid- β (A β) is a key factor in the pathogenesis of Alzheimer's disease (AD). A crucial question for the treatment of AD is whether reducing brain A β burden can reverse already established neural circuit and memory dysfunctions. A clinically interesting target for such treatment is the β -secretase BACE, one of the enzymes involved in the generation of A β . However, while BACE inhibition has been shown to reduce pathological A β levels in the brain, the pathophysiological consequences of that treatment remain unknown. Here, by employing in vivo calcium fluorescence imaging in a mouse model of AD, we demonstrate that the pharmacological inhibition of BACE activity can rescue neuronal hyperactivity, impaired long-range circuit function and memory defects. Thereby, our findings provide experimental evidence for the benefits of BACE inhibition in the effective treatment of a wide range of AD-related pathophysiological and behavioral impairments.

Deep two-photon calcium imaging in vivo

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Two-photon calcium brain imaging is widely used for functional recordings from dense neuronal populations with single-cell resolution and single action-potential sensitivity in vivo. However, these recordings are often restricted to superficial structures of biological tissues, because of scattering and aberrations of the two-photon excitation light. A major problem is the gradual increase in background fluorescence when increasing the imaging depth. Therefore, in case of the densely labeled cell populations in the mouse cortex in vivo, for example, only upper cortical layers can be well resolved. To enhance the imaging depth, various approaches were implemented in the past, including adaptive optics to reduce optical aberrations, regenerative amplifiers to increase laser light peak intensities and three-photon excitation of fluorescent indicators. Most of these techniques involve elaborate technical modifications of the multi-photon microscope or time expensive labeling procedures. Here, we present a deep imaging approach that can be readily used in standard two-photon microscopes. The method is based on the use of the red-shifted calcium indicator dye Cal-590, with reduced scattering effects and increased depth penetration. We show that it is applicable to population imaging with single cell resolution in all six cortical layers of the mouse brain (Tischbirek et al., "Deep two-photon brain imaging with a red-shifted fluorometric Ca²⁺ indicator", PNAS, 2015). An essential feature of the method is the spatial restriction of dye delivery to a small volume within the target layer of the mouse cortex to minimize out-of-focus fluorescence (Birkner et al., "Improved deep two-photon calcium imaging in vivo", manuscript submitted). In order to increase the efficiency of fluorophore excitation and to reduce the risk of photodamage, we recommend the use of femtosecond lasers that produce light with ultra-short pulse widths. We anticipate that the method is a versatile tool not only for the in vivo analysis of mouse brains but may be particularly useful in species with larger brain structures, such as non-human primates.

Symposium

S31: Transport mechanisms at the blood-brain barrier

- [S31-1](#) Drug Delivery to the brain by colloidal carriers
Gert Fricker
- [S31-2](#) Nanomedicine for efficient chemotherapy of brain tumours: From bench to bedside
Svetlana Gelperina, Joerg Kreuter
- [S31-3](#) Claudins and claudin mimetics - tight junction proteins in normal and ischemic blood-brain barrier
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- [S31-4](#) Interaction and causal relationship of blood-brain barrier damage and CNS disease
Anne Mahringer, Gert Fricker
- [S31-5](#) Claudin peptidomimetics to modulate the blood-brain barrier for enhanced drug delivery
Sophie Dithmer, Christian Staat, Carolin Müller, Nora Gehne, Min-Chi Ku, Andreas Pohlmann, Hartwig Wolburg, Lars Winkler, Ingolf E. Blasig
- [S31-6](#) Guiding nanoparticles' design by *in vivo* visualization and quantification of their blood-brain barrier passage
Qing You, Talea Hopf, Petra Henrich-Noack, Werner Hintz, Bernhard A. Sabel

Drug Delivery to the brain by colloidal carriers

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Most drugs do not reach the CNS because they do not cross the blood- brain barrier (BBB). It is sealed by extremely tight junctions and equipped with export-proteins recognizing diverse substrates and driving them back into blood circulation. Nevertheless, there is a very urgent medical need to overcome the BBB.

Here we describe the use of surface modified biodegradable poly(butylcyanoacrylate) nanoparticles. The nanocarriers were characterized by size, polydispersity and zeta-potential. Their transport across the BBB was studied in isolated cells and isolated and functional intact brain capillaries as well as in vivo in rats by confocal laser microscopy. When pure drugs were given to BBB cells or animals no uptake could be detected. "Naked" nanocarriers showed also no brain uptake. Drug loaded and surface modified carriers were taken up by cells in vitro and into brain in a time dependent manner. 2 hours after administration, an homogeneous distribution of nanoparticles could be detected in brain tissue.

Results with itroconazol were of particular interest as drug nanocrystals were coated with polymer and surfactant, thus achieving brain concentrations corresponding to 3-4% of the given dose.

Assessment of cytotoxicity and acute inflammatory signals in rats and in human blood samples gave no evidence for adverse reactions versus particles. The results indicate that surface modification of nanocarriers with distinct surfactants or target-seeking molecules is essential for effective passage across the BBB. Thus, therapeutic levels of otherwise not effective drugs can be reached inside the brain.

Nanomedicine for efficient chemotherapy of brain tumours: From bench to bedside

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Brain tumours, and especially glioblastomas, belong to the most aggressive human cancers associated with a very unfavorable prognosis for the patients. Treatments of choice are neurosurgery and radiotherapy, while the contribution of chemotherapy is only modest, which is largely due to the ineffective drug delivery to the brain limited by the blood–brain barrier (BBB). Thus poor brain uptake of doxorubicin (DOX) is perhaps the main reason why this potentially active anti-tumour antibiotic is not used for the chemotherapy of glioblastoma.

The nanoparticle-based formulation of doxorubicin (DOXN-TL-01) is a novel drug candidate for systemic chemotherapy of glioblastoma. The technology is based on brain delivery by poly(lactide-co-glycolide) (PLGA) nanoparticles overcoated with poloxamer 188. The surface-modified PLGA nanocarriers represent a self-assembling delivery system acquiring biological vectors – apolipoproteins from the blood, which enables their passage across the BBB and doxorubicin delivery to the tumour inside the brain.

Preclinical studies demonstrated that DOXN-TL-01 enabled considerable growth inhibition of the intracranially implanted 101.8 glioblastoma in rats and long-term remission in >20% animals, whereas the conventional formulation was only marginally effective [1]. The anti-tumour effect was also confirmed by histology. The microscopical studies revealed the effective intratumoral penetration and accumulation of the nanoparticles. Importantly, DOXN-TL-01 exhibited a favourable toxicological profile. The most important finding is the reduction of cardiotoxicity, evidenced by both functional and histological assessment. The lower toxicity of the nanoparticle formulation is most probably explained by the altered biodistribution of the drug mediated by the nanoparticles.

The laboratory technique of the nanoparticle preparation was successfully optimized, scaled-up, and transferred to industry (pilot production).

Phase I dose escalation study of DOXN-TL-01 in patients with advanced solid tumours (including GBM) sponsored by Drugs Technology Ltd. is currently on-going in Russia. The drug is well tolerated and does not cause any dose-limiting toxicity at the dose levels studied so-far (up to 60 mg/m²).

This ability of the PLGA NPs to enable brain delivery of drugs that cannot circumvent the BBB by simple intravenous infusion is representing a major breakthrough. Overall this technology holds great promise for the treatment of severe CNS diseases such as glioblastomas.

Claudins and claudin mimetics - tight junction proteins in normal and ischemic blood-brain barrier

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The blood-brain barrier (BBB) controls cerebral compound exchange and limits drug delivery to the brain. The role of the BBB in stroke and modulation of the BBB are not understood. The BBB-forming endothelium, paracellularly sealed by tight junction (TJ) proteins, ensures brain homeostasis and proper metabolite exchange. As far as known, claudin-5 (Cldn5) dominates BBB's TJ function. Contribution of other TJ proteins is unclear. We therefore aim at elucidation of the structure and function of TJs and TJ proteins upon stroke and the administration of claudin mimetics both under normal and pathological conditions. We found that Cldns 5, 3 and 1 contribute to the intactness of the BBB under physiological and pathological conditions, protect the BBB in stroke but prevent detumescence of the infarcted area, hence worsening infarct outcome. Thus, modulation of Cldns tightening the BBB might help to improve stroke recovery as well as cerebral drug delivery.

Interaction and causal relationship of blood-brain barrier damage and CNS disease

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Localised at the brain microvessel endothelium, the blood-brain barrier (BBB) provides a precise homeostatic neuronal environment and means a key determinant in drug transport to the brain. Solute carriers, efflux transporters (*P-glycoprotein (P-gp)*, *Breast Cancer Resistance Protein (Bcrp)*, *Multidrug Resistance Protein 4 (Mrp4)*), tight junctions (*TJs*, *Occludin*, *Claudin-5*, *Zonula occludens (ZO-1)*) as well as endocytotic processes selectively deliver essential nutrients to the brain or remove neurotoxic agents. These BBB elements respond to a variety of regulatory signals making them susceptible to profound changes that occur during CNS diseases or pharmacotherapy [1].

In Parkinson's Disease, alpha-Synuclein (α S) is a 14.4 kDa neuropeptide that forms neurotoxic deposits in dopaminergic neurons and is prone to aggregation into fibrils or Lewy bodies [2]. Particularly, the A53T mutant conformation leads to an early-onset of motor dysfunction and cognitive impairment symptoms. It has been shown recently that α S is present in cerebral blood vessels of cerebral amyloid angiopathy patients as well as in the cerebrospinal fluid (CSF) and blood plasma [3,4].

The following project provides insights into changes of BBB elements evoked by α S in Parkinson's Disease and allows conclusions on the involvement of pathophysiologically altered BBB clearance mechanisms in α S brain accumulation. Their restoration to healthy control levels could imply new targets in the therapy of Parkinson's Disease and extend established neurologic treatments to a vascular approach. A biphasic effect of both human native and A53T mutant α S monomers on the expression and function of the efflux transporters P-gp, Bcrp and Mrp4 as well as of TJs in an *in vitro* and *ex vivo* model of porcine brain capillary endothelial cells, isolated rat brain capillaries as well as in A53T α S transfected rats (*SD-Tg(SNCA*A53T)268Cjli*) was determined in the present study: Both native and mutant α S isoforms significantly increased P-gp, Bcrp and Mrp4 mRNA, protein-expression and -function at lower concentrations after short-term incubation which turned into a decline at higher concentrations and after exposure for 48 to 72h (1-1000ng/ml, 1-72h); this process was accompanied by an initial tightening of the BBB at low α S concentrations but followed by a gradual opening at higher concentrations after long-term incubation (*Occludin*). In parallel to the up-regulation of the efflux transporters an increased RAGE (*receptor of advanced glycation end-products*) expression was observed, which emerged together with the secretion of inflammatory TNF and the induction of NF- κ B. Additionally, low α S concentrations caused a transient reduction (1-24h) of LRP1 (*low density lipoprotein receptor-related protein 1*) expression in capillary endothelial cells.

Transport experiments across the BBB *in vitro* indicated an increased efflux rate and extent of both native and mutant α S monomers from the brain to the blood compartment relative to their uptake. This was also confirmed by the calculation of $K_{p,uu}$ *in vitro*. Inhibitors of clathrin-mediated endocytosis decreased the passage from brain to blood and vice versa implicating a receptor-mediated mechanism (e.g. RAGE, LRP1). Last, α S uptake was higher in Parkinson rats after tail vein injection which can be linked to the pathological modifications at the BBB.

1. Danemann R., Prat A.: The Blood-Brain Barrier (Cold Spring Harb. Perspect. Biol.) **2015**.
2. Peelaerts W. et al.: Nature **2015**, 522(7556): 340-344.
3. Tamo W. et al.: Neurosci. Lett. **2005**, 326: 5-8.
4. Simonsen A.H. et al.: Biomark. Med. **2016**, 10(1): 19-34.

Claudin peptidomimetics to modulate the blood-brain barrier for enhanced drug delivery

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The blood-brain barrier (BBB) is still a major challenge for successful delivery to the brain for the vast majority of drugs. Especially, the treatment of brain tumours is limited due to the sealing function of brain capillary endothelial cells forming the BBB. The paraendothelial diffusion of macromolecules and solutes is limited by tight junctions (TJs), a multiprotein complex composed of claudin-1, -3, -5, -12. Claudin-5 is thought to be the key TJ protein tightening the BBB paracellularly. Therefore, claudin-5 has been selected as target for BBB modulation. For this reason, drug enhancer peptides (peptidomimetics) were designed to modulate transiently claudin-5 and, thereby, to permeabilize the BBB. By combining biochemical protein/peptide interaction and tissue culture methods, we identified, validated and optimized peptide sequences modulating claudin-5 containing barriers. The claudin-5 targeting peptides decreased the transcellular electrical resistance of immortalized brain endothelial cell layers (bEND.3), and increased the permeability of doxorubicin, a cytostatic drug, through MDCK-II cell monolayers stably expressing YFP-claudin-5. Furthermore, the expression level of junctional proteins (e.g. claudin-5, occludin, zonula occludens protein-1), and the amount of claudin-5 at cell-cell contacts of endothelial cells was decreased after peptide treatment. This is accompanied by the findings obtained by freeze-fracture electron microscopy showing a loss of particles in tight junction strands at the exoplasmic face of bEND.3 cells. Structural investigations of the peptidomimetics revealed that the C-terminal region building up an α -helix as well as the N-terminal β -sheets, being essential in contributing to the barrier opening function of the peptidomimetics. All tested peptides showed no signs of toxicity in vitro and in vivo (i.v. injection). Permeability measurements in mice proved enhanced permeation of Na-fluorescein (376 Da) through the BBB, which was confirmed by magnet resonance imaging (Gd-DTPA uptake, 547 Da). In summary, we identified new peptides holding the potential to enhance brain delivery of small molecules through the BBB.

Guiding nanoparticles' design by *in vivo* visualization and quantification of their blood-brain barrier passage

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The blood-brain barrier (BBB) allows the central nervous system (CNS) to keep its physiological milieu stable and it protects the brain from toxic substances. However, it is also an obstacle for drugs and makes it therefore very difficult to target the CNS pharmacologically. Here polybutylcyanoacrylate (PBCA)-nanoparticles as promising drug carriers come into play. The systematical investigation of the mechanisms underlying the particles' ability to cross the BBB will lead to a better understanding of the important factors mediating the passage and enable us to purpose-design PBCA-nanoparticles for optimized BBB transport efficiency. For this purpose an efficient, reliable and meaningful biological model is necessary. As retina is part of brain and blood-retina barrier (BRB) is essentially the same as BBB, we use a confocal laser scanning microscope to image the retina of rats non-invasively after injecting the fluorescence-labeled nanoparticles into the tail vein. The fluorescent signals in retina tissues and blood vessels can be visualized and measured in real-time, and repeatedly over an extended period of time in the same living rat. In our previous experiments, surfactant was proved to be the key factor determining the BBB passage of PBCA-nanoparticles. Thus, we tested various different biochemicals and found the optimal combination of DEAE-dextran and surfactant modification. We then further studied the BBB passage efficiency of DEAE-nanoparticles by systematically changing size and surface properties. We found that varying nanoparticle size and surface property had a significant impact on the pharmacokinetic profiles. Comparing our results with the literature data, it indicates that particles may be taken up either by a caveolar endocytosis or clathrin-dependent mechanism. These results can guide the optimization of PBCA-nanoparticles and provide clues for future research on their molecular mechanism.

Symposium

S32: The longitudinal course of psychosis - clinical and neurobiological aspects

- [S32-1](#) Disease trajectories in schizophrenia and bipolar disorder and the genome-environment interface
Monika Budde, André Fischer
- [S32-2](#) fMRI findings in the longitudinal course of psychosis
Sarah Trost, Sarah Wolter, Anja Richter, Maria Keil, Peter Dechent, Oliver Gruber
- S32-3 The schizophrenia risk gene *tcf4* controls and neuronal plasticity
Nirmal Kannayian - No abstract available
- [S32-4](#) Genotype-phenotype relationships of the longitudinal course of psychosis – statistical aspects
Heike Bickeböller, Sergi Papiol, Thomas G Schulze

Disease trajectories in schizophrenia and bipolar disorder and the genome-environome interface

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Bipolar disorder, schizoaffective disorder and schizophrenia are severe mental illnesses that share – at least in parts – psychopathological features and an underlying polygenic nature. One characteristic of all three diagnoses is the highly variable disease course and outcome. This heterogeneity is one of the biggest challenges in studying the underlying biological mechanisms. We have therefore embarked upon a project in which we seek to delineate different course types in a large longitudinal sample of deeply phenotyped patients using cluster analysis. We will present analyses that investigate how these different course clusters are associated with biological markers at the genomic (e.g. polygenic risk scores) and transcriptomic (e.g. microRNAs) level.

fMRI findings in the longitudinal course of psychosis

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The functional outcome and amount of disability in the longitudinal course of affective and psychotic disorders highly varies between affected individuals.

Here, we present first neuroimaging findings associated with clinical outcome parameters (e.g. psychopathology, psychosocial functioning, residual symptoms) during a 2-years longitudinal fMRI investigation of bipolar and schizophrenic patients. Our results show functional alterations in neuronal networks associated with disease progress in the longitudinal course and provide evidence for potential neuroimaging biomarkers with reference to disease outcome parameters.

Genotype-phenotype relationships of the longitudinal course of psychosis – statistical aspects

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The genetics of psychosis is far from being understood. One reason for this is that the longitudinal course of psychosis has not been investigated in detail. The networks KFO241 and PsyCourse have already collected over 1000 patients with the course of psychosis assessed at several points in time. These encompass many different phenotypes and measurement scales on the phenomic side. This alone allows the investigation of phenomic trajectories. Relating these trajectories to genomic data requires on the one side careful consideration of the distribution of the phenotypes and of their longitudinal distributional changes and on the other hand novel statistical tools that can e.g. question the implication of a biological pathway as a whole in the change of course in psychosis. Different statistical approaches and applications within our networks will be discussed.

Symposium

S33: The multiple neural codes of the retina

- [S33-1](#) Functional diversity in the mouse retina
Thomas Euler, Katrin Franke, Philipp Berens, Miroslav Román Rosón, Timm Schubert, Matthias Bethge, Tom Baden
- [S33-2](#) Signal gating and neural coding in the retina under saccadic scene changes
Tim Gollisch, Vidhyasankar Krishnamoorthy, Michael Weick
- [S33-3](#) Interplay of excitation and inhibition decorrelates visual feature representation in the mammalian inner retina
Katrin Franke, Philipp Berens, Timm Schubert, Matthias Bethge, Thomas Euler, Tom Baden
- [S33-4](#) Reading the population code of the retina
Olivier Marre
- [S33-5](#) Decorrelation of retinal response to natural scenes by eye movements
Ronen Segev
- [S33-6](#) Towards the activation of physiological retinal ganglion cell spiking by electrical stimulation
Florian Jetter, Larissa Höfling, Günther Zeck

Functional diversity in the mouse retina

Thomas Euler^{1,2,3}, Katrin Franke^{2,3}, Philipp Berens^{1,2,3}, Miroslav Román Rosón^{2,3},
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Visual processing begins in the retina: within only two synaptic layers, multiple parallel channels emerge, which relay highly processed visual information to different parts of the brain. The origin of this functional diversity lies in the retina's second synaptic layer, the inner plexiform layer (IPL), where bipolar cells, amacrine cells and retinal ganglion cells (RGCs) form complex interconnected networks. We use two-photon (2P) microscopy to image light stimulus-evoked activity at different levels of the mouse retina to study how visual information channels are generated in the retinal network.

Using 2P calcium imaging, we simultaneously recorded from all RGCs at one retinal location to obtain a complete sample of the visual information sent to the brain and to understand how the representation of spatio-temporal information in a local image patch is distributed across RGC types (Baden, Berens, Franke et al., 2016). This resulted in database of >11,000 RGC responses to a set of visual stimuli, including information about genetic and immunohistochemical signatures. The RGCs were then clustered into functional types, employing sparse principal component analysis and unsupervised Mixture of Gaussian clustering. We found that the functional diversity in retinal output is much larger than previously thought, resulting in an estimate of approx. 40 RGC types. To yield a better understanding how this diversity is generated, we next recorded the excitatory drive to the RGCs by imaging light stimulus-driven glutamate released from bipolar cell (BC) terminals in the IPL (Franke, Berens et al., in revision). The resulting dataset consists of 13,000 BC terminals and was analysed using similar clustering approaches as for the RGCs. We found that functional diversity in BC output is generated by the interplay of dendritic excitatory inputs and axonal inhibitory inputs. The resultant centre and surround components of BC receptive fields interact to decorrelate BC output in the spatial and temporal domain. Our findings suggest that decorrelation of parallel visual pathways begins already at the second synapse of the mouse visual system.

In my presentation, I will put these results into a more general context and discuss some of the principles that give rise to the functional diversity of mouse retinal output.

Signal gating and neural coding in the retina under saccadic scene changes

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Natural vision is partitioned into brief episodes of fixation, separated by rapid shifts in the direction of gaze, called saccades. The saccadic nature of vision strongly structures the visual stimuli that impinge on the retina, interspersing nearly static image presentations with global motion signals. Previous work has shown that saccades can either activate or suppress responses in different retinal ganglion cells. Little is known, however, about how saccades affect the processing and encoding of the visual information that is embedded in this sequence of fixations and global motion. In order to address this question, we have recorded the activity of ganglion cells in isolated mouse retina under saccade-like shifts of a spatial grating and analyzed how the responses depend on the grating positions prior to and after the saccade as well as on the motion signal during the saccade. The recorded ganglion cells showed a variety of distinct response types. Some of these responses were in stark contrast to standard receptive-field-based models of ganglion cells. For example, we found cells that displayed distinctive response patterns when pre- and post-saccadic grating positions were either different or identical. We further investigated the origin of this response specificity by testing the effects of different pharmacological blockers and by intracellularly recording the excitatory and inhibitory inputs currents in certain ganglion cells. These investigations revealed that saccadic image transitions can trigger specific inhibitory interactions that gate the flow of different types of signals through the retina. This signal gating endows the cells with response features that distinctly differ from those commonly observed with stationary stimulus statistics, such as white noise.

Supported by the DFG (SFB 889, C1).

Interplay of excitation and inhibition decorrelates visual feature representation in the mammalian inner retina

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The retina decomposes the visual input into parallel feature channels for transmission to the brain. Already at the first synapse, ~14 bipolar cell (BC) types systematically transform the spatio-temporal photoreceptor activation pattern into different parallel pathways and provide the glutamatergic drive for downstream retinal circuits (reviewed in Euler et al., 2014). In mouse, the set of BC types is anatomically well characterised (Ghosh et al., 2004; Wässle et al., 2009) and their exact number is known (Helmstaedter et al., 2013; Kim et al., 2014; Greene et al., 2016; Shekhar et al., 2016). However, a systematic understanding of their functional diversity and its origin within the retina is lacking. Here, we present a detailed characterisation of the complete glutamatergic output of mouse BCs at the level of individual axon terminals.

We used an AAV transduction strategy to express the fluorescent glutamate sensor iGluSnFR (Marvin et al., 2013) in the transgenic mouse line ChAT:Cre x Ai9tdTomato. Light stimulus-evoked glutamate release was recorded using two photon imaging in the whole-mounted retina at the level of individual processes in the inner plexiform layer (IPL). A correlation-based algorithm was applied to place regions of interest (ROIs), which were restricted in size to approximately match individual BC terminals. By using calcium imaging of individual BC axon terminals with the GCaMP6f biosensor (Chen et al., 2013), we verified our ROI algorithm and confirmed that each ROI captured the light-driven glutamate signal of at most one BC axon terminal. Drawing upon available EM-data (Kim et al., 2014; Greene et al., 2016) and recorded depth information for each ROI, we implemented a probabilistic clustering framework for separating functional response profiles of >13,000 ROIs (37 mice) into functional clusters. Each cluster was linked to an anatomically known BC type.

This approach yielded a functional fingerprint of every anatomical BC type in the mouse retina, including a detailed account of their response kinetics, receptive field (RF) structure and centre-surround properties. We found that the overall functional heterogeneity within individual IPL strata was larger than previously thought and showed that this functional diversity is generated by the balanced interplay of dendritic excitatory inputs and axonal inhibitory inputs. Here, GABAergic amacrine cells (ACs) mediated the BC surround and provided decorrelating inhibitory inputs. In contrast, glycinergic ACs mainly shaped BC function indirectly by gating the GABAergic network. Furthermore, we demonstrated that centre and surround components of BC RFs interact to decorrelate BC output not only in the spatial, but also in the temporal domain. Our findings highlight the importance of inhibitory circuits in generating functionally diverse excitatory pathways and suggest that decorrelation of parallel visual pathways begins already at the second synapse of the mouse visual system.

Reading the population code of the retina

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In a neural map, cells of the same subtype perform the same computation in different places of the visual field. How these different cells code together a complex visual scene is unclear. It is commonly assumed that they will code for a same feature to form a feature map, but this has rarely been observed directly. Using large-scale recordings in the retina, we show that a homogeneous population of fast OFF ganglion cells encode simultaneously two radically different features of a visual scene. Cells close to a moving object coded linearly for its position. More distant cells remained largely invariant to its position and responded non-linearly to speed changes. Cells switched from one computation to the other depending on the stimulus. Therefore ganglion cells of a single type do not code for one, but two features simultaneously. This richer, flexible neural map might also be present in other sensory systems.

Decorrelation of retinal response to natural scenes by eye movements

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Fixational eye movements are critical for vision since without them the retina adapts fast to a stationary image and the entire visual perception fades away in a matter of seconds. Yet, the connection between fixational eye movements and retinal encoding is not fully understood. To address this issue, it was suggested theoretically that fixational eye movements are required to reduce the spatial correlations which are typical for natural scenes. The goal of our study was to put this theoretical prediction under experimental test. Using a multi electrode array, we measured the response of the tiger salamander retina to movies which simulated two types of stimuli: fixational eye movements over a natural scene and flash followed by static view of a natural scene. Then we calculated the cross-correlation in the response of the ganglion cells as function of receptive fields distance. We found that when static natural images are projected, strong spatial correlations are present in the neural response due to correlation in the natural scene. However, in the presence of fixational eye movements, the level of correlation in the neural response drops much faster as function of distance which results in effective decorrelation of the channels streaming information to the brain. This observation confirms the prediction that fixational eye movement act to reduce the correlations in retinal response and provides better understanding of the contribution of fixational eye movements to the information processing by the retina.

Towards the activation of physiological retinal ganglion cell spiking by electrical stimulation

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Motivation: Retinal ganglion cells (RGC) respond differentially to incremental or to decremental luminance changes (ON and OFF RGC classes), to chromatic content and to spatio-temporal changes of the visual input. After photoreceptor-degeneration, the surviving ganglion cells in blind retinas remain intact and show a functionally diverse spiking pattern [1]. However, the activation of specified ganglion cell output patterns by artificial electrical stimulation is currently not feasible. This might be attributed to the lack of large-scale screening of physiological and artificial stimuli and simultaneous recording of the induced activity.

Approach: Here we address the question, to what degree light-activated ganglion cell patterns in the mouse retina can be reproduced by appropriate electrical stimulation. Towards this goal we interface *ex vivo* mouse retina (C57/Bl6J) in epiretinal configuration to a CMOS-based microelectrode array comprising 4225 recording sites and 1024 stimulation electrodes. The high density of recording electrodes enables high accuracy of the spike sorting [2]. We present flickering light stimuli of different wavelength (range 405 – 635 nm) using a customized illumination system. Ganglion cell responses are classified with respect to response polarity and transience [3]. Continuous electrical stimulation is applied via the capacitive stimulation sites [4]. Artefact – free recording of the stimulated ganglion cell spiking is performed on all recording sites.

Results: We identify light stimulated ganglion cell activity from more than 100 RGCs within a recording area of 1mm². Fewer RGCs were activated by short wavelength (405 nm) or by long-wavelength (635 nm) stimuli. Electrical continuous-wave stimuli activated reliably 90% of light-activated RGCs. Ten percent of electrically activated RGCs showed no light response. As suggested by Freeman et al.[5], we could sequentially activate ON and OFF RGCs using sinusoidal stimulation. The time delay between the spiking of ON and OFF RGCs was adjusted by the stimulation frequency and compared to flickering light stimulation.

Conclusion: Here we present a strategy how to evaluate physiological and artificial stimulation of the retinal output by screening light- and electrically induced activity in more than one hundred RGCs within the same retinal portion using a rich stimulus set.

References:

- [1] Menzler J. and Zeck G., J.Neurosci. 2011
- [2] Leibig C. et al., J.Neurosci. Methods 2016
- [3] Farrow K. and Masland R.H., J.Neurophysiol. 2011
- [4] Bertotti G. et al., IEEE BIOCAS 2014
- [5] Freeman D. et al., J.Neurophysiol. 2010

This work was supported by a BMBF Grant (no. 031L0059A) to F.J. and G.Z.

Symposium

S34: Glial cells in de- and remyelination

[S34-1](#) Mechanisms of myelin breakdown in demyelinating diseases

Mikael Simons

[S34-2](#) NG2-glia in health and disease: Their role in the adult brain

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[S34-6](#) Functional heterogeneity of OPCs in the central nervous system

Sarah Förster, Abbe H Crawford, Christopher J Heath, Richa B Tripathi, Tim J Bussey, William D Richardson, Robin JM Franklin

Mechanisms of myelin breakdown in demyelinating diseases

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Breakdown of myelin sheaths is a pathological hallmark of several autoimmune diseases of the nervous system. We have employed autoantibody-mediated animal models of demyelinating diseases to target myelin and found that myelin lamellae are broken down into vesicular structures at the innermost region of the myelin sheath. We demonstrated that myelin basic proteins, which form a polymer in between the myelin membrane layers, are targeted in these models. Elevation of intracellular Ca²⁺ levels resulted in MBP network disassembly and myelin vesiculation. We propose that the aberrant phase transition of MBP molecules from their cohesive to soluble and non-adhesive state is a mechanism triggering myelin breakdown in demyelinating diseases.

NG2-glia in health and disease: Their role in the adult brain

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Glial cells in the adult brain are very diverse and some of them represent the stem and progenitor cells of the CNS. My talk will focus on the adult oligodendrocyte progenitor cells (OPCs), also known as NG2-glia, in the intact and injured mouse brain. The widespread interest in this glial cell population raises from their unique properties, as adult NG2-glia represent the only proliferating cell type in the adult brain parenchyma outside the neurogenic niches and continuously generate -in a region specific manner- mature, myelinating oligodendrocytes. However, their functions in the adult CNS and the mechanisms regulating their behavior under both physiological and pathological conditions are still not resolved. Additionally, it is still widely unknown whether NG2-glia comprise a homogeneous or heterogeneous population. To tackle these questions, we used various tools such as transplantation experiments, conditional depletion of proliferating NG2-glia, proteomic and transcriptomic analysis as well as in vivo live imaging of these cells in the adult mouse cerebral cortex. By these techniques we were able to reveal new insights into the functional role of NG2-glia in the intact and injured brain.

Role of astrocytes in de- and remyelination

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The role of astrocytes in demyelinating diseases during de- and remyelination is only poorly characterized. In order to study the contribution of astrocytes during these processes we have employed transgenic animals where activated, GFAP-expressing astrocytes can be ablated. Using these animals we have ablated astrocytes during cuprizone-induced demyelination and the following remyelination. During demyelination the absence of astrocytes led to a failure of timely removal of damaged myelin associated with retarded recruitment of microglia. CCL10 was identified as a possible signal mediating the communication between astrocytes and microglia. The delayed removal of damaged myelin led also to a delay in remyelination. Ablating astrocytes during remyelination only also impaired the process of remyelination. These studies demonstrate the importance of astrocytes during both demyelination and remyelination.

Modulating glial cells in autoimmune encephalomyelitis – on the way to translational medicine?”

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Glial participate in degeneration and repair during autoimmune demyelination of the nervous system. In multiple sclerosis (MS), microglia and astrocyte may play a detrimental or protective role depending on the micromilieu und cellular polarization. In contrast, oligodendroglial cells are culprits of the disease process where inflammatory mechanisms may lead to oligodendroglial apoptosis via tumor necrosis alpha and Fas-dependent mechanisms. Consequently remyelination in MS is limited by an exhaust of oligodendroglial precursor cells over the course of the disease and an differentiation block oligodendroglial cells as well as the expression of molecules inhibiting the interaction between the remyelinating oligodendrocyte and the axon.

Thus, a better understanding of mechanisms of oligodendroglial cell death and differentiation i neuroinflammation is of utmost importance for the design of better therapies aiming at repair in MS.

New targets for blocking oligodendrocyte apoptosis comprise proteins of the 14-3-3 family which are involved in the regulation of protein-protein interaction and have been implicated in neurodegeneration and 14-3-3 proteins are also expressed in cells of the oligodendroglial lineage. 14-3-3 gamma knockout mice display a more severe course of experimental autoimmune encephalomyelitis associated with enhanced oligodendrocyte apoptosis and myelin loss without interfering with the immune response. Hence, small molecule activators of 14-3-3 protein pathways may constitute a protective therapy approach in neuroinflammation.

In addition, the relation of new goal MS therapies to cells of the oligodendroglial lineage is discussed including dimethyl fumarate and fingolimod. Finally, regenerative approaches include the blockade of inhibitory pathways like a monoclonal antibody targeting LINGO-1 (opicinumab) which has just completed phase II trials in MS.

Impaired Schwann cell autophagy in a late onset motoneuron disease

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Mutations in the human *PLEKHG5* gene are associated with several motoneuron diseases, such as an intermediate form of the Charcot-Marie-Tooth disease (CMT), distal spinal muscular atrophy type IV (DSMA-IV) and amyotrophic lateral sclerosis (ALS). We generated *Plekhhg5* deficient mice to study the function of Plekhhg5 within the nervous system. These mice developed a late onset motoneuron disease, characterized by severe hindlimb paralysis, progressive loss of motoneurons and an altered myelination within peripheral nerves.

In particular we observed axons with myelin infoldings, which progressed with age. G-ratio analysis indicates slight hypermyelination in small caliber axons. Moreover, axonal diameter was significantly reduced in mutant mice.

With *Plekhhg5* being expressed not only in neurons but also in Schwann cells, we cultivated primary Schwann cells to search for cell autonomous effects contributing to the disease phenotype. We observed reduced amount of autophagosomes in Schwann cells lacking *Plekhhg5*. Schwann cell autophagy was recently described to participate in degradation of the myelin sheath upon axonal stress. In an *ex vivo* demyelination assay, we detected reduced demyelination upon *Plekhhg5* deficiency indicating contribution of impaired Schwann cell autophagy to the phenotype observed and possibly to human *Plekhhg5*-associated motoneuron diseases.

Functional heterogeneity of OPCs in the central nervous system

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In the mouse embryonic forebrain OPCs are generated in consecutive waves from distinct brain regions along a spatiotemporal gradient, with ventral OPCs emerging before dorsal OPCs. Although these different OPC populations functionally compensate during development, they persist in the brain throughout life. To investigate whether ventrally and dorsally derived OPCs fulfil different functions in the adult brain, dorsally derived OPCs were ablated using a Sox10-driven diphtheria toxin A mouse model. As dorsally derived OPCs mainly populate the cortex, motor functional and cognitive abilities after the ablation of dorsally derived OPCs were assessed. Mice ablated of dorsally derived OPCs show significant impairment in motor function and cognition. These disabilities are not due to a decrease in overall brain white matter content shown by magnetic resonance imaging. Accordingly, assessing the differentiation capabilities of ventrally and dorsally derived oligodendrocyte lineage cells in vivo demonstrated that after an initial delay in differentiation of dorsally derived OPCs early after birth, ventrally and dorsally derived OPCs give rise to similar numbers of mature oligodendrocytes in the adult brain. In conclusion, our results point towards distinct roles for ventrally and dorsally derived OPC in executing specific brain functions in the adult brain. A more detailed analysis is of signalling pathways differentially regulated in ventrally and dorsally derived OPCs, and myelin structures formed by oligodendrocytes derived from the distinct OPC populations is ongoing, which will help further uncover the underlying difference of the two developmentally distinct OPC populations.

Symposium

S35: Use it or lose it - cellular and molecular mechanisms of synapse remodeling in developmental plasticity

- [S35-1](#) Molecules of the excitatory postsynapse govern the duration of plastic phases during brain development
Oliver Marcus Schlüter
- [S35-2](#) Experience-dependent equilibration of AMPAR-mediated synaptic transmission during the critical period
Weifeng Xu, Kyung Seok Han, Samuel Cooke
- [S35-3](#) Lateral geniculate neurons projecting to mouse visual cortex show robust ocular dominance plasticity
Juliane Jäpel, Mark Hübener, Tobias Bonhoeffer, Tobias Rose
- [S35-4](#) Plasticity for fine-tuning developing cortical circuits with single synapse precision
Christian Lohmann
- [S35-5](#) Microglia – a critical element of cortical plasticity
Ania Katarzyna Majewska
- [S35-6](#) Activity-dependent apoptosis shapes the structural maturation of the cerebral cortex in an area-dependent manner
Oriane Blanquie, Jenq-Wei Yang, Werner Kilb, Salim Sharopov, Anne Sinning, Heiko Luhmann

Molecules of the excitatory postsynapse govern the duration of plastic phases during brain development

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Refinement of synaptic connections takes place during restricted time windows of brain cortex development. AMPA type glutamate receptor (AMPA) lacking (silent) synapses are abundant at the beginning of structured sensory innervation of the primary visual cortex. These synapses form morphological connections between neurons, but do not or only little contribute to synaptic transmission and thus the information flow. Visual experience, likely triggering associative plasticity, governs the maturation of silent synapses by incorporating AMPARs and thus stabilizes the information flow across these matured connections. PSD-95 is a signaling scaffolds of the postsynaptic density, which regulates AMPAR synaptic incorporation. Experience-dependent silent synapse maturation is impaired in PSD-95 knock-out mice and half of the synapses prevail silent lifelong. The juvenile form of ocular dominance plasticity in normal reared mice is restricted to its critical period during the plastic phase of visual cortex development. In PSD-95 knock-out mice, the critical period does not close and the visual cortex remains plastic lifelong. Silencing PSD-95 expression after the closure of the critical period, reinstates silent synapses and the juvenile form of ocular dominance plasticity, indicating that PSD-95-dependent progressive maturation of silent synapses terminates this plastic phase of visual cortex development.

Experience-dependent equilibration of AMPAR-mediated synaptic transmission during the critical period

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Experience-dependent refinement of synaptic connections is essential for functional optimization of neural circuits. However, how sensory experience sculpts the excitatory synaptic transmission mediated by AMPARs is poorly understood. Here, we show that despite substantial remodeling of synaptic connectivity, AMPAR-mediated synaptic transmission remains at equilibrium during the critical period in the mouse primary visual cortex. The maintenance of this equilibrium requires neurogranin (Ng), a postsynaptic calmodulin-binding protein important for synaptic plasticity. With normal visual experience, loss of Ng decreased AMPAR-transmitting synapse numbers, prevented AMPAR-silent synapse maturation, and increased spine pruning. Importantly, visual deprivation halted synaptic deficits caused by loss of Ng, revealing that Ng coordinates experience-dependent AMPAR-silent synapse maturation and synapse pruning. Loss of Ng also led to sensitized functional synaptic long-term depression (LTD), reduced cortical visual responsiveness, and impaired visually-guided behavior. Our synaptic interrogations reveal that experience-dependent coordination of AMPAR-silent synapse maturation and synapse pruning hinges upon Ng-dependent mechanisms for constructive synaptic refinement during the critical period.

Lateral geniculate neurons projecting to mouse visual cortex show robust ocular dominance plasticity

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Ocular dominance (OD) plasticity, the shift in eye-specific responsiveness after chronic closure of one eye, is a widely studied model of experience-dependent plasticity. Most theories of OD plasticity assume that the synaptic changes in response to monocular deprivation (MD) are exclusively cortical. This assumption is largely based on early cross-sectional recordings from cells in the lateral geniculate nucleus (LGN) that did not reveal prominent functional changes (e. g. Wiesel & Hubel, 1963). Yet, none of the subcortical measurements so far have been performed chronically with single-cell resolution, leaving the possibility that subtle changes may have been overlooked. Additionally, recent studies provide evidence that a significant fraction of LGN cells responds robustly to stimulation of both eyes in rodents and primates (Howarth et al., 2014; Zeater et al., 2015), potentially providing the basis for competitive plasticity. This led us to re-examine the question of subcortical experience-dependent plasticity in the LGN by chronically following the activity of the same thalamocortical (TC) afferents in layer I of the binocular visual cortex of adult mice during OD plasticity.

We conditionally expressed the genetically encoded Ca²⁺ indicator GCaMP6m in LGN neurons of partially thalamus-specific Scnn1a-Tg3-Cre mice using AAV-mediated transduction. We repeatedly measured the responses of TC afferents to visual stimulation both before and after MD using chronic two-photon Ca²⁺ imaging. The eye-specific tuning of the same identified boutons imaged over weeks was largely stable under baseline conditions. To our surprise, however, we found that LGN axons and boutons show prominent OD plasticity after MD. Similar to the changes that we have observed in visual cortex (Rose et al. 2016), a large fraction of individual TC boutons reduced their response to deprived eye stimulation and increased their responsiveness to open eye stimuli. This clear expression of thalamic OD shifts argues against an exclusively cortical expression of OD plasticity which should be taken into account by future models.

Howarth, M., Walmsley, L., and Brown, T. M. (2014). Binocular integration in the mouse lateral geniculate nuclei. *Current Biology*, 24(11):1241–1247.

Rose, T., Jaepel, J., Hübener, M., and Bonhoeffer, T. (2016). Cell-specific restoration of stimulus preference after monocular deprivation in the visual cortex. *Science*, 352(6291):1319–1322.

Wiesel, T. and Hubel, D. (1963). Effects of visual deprivation on morphology and physiology of cells in the cat's lateral geniculate body. *Journal of Neurophysiology*, 26:978–93.

Zeater, N., Cheong, S. K., Solomon, S. G., Dreher, B., and Martin, P. R. (2015). Binocular Visual Responses in the Primate Lateral Geniculate Nucleus Binocular Visual Responses in the Primate Lateral Geniculate Nucleus. *Current Biology*, 25(24):3190–3195.

Plasticity for fine-tuning developing cortical circuits with single synapse precision

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Already early in development, before the senses are functional, spontaneous neuronal activity occurs in emerging circuits and determines neuronal connectivity. While experimentally blocking or perturbing spontaneous activity has demonstrated its importance for network development, the plasticity mechanisms underlying activity dependent wiring on the level of individual synapses are still unclear. We use time-lapse microscopy and electrophysiological recordings to monitor functional neuronal connectivity with single synapse resolution in vitro and in vivo. Mapping spatio-temporal patterns of synaptic activity and plasticity across large populations of synapses of individual neurons showed that functionally correlated synapses are clustered along developing dendrites. Pharmacological experiments demonstrated that clustering is dependent on spontaneous activity. Our analysis of spontaneous activity changes at hundreds of individual synapses in vitro and in vivo revealed a general, yet very simple, plasticity rule: synapses that become desynchronized with neighboring synapses undergo synaptic depression, whereas synapses that are locally synchronized become stabilized. This local “out of sync – lose your link” plasticity mechanism can cluster synaptic inputs efficiently. Thus, spontaneous activity fine-tunes neuronal connectivity with single synapse precision.

Microglia – a critical element of cortical plasticity

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In the brain, neurons carry out the complex computations that allow proper cognitive function. These neurons are connected together into networks through connections called synapses. The ability to tune these synapses, and thus alter neural networks is critical to both the normal development of brain circuitry and brain function throughout life, underlying processes such as learning and memory. Microglia are immune cells that infiltrate the brain early in development before the formation of the blood brain barrier. They have critical roles during brain injury, infection or disease. However, new data has thrust these non-neural cells into the spotlight as regulators of synapses. We show that microglia display dynamic interactions with synapses in the non-pathological brain, and contribute to experience-dependent plasticity in the visual cortex in vivo. We found that manipulations of visual experience elicit a remarkably rapid behavioral response in microglia which is distinct from their inflammatory response, and includes an increase in phagocytosis that corresponds to the early phase of plasticity when synapses are lost in this model and when microglia increase their synaptic interactions. We also describe a role for P2Y₁₂, a purinergic receptor expressed exclusively in microglia in the brain, in visual plasticity. Our findings suggest that microglia play an important role in synaptic plasticity, and use a subset of their pathological molecular repertoire to implement plastic changes in the non-pathological brain.

Activity-dependent apoptosis shapes the structural maturation of the cerebral cortex in an area-dependent manner

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During early postnatal development, a wave of programmed cell death is responsible for the loss of up to 50% of neurons. This physiological process ensures an accurate balance between excitatory and inhibitory neurons and determines the ultimate number of neurons. In the central nervous system, increase in spontaneous activity is commonly associated with a decreased number of neurons undergoing apoptosis whereas a reduction of electrical activity exacerbates the rate of programmed cell death. However, the precise mechanisms underlying this activity-dependent process are still unclear. Here, we ask whether developmental apoptosis differentially affects neocortical areas, thus taking part in the structural and functional maturation of the rodent cerebral cortex.

Using immunocytochemistry and *in vivo* extracellular recordings, we report that the developing cerebral cortex displays both area-specific levels of activity and area-specific densities of degenerating neurons and that these two parameters negatively correlate. Through electrophysiological and pharmacological modification of activity levels, we next show that activity patterns recorded in the investigated functional cortical areas of anaesthetized mice contribute to the regional pattern of apoptosis, indicating that endogenous early cortical activity participates in tuning the density of apoptotic neurons. Further, unilateral whisker deafferentation increases the density of apoptotic cells in the corresponding barrel cortex, revealing that peripheral inputs provide an additional pro-survival signal to cortical neurons.

All together, our results provide evidence that intrinsically-generated activity and periphery-driven activity interplay to control the extent of cortical apoptosis in an area-dependent manner, thus shaping the structural maturation of the cerebral cortex. Since both developmental apoptosis and early activity patterns are conserved in humans, these results provide important insight in neurodevelopmental disorders involving a perturbation of activity during early life.

Symposium

S36: Novel local mechanisms of motor control

- [S36-1](#) Modular microcircuits underlying gear changes during locomotion
Abdel El Manira
- [S36-2](#) Two discrete pathways responsible for the intrasegmental coordination of limb movements in the abdominal ganglia of crayfish
Carmen R Smarandache-Wellmann
- [S36-3](#) Motor Control of *Drosophila* Courtship Song and Flight
Angela O`Sullivan, Anne C. von Philipsborn
- [S36-4](#) A local command neuron and the control of leg searching movements in the stick insect
Eva M Berg
- [S36-5](#) Regulation of locomotor network performance by the sodium pump in *Xenopus* frog tadpoles
KeithThomas Sillar, HongYan Zhang, Laurence David Picton
- [S36-6](#) Descending modulation of thoracic motor activity in the stick insect
Thomas Stolz, Max Diesner, Susanne Neupert, Joachim Schmidt

Modular microcircuits underlying gear changes during locomotion

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A salient feature of neural circuit for motor behavior is to produce movements with variable speed and force as circumstances demand. How this variability is encoded within motor circuits is the focus of our research. We use the adult zebrafish as a model system to decipher the organization of spinal circuits underlying the change of gears to control the locomotor speed. Our results reveal an important principle of organization of the spinal locomotor circuits that accounts for the orderly activation of slow, intermediate, and fast motor units at different speeds of locomotion. In contrast to what has previously been assumed, we show that the locomotor network does not consist of a single unit, but can be deconstructed into three microcircuit modules. Each module comprises a distinct subclass of excitatory premotor (V2a) interneurons that drive locomotion and make selective monosynaptic connections with slow, intermediate, or fast motoneurons. This modular organization of V2a interneurons-motoneurons combined with their overlapping activation during swimming ensures a smooth transition between locomotor speeds during acceleration or deceleration by sequentially engaging or disengaging the successive microcircuits. Such a multiple microcircuit organization may represent a general feature of vertebrate locomotor networks that endows them with an intrinsic mechanism to increase speed and force of movements.

Two discrete pathways responsible for the intrasegmental coordination of limb movements in the abdominal ganglia of crayfish

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Coordination of central pattern generators (CPG) is an important feature accomplished by the nervous system. I am interested in the cellular mechanisms and connectivity of neurons responsible for coordination of CPGs. For this I study the crayfish swimmeret system, which is used for forward swimming. The central nervous system of crustaceans consists of a ganglia chain. The swimmerets of the crayfish occur in pairs on the second to the fifth abdominal segment, with one limb on each side of the abdomen.

Each swimmeret movement is controlled by a neuronal microcircuit located in its hemisegment, within this the CPG consists of a set of two non-spiking interneurons which form a half center oscillator. A separate neuronal network coordinates the movement of four swimmeret pairs. Their metachronal movement is observed in vitro and in vivo, such that the swimmeret movement starts in the last segment and the anterior swimmerets always follow with a latency of a quarter cycle.

The neuronal network for intersegmental coordination consists of exactly three neurons in each hemisegment: Two spiking coordinating neurons, ASC_E and DSC, and one non-spiking local interneuron, ComInt1. ASC_E and DSC encode information about the activity state of their microcircuit, driven by the CPG interneurons. Coordinating information from one microcircuits is sent to all neighboring hemisegments arriving with a gradient of synaptic strength in ComInt1. The neighboring coordinating neurons always elicit larger depolarizations in ComInt1 than those which are more remote. To close the loop ComInt1 decodes the coordinating information and synchronizes the activity of the microcircuits through its electric synapse to one specific CPG interneuron.

The swimmerets of crayfish in one segment move mostly in phase, which is rather unusual for leg movement in invertebrates. Recently we found two distinct pathways which couple the two segmental oscillators to be active in phase. The oscillators on the ipsilateral side (the intersegmental coupling) are weakly coupled and so are the intrasegmental oscillators. At least two inputs guarantee their in-phase activity. The first pathway is formed by the coordinating neurons. We could demonstrate that ASC_E and DSC do not only elicit excitatory postsynaptic potentials (EPSPs) in the ipsilateral ComInt1, but also in the contralateral ComInt1. Although contralateral inputs are smaller than the one from the neighboring ipsilateral Coordinating Neurons, they still seem to be strong enough to give excitatory input into the CPGs to be active in phase. The second pathway goes via a three synaptic pathway, including two electrical synapses. One specific motor neuron in each hemiganglion receives rhythmic input from one CPG neuron, while it is electrically coupled with the other CPG neuron by a rectifying synapse. This rectifying synapse is important, because this specific motor neuron is also coupled to its homologue counterpart on the contralateral side via an electrical synapse. So when one neural oscillator is active on one side, the motor neuron excites the homologue motor neuron from the other side, and over the rectifying synapse also the CPG neuron. This complicated pathway adds to the first one, and ensures the in phase activity under normal circumstances.

Support: DFG SM206/3-1, UoC ZUK 81/1

Motor Control of *Drosophila* Courtship Song and Flight

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A major objective of neuroscience is to comprehend how neural circuits guide behaviour. Many biological motor systems are multifunctional and can switch between different modes of operation to perform multiple behaviours. *Drosophila melanogaster* use their wings not only to fly but also to execute the courtship song, a vital step of mating behaviour. The motor patterns for song and flight differ considerably. My research explores how this multifunctionality is achieved on the neuronal circuit level. We use the split GAL4/UAS system for restricted genetic access to motor neurons innervating flight muscles as well as candidate upstream interneurons. By silencing or activating these neurons, in conjunction with behavioural assays their function can be identified. Immunostaining techniques, 3D image registration and reconstruction permit generation of an anatomical atlas of the wing neuropil in the ventral nerve cord. Furthermore, optogenetic activation of courtship-initiating neurons coupled with GCaMP imaging of the flight control muscles has revealed the pattern of muscle activity during both flight and song, providing novel insight into wing motor control. Further knowledge on wing motor control aims to elucidate basic principles of how the nervous system initiates and maintains distinct and mutually exclusive motor patterns employing the same neuronal components.

A local command neuron and the control of leg searching movements in the stick insect

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Animals that move through the environment constantly need to adapt their movements to the surrounding conditions. To this end, many animals use their tactile senses to gain information about their surroundings, e.g. by palpating objects with their hands [1], whiskers [2], or antennae [3]. Similar, stick insects, when losing ground contact with one of their legs perform rhythmic searching movements with the respective leg to find a new foothold. Once stick insects touch an object with their leg the movements are modified in order to grasp the object [4] or –if the object disappears before it can be grasped- to center the SMs on the former position of the object [5]. Thus, searching movements provide an experimental accessible behavior to study the generation and modification of a rhythmic motor behavior. Additionally, the switch from multi-legged walking or standing to single-legged searching (and back) provides the opportunity to investigate the neuronal mechanisms underlying the transition between two different behaviors.

For walking, the kinematics and underlying motor- and premotor neuron activity have already been investigated [6-9]. Likewise, the kinematics of searching movements and the underlying motoneuronal activity have been described [10,11]. However, neither the activity of premotor interneurons during rhythmic searching movements, nor their potential contribution to a modification of leg movements has been the objective of research. Therefore, I investigated the activity of non-spiking interneurons (NSIs) -a subpopulation of local thoracic interneurons- during searching movements. I simultaneously recorded the intracellular activity of individual NSIs in the semi-intact behaving animal, electrical muscle activity of the four main leg muscles and movement trajectories.

I found that the membrane potential of multiple identified premotor NSIs was rhythmically modulated during leg searching movements. NSIs received alternating synaptic excitation and inhibition riding atop a tonic depolarization. When I artificially changed the membrane potential of single NSIs by tonic current injection throughout ongoing searching movements, I could induce changes in specific searching movement parameters. These parameters were movement amplitude, velocity, position and interjoint coordination. Individual NSIs repeatedly affected the same parameters across animals. Most notably, one NSI, I4, was sufficient and necessary for the generation of searching behavior. I4 could not initiate or stop walking behavior. This “command neuron” function for searching was present when the leg was in midair but was lost when the leg contacted the ground. It thus provides a substrate for mediating the switch between searching and walking behavior based on environmental conditions.

Overall, I show that NSIs are an important element for the control of searching behavior and can serve as neural substrate to effect modifications of searching behavior.

[1] Klatzky and Lederman 1990, *Dextrous Robot Hands*, Springer-Verlag New York Inc.

[2] Diamond et al. 2008, *Nat Rev Neurosci* 9, 601-12

[3] Dürr 2001, *J Exp Biol* 204, 1589-1604

[4] Bässler et al 1991, *Biol Cybern* 65, 507-14

[5] Berg et al 2013, *J Exp Biol* 216, 1064-74

[6] von Uckermann and Bueschges 2009, *J Neurophysiol* 102, 1956-1975

[7] Rosenbaum et al 2010, *J Neurophysiol* 104:1681-1695

[8] Fischer et al 2001, *J Neurophysiol* 85:341-353

- [9] Schmidt et al 2001, J Neurophysiol 85: 354-361
- [10] Schmidt et al 2001, J Neurophysiol 85, 354-61
- [11] Fischer et al 2001, J Neurophysiol 85, 241-353

Regulation of locomotor network performance by the sodium pump in *Xenopus* frog tadpoles

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Na⁺/K⁺ ATPases (aka sodium pumps) are ubiquitously expressed membrane proteins responsible for establishing sodium and potassium ion gradients across cell membranes. These proteins continuously pump 3 Na⁺ out for 2 K⁺ in per pump cycle and this ratio generates a pump current that establishes and maintains the resting membrane potential of neurons. However, the pumps are also able to detect rises in intracellular sodium, for example following intense neuronal firing, and increase their activity so as to homeostatically restore ionic equilibrium.

In this talk I will review recent evidence that activity-dependent changes in sodium pump currents regulate the output of spinal locomotor networks, such as the CPG underlying rhythmic swimming movements in *Xenopus* frog tadpoles¹. Fictive swim episode duration linearly correlates with inter-swim interval if episodes are evoked within ~1 minute of each other: shorter intervals produce shorter episodes, an effect that is blocked by low concentration of ouabain. This novel form of short term motor memory results from a pump-mediated ultraslow afterhyperpolarization (usAHP) of up to 10 mV. The usAHP lasts approximately 60 seconds, is spike-dependent, sensitive to low concentrations of ouabain, and it is not associated with a change in membrane conductance. The effect on swimming is a consequence of the de-inactivation of an A-type K⁺ current caused by the hyperpolarization, which in turn delays the first spike in a swim burst and so reduces excitability in the network as a whole².

The usAHP is only expressed in a sub-set of spinal CPG neurons, including about 50% of motoneurons, commissural and ascending interneurons. However, it is absent in descending excitatory interneurons which presumably enables the network to produce some cycles of swimming even when the inter-swim interval is short. Our data support the idea that this heterogeneity of usAHP expression results from the presence of two types of sodium pump in spinal neurons; neurons lacking a usAHP may express only 1 subunits containing pumps, which have a high affinity for intracellular sodium and are maximally activated at rest, while neurons displaying a usAHP express the 3 subunit which has a higher affinity for sodium, is blocked by low concentrations of ouabain and only recruited following the large increases in intracellular sodium that accompany high frequency firing. Finally, it is widely known that sodium pumps can be phosphorylated at sites located on the intracellular domain of the protein³. We have addressed the possibility that the pumps are targets for neuromodulation and show that dopamine and nitric oxide, both known intrinsic modulators of tadpole locomotor network output, affect the amplitude of the usAHP. Nitric oxide reduces and dopamine enhances the usAHP.

In summary, sodium pumps play a dynamic, activity-dependent role in regulating spinal locomotor network output in *Xenopus* tadpoles and represent important targets for intrinsic modulatory control pathways. They provide the network with a flexible form of short-term motor memory that links future to past locomotor behaviour.

1. Zhang H, Sillar KT (2012) Current Biology 22:526-531.

2. Zhang, H. Y., Picton, L., Li, W. C., & Sillar, K. T. (2015). *Scientific Reports* 5: 16188.
3. Therien a G & Blostein R (2000). *Am J Physiol Cell Physiol* 279, C541–C566.

Descending modulation of thoracic motor activity in the stick insect

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Neuromodulators are instrumental in the selection of task-specific motor output in animals. The biogenic amine octopamine is a key modulator of insect thoracic locomotor networks. In inactive stick insects, for example, octopamine alters the response properties of a leg-proprioceptive feedback system towards those that characterize the active state of animals (Büsches et al. 1993). Furthermore, octopamine increases a tonic depolarization, ubiquitous in mesothoracic leg motoneurons during walking (Westmark et al. 2009). Until now, the identity of octopaminergic neurons modulating thoracic motor activity has remained elusive. In insects, octopamine can be released from dorsal unpaired median (DUM) neurons. Six DUM neurons with somata located in the posterior part of the locust subesophageal ganglion have axons that are bilaterally descending (abbreviated DUM-SD) to thoracic ganglia (Bräunig and Burrows, 2004). We hypothesize that presumably homologous neurons in the stick insect might be candidates for the modulation of thoracic motor activity. Using semi-intact preparations and intracellular recordings, we observed the generation of action potentials in DUM-SD neurons during stance phases, when animals were stepping with a single middle leg and during restrained six-legged walking. Mechanical stimulation by passive movement of legs was excitatory to DUM-SD neurons. In contrast, pharmacologically evoked activity of central pattern generating neurons (CPGs) had no effect on DUM-SD neuron activity. Thus, the excitatory input to DUM-SD neurons during walking most likely arises from leg sensory organs rather than from coupling to CPG activity. In order to test a possible role of DUM-SD neurons in the modulation of thoracic motor activity, we studied the effect of DUM-SD neuron activity on reflex responses evoked by stimulation of the mesothoracic femoral chordotonal organ (fCO). We observed two major effects: 1. Stimulation of some DUM-SD neurons decreased resistance reflex responses in middle leg *extensor tibiae* motoneurons. 2. Spike activity in other DUM-SD neurons induced an increase in *extensor tibiae* motoneuron activity. Additionally, it increased the likelihood for the occurrence of assistance reflex responses during fCO stimulation. Experiments using MALDI-TOF MS indicate that the somata of both DUM-SD neuron types mediating excitatory as well as inhibitory effects on *extensor tibiae* motoneuron activity contain octopamine. Thus, individual octopaminergic neurons appear to differentially modulate a specific motor behavior, rather than promoting a general state of arousal.

The project is supported by DFG Grant Schm 1084/3-1.

Poster Topics

- [T1](#) Stem cells, Neurogenesis and Gliogenesis
- [T2](#) Axon and Dendrite Development, Synaptogenesis
- [T3](#) Developmental Cell Death, Regeneration and Transplantation
- [T4](#) Neurotransmitters, Retrograde messengers and Cytokines
- [T5](#) G Protein-linked and other Receptors
- [T6](#) Ligand-gated, Voltage-dependent Ion Channels and Transporters
- [T7](#) Synaptic Transmission, Pre- and Postsynaptic organization
- [T8](#) Synaptic Plasticity, LTP, LTD
- [T9](#) Glia, Glia-Neuron Interactions
- [T10](#) Aging and Developmental Disorders
- [T11](#) Alzheimer's, Parkinson's and other Neurodegenerative Diseases
- [T12](#) Neuroimmunology, Inflammation, and Neuroprotection
- [T13](#) Cognitive, Emotional, Behavioral State Disorders and Addiction
- [T14](#) Vision: Invertebrates
- [T15](#) Vision: Retina and Subcortical Pathways
- [T16](#) Vision: Striate and Extrastriate Cortex, Eye Movement and Visuomotor Processing
- [T17](#) Auditory Mechanoreceptors, Vestibular, Cochlea, Lateral Line and Active Sensing
- [T18](#) Auditory System: Subcortical and Cortical Processing
- [T19](#) Chemical Senses: Olfaction, Taste, Others
- [T20](#) Somatosensation: Touch, Temperature, Proprioception, Nociception

[T21](#) Motor Systems

[T22](#) Homeostatic and Neuroendocrine Systems, Stress Response

[T23](#) Neural Networks and Rhythm Generators

[T24](#) Attention, Motivation, Emotion and Cognition

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Poster Topic

T1: Stem cells, Neurogenesis and Gliogenesis

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- [T1-4A](#) Developmental transcriptomics reveals an unexpected role of Hunchback in retinal glia cell formation in *Drosophila melanogaster*
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- [T1-1B](#) DOT1L and Histone H3 lysine 79 methylation determine cortical and hippocampal development by controlling neural progenitor proliferation and cell fate
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- [T1-2B](#) Fluid mechanical forces induced by Reelin determine the shape and directionality of migrating hippocampal neurons
Shaobo Wang, Peter Wulf, Shanting Zhao, Xuejun Chai, Jiawei Li, Jeremie Lau, Antonio Virgilio Failla, Bernd Zobiak, Mirjam Sibbe, Gary L. Westbrook, Michael Frotscher, David Lutz
- [T1-3B](#) Functional analysis of post-translational modifications of Brn2 relevant for proper cortex formation
Theres Schaub, Mateusz Ambrozkiwicz, Victor Tarabykin
- [T1-4B](#) In vivo cell fate imaging: generating the timeline of neural differentiation
Stefanie Vogel, Markus Aswendt, Cordula Schäfer, Kat Folz-Donahue, Christian Kukat, Marc Ehrlich, Holm Zaehres, Mathias Hoehn
- [T1-5B](#) Loss of entire multi-subunit BAF (mSWI/SNF) complexes impairs global epigenetic programs in forebrain development
Tran Tuoc, Ramanathan Narayanan, Cemil Kerimoglu, Mehdi Pirouz, Kamila Kiszka, Linh Pham,

Robin Wagener, Joachim Rosenbusch, Michael Kessel, Andre Fischer, Anastassia Stoykova, Jochen Staiger

- [T1-1C](#) Molecular profiling of peripheral glial subtypes
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- [T1-2C](#) Neural stem cells of rat hippocampus lack the expression of KV10.1 channels: implications for a safe neurogenesis.
Cilene Lino de Oliveira , Sabine Martin, Sunke L Mortensen, Fernanda R Gomes, Luis Pardo, Elaine Del Bel, Walter Stuehmer
- [T1-3C](#) Patient-derived Pluripotent Stem Cells for the Analysis of Schizophrenia in 3D Cerebral Organoids
Matthias Jung, Jovita Schiller, Anne Puls, Albrecht Klemenz, Ina Giegling, Dan Rujescu
- [T1-4C](#) Regulation of Aberrant Adult Hippocampal Neurogenesis by microRNAs After Mild Kainic Acid-Induced Status Epilepticus: Effect on Gliogenesis and Reactive Neural Stem Cells.
Carlos P. Fitzsimons, Pascal Bielefeld, Sedef Karayel, Alisa Alisa Tiaglik, Marijn Schouten, Paul J. Lucassen, Juan M. Encinas
- [T1-5C](#) Spontaneous calcium oscillations modulated by P2Y2 receptor and L type calcium voltage gated channel activity in neurogenesis: a novel approach for studying cell fate determination
Henning Ulrich, Talita Glaser, Àgatha Oliveira, Hiromi Shimojo, Juliana Corrêa-Velloso, Claudiana Lameu, Ryoichiro Kageyama
- [T1-1D](#) The abnormal communication between neuron and oligodendrocyte disrupts myelination in NPC1 deficient mice
Fan Yang, Xiao Feng, Arndt Rolfs, Jiankai Luo
- [T1-2D](#) The Chondroitin Sulfate Code Hypothesis and FGF Signaling in the Neural Stem Cell Niche of the Developing Mouse Forebrain
Alexander von Holst, Denise Harrach
- [T1-3D](#) The contribution of Dgcr8 to mouse corticogenesis and neocortex expansion
Davide De Pietri Tonelli, Nadin Hoffmann, Federica Marinaro
- [T1-4D](#) The role of foxQ2 in insect central complex development
Gregor Bucher, Bicheng He, Marita Büscher
- [T1-5D](#) The serine protease inhibitor neuroserpin regulates developmental neurogenesis, synaptic plasticity, learning and social behaviour
Giovanna Galliciotti, Melanie Neumann , Rebecca Reumann , Ricardo Vierk , Lepu Zhou , Frederice Gries , Diego Sepulveda-Falla , Michaela Schweizer , Fabio Morellini , Chiara Nicolini , Margaret Fahnestock , Gabriele Rune , Markus Glatzel
- [T1-6D](#) Functional analysis of Lin41 in the adult stem cell niche: repurposing of a pluripotency factor in ependymal cells?
Claudia Marini, Elisa Cuevas, F. Gregory Wulczyn

A CXCL12 feedback signal from mature granule neurons to neuronal progenitors anchors neuroblasts in the subgranular zone of the dentate gyrus

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Previously, we reported that conditional deletion of chemokine receptor CXCR4 in adult neural stem cells perturbs neurogenesis in the dentate gyrus (*Schultheiss et al., Hippocampus 2013*). While CXCR4 is expressed in neural stem cells, neuronal progenitors and immature neurons, CXCL12 is expressed in mature granule neurons and endothelial cells. This constellation opens the possibility that mature granule neurons liberate CXCL12 to provide feedback to neuronal precursors. To test this hypothesis, we combined *Tbr2^{Cre}* and *Cxcl12^{LoxP}*, which deletes *Cxcl12* from granule neurons and other glutamatergic populations in the cerebral cortex. In conditional *Cxcl12* knockout (*Cxcl12cKO*) mice, the number of neural stem cells was not changed. However, NeuroD-positive neuronal progenitors were reduced in number and abnormally dispersed into the GCL. Immunostaining for doublecortin confirmed precursor dispersion and revealed increased dendritic sprouting of immature neurons in the mutants. A BrdU pulse-chase experiment from P28 to P56 showed a reduced number of BrdU-positive neurons in *Cxcl12cKO* mice. Taken together, our data indicate that loss of neuronal CXCL12 affects neurogenesis at the neuronal progenitor/ immature neuron stages. We propose that granule cell-derived CXCL12 anchors neuroblasts in the neurogenic niche, thus ensuring that neuronal differentiation takes place in the appropriate microenvironment.

Aperiodic light environment suppresses the dendrite maturation and neurogenesis in adult Indian house crows, *Corvus splendens*

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Several lines of evidence suggest the role of neurogenesis in the hippocampus and pallium in the learning and memory in birds. In a previous study, we have shown decreased tyrosine hydroxylase (TH) activation in parallel with declined cognitive performances in corvids under constant light (LL). This study aimed to investigate the effects on neurogenesis and dendrite complexity in the hippocampus and pallium in Indian house crows (*Corvus splendens*) exposed LL, with controls on 12 h light per day. In both these brain regions, the Sholl analysis of doublecortin (DCX) immunoreactive (ir) neurons revealed a significant decline in the numbers and dendritic complexity of newborn neurons under LL. Further, we found close proximity between DCX-ir and TH-ir, as shown by the double-label immunohistochemistry. Overall, these results show the effects of aperiodic LL environment on different aspects of the neurogenesis, including the survival, dendrite maturation, and integration of new neurons into a functional neuronal circuitry mediated by neurotransmitter, dopamine.

Cooperative functions of Bcl11a/Ctip1 and Bcl11b/Ctip2 in neocortex development

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The C₂H₂ zinc-finger transcription factors Bcl11a and Bcl11b play important roles in neocortical development. We and others recently showed that Bcl11a is necessary for migration and survival of upper-layer cortical projection neurons as well as subtype specification of deep-layer neurons (Wiegrefe et al., 2015, Woodworth et al., 2016). Bcl11b is required for differentiation of deep layer neurons (Arlotta et al., 2005). Bcl11a is broadly expressed in upper (II-IV) and deep (V-VI) cortical layers whereas Bcl11b expression is restricted to deep cortical layers and overlaps to a large extent with Bcl11a expression. To analyze whether both genes serve cooperative and/or redundant functions in neocortical development we analyzed forebrain-specific conditional Bcl11a/Bcl11b double mutant mice. Our phenotypic analysis demonstrates major alterations of the normal architecture of the neocortex suggesting that both genes, together, execute fundamental functions in neocortical development. Detailed mechanistic phenotype analysis of Bcl11a/Bcl11b double mutants will be presented.

Wiegrefe, C., Simon, R., Peschkes, K., Kling, C., Strehle, M., Cheng, J., Srivatsa, S., Liu, P., Jenkins, N.A., Copeland, N.G., Tarabykin, V., and Britsch, S. (2015). Bcl11a (Ctip1) Controls Migration of Cortical Projection Neurons through Regulation of Sema3c. *Neuron* 87, 311-325.

Woodworth, M.B., Greig, L.C., Liu, K.X., Ippolito, G.C., Tucker, H.O., and Macklis, J.D. (2016). Ctip1 Regulates the Balance between Specification of Distinct Projection Neuron Subtypes in Deep Cortical Layers. *Cell Rep* 15, 999-1012.

Arlotta, P., Molyneaux, B.J., Chen, J., Inoue J., Kominami, R., and Macklis J.D. (2015). Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. *Neuron* 45, 207-221.

Developmental transcriptomics reveals an unexpected role of Hunchback in retinal glia cell formation in *Drosophila melanogaster*

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Although eye development is one of the best studied processes in the model system *Drosophila melanogaster*, a comprehensive understanding of all gene products and their interactions is still missing to date. Therefore, we used stage specific genome wide expression profiling (RNAseq) to reconstruct the developmental transcriptome of *D. melanogaster* eye and head formation.

This analysis revealed that many genes activated late during larval eye development are regulated by the transcription factor Hunchback (Hb). A thorough expression analysis showed that *hb* is expressed in retinal subperineural glia cells (carpet cells). Loss of function experiments were subsequently performed to elucidate the role of Hb during carpet cell development. We show that Hb is necessary for proper carpet cell formation and/or migration and retinal axon guidance. We could also show that Hb is important for normal blood-brain-barrier development.

These unexpected roles of Hb in visual system and brain development have not been described so far.

Activity-dependent changes underlying altered human neural progenitor differentiation in fragile X syndrome, a variant of autism spectrum disorder

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Autism spectrum disorder (ASD) consists of a range of heterogeneous group of disorders. Fragile X syndrome (FXS) is a monogenic variant of ASD and the most common form of inherited intellectual disability. FXS is caused by the absence of functional FMR1 protein (FMRP) that is needed for normal neurogenesis and functional maturation of synapses and neuronal networks. The absence of FMRP results in alterations of neural progenitor fate determination and differentiation. Defects of both inhibitory and excitatory transmission impair functional connectivity and lead to hyperexcitability in brain of FXS mouse. Maturation of glutamate receptor signaling is affected and an abnormally large proportion of NMDA-only neurons has been found at the close of the critical period in the somatosensory cortex of the developing FXS mouse brain.

We have studied mechanisms underlying aberrances of FXS neurogenesis by investigating the differentiation and functional maturation of human neural progenitors lacking FMRP.

We reprogrammed somatic cells of males diagnosed with FXS and healthy controls to induced pluripotent stem (iPS) cell lines. Transcriptional silencing of the FMRI gene was confirmed by real time PCR in FXS cell lines. Human iPS cells were differentiated to neuronal lineages using dual SMAD inhibition. Differentiation of neuronal progenitors was studied by immunocytochemistry and fura2-AM based intracellular calcium recordings. We observed that the intracellular calcium responses to depolarization with high extracellular potassium were augmented in neural progenitors lacking FMRP. We exposed cells to specific ligands of glutamate receptors and observed abnormalities in the amplitudes of calcium responses to glutamate receptor agonists and enhanced differentiation of glutamate-responsive neuronal cells. Furthermore, we identified altered differentiation of a subpopulation of glutamate-responsive cells in human FXS progenitors. The FXS-specific changes were similar in mouse neural progenitors derived from the mouse model of FXS although species-specific differences existed during differentiation of progenitors.

Our results demonstrate early defects during differentiation of patient-specific FXS neural progenitors to glutamatergic neurons when compared with normal controls. Improved understanding of the pathophysiology of FXS may open new avenues for novel treatment options for neurodevelopmental disorders.

DOT1L and Histone H3 lysine 79 methylation determine cortical and hippocampal development by controlling neural progenitor proliferation and cell fate

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DOT1L histone methyltransferase of histone H3 lysine 79 plays an important role in different cellular processes such as DNA damage response, gene expression and cell cycle regulation. Aberrant H3K79 methylation (H3K79me) is observed in humans with neural tube defects indicating the dependence of correct neural development on H3K79me patterning. DOT1L activity influences progenitors in different neuroanatomical locations, including spinal cord, cerebellum and telencephalon. H3K79me modulation upon Af9/Mllt3 knockout leads to impaired Tbr1 expression in vivo. In vitro, DOT1L activity prevents cell death through repression of the unfolded protein response and promotes proliferation of cortical progenitors.

Conditional DOT1L depletion through Foxg1-cre in the mouse telencephalon resulted in a misstructured hippocampus and cortical plate and DOT1L depletion altered neural cell proliferation not only in vitro but also in vivo. Alterations in the cell cycle caused an imbalanced neural progenitor cell fate and impaired layering of the cortical plate. To unravel the mechanism of DOT1L in regulating progenitor proliferation and neural cell fate we applied genome-wide analysis of E14.5 dorsal telencephalon expression profiles using RNAsequencing together with H3K79me2 profiling using ChIPsequencing. We revealed increased expression of layer I, V and VI transcribed genes, and decreased transcription of specific progenitor and layer II, III, and IV genes indicating premature neuronal differentiation upon DOT1L knockout. We identified key transcription factors and signalling pathways affecting neural proliferation and cell fate determination as putative direct DOT1L/H3K79me target genes. Our findings delineate DOT1L and the H3K79me modification as novel master regulator of cortical and hippocampal development.

Fluid mechanical forces induced by Reelin determine the shape and directionality of migrating hippocampal neurons

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Driven by various forces migrating neurons travel with a defined speed and optimised morphology to reach their destinations. It is unclear why neurons change their shape during migration and arrange in layers. Here, we use fluid mechanical laws at low Reynolds numbers to define and assess the drag forces acting on newborn granule cells (GCs) as they move towards the attractor molecule Reelin to laminate the mouse hippocampal dentate gyrus (DG). Our analysis of the migratory behaviour in the presence and absence of Reelin suggests a fundamental principle of neuronal migration, in which optimised cell morphology depends on the Reelin-mediated drag force, reduced drag coefficients, and the spatiotemporal synchronisation of motility. Geometry-based numerical simulation using calculated fluid dynamical parameters of migrating neurons allowed for validation and a first quantification of the fluid dynamical forces acting on neurons and determining their migratory behaviour.

Functional analysis of post-translational modifications of Brn2 relevant for proper cortex formation

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The mammalian cortex is formed sequentially into six different layers, each containing a characteristic distribution of neuronal cell types and connections with other cortical and subcortical regions.

The successive generation of the different excitatory pyramidal neuron subtypes starts with deep layer neurons, followed by upper layer neurons [1] and a characteristic set of proteins and transcription factors can be detected in each of the layers.

Whereas the presence of zinc-finger transcription factors as Fezf2 or Ctip2 in deeper layers and the DNA-binding proteins Brn2 or Satb2 in upper layers is well documented, little is known about their post-translational modifications in different layers.

As activity, interaction or localization of proteins is heavily influenced and regulated via enzymatically modifications after translation[2-5], the evaluation of the actual protein state in individual layers is important to be capable to explain and unveil the cell signaling network that characterizes each area.

Here, for the first time, we systematically analyze the influence of individual modifications for the transcription factor Brn2 (POU3F2 gene product) in vivo and in vitro.

Using a variety of point mutations and examining their effects during cortex formation via in-utero-electroporation and a FACS-based deeper versus upper layer differentiation assay, as well as performing immunoprecipitation analysis of subcellular Brn2-fractions, we enlighten the role of specific post translational modifications for the functionality of this protein.

Our work permits not only important data to specify the actual state of the protein at several time points during embryonic cortex formation, it also unveils necessary interactions with other enzymes.

Taken together, our analysis extends our knowledge about activation and recruitment of Brn2 in order to become one of the most important transcription factors during cortex development.

1. Angevine JB, Jr., Sidman RL (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* 192: 766-768.
2. Berlin I, Denat L, Steunou AL, Puig I, Champeval D, et al. (2012) Phosphorylation of BRN2 modulates its interaction with the Pax3 promoter to control melanocyte migration and proliferation. *Mol Cell Biol* 32: 1237-1247.
3. Kaminska B, Kaczmarek L, Zangenehpour S, Chaudhuri A (1999) Rapid phosphorylation of Elk-1 transcription factor and activation of MAP kinase signal transduction pathways in response to visual stimulation. *Mol Cell Neurosci* 13: 405-414.
4. Vallejo D, Codocedo JF, Inestrosa NC (2016) Posttranslational Modifications Regulate the Postsynaptic Localization of PSD-95. *Mol Neurobiol*.
5. Gwizdek C, Casse F, Martin S (2013) Protein sumoylation in brain development, neuronal morphology and spinogenesis. *Neuromolecular Med* 15: 677-691.

In vivo cell fate imaging: generating the timeline of neural differentiation

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Introduction

In several pre-clinical studies, the integration of implanted neural stem cells (NSCs) and their differentiation into the neural lineage was shown together with therapeutic improvement. NSCs generated from induced pluripotent stem cells (iPSCs) hold great promise as a therapeutic intervention for several neurodegenerative diseases [1]. However, these findings are typically derived from post-mortem histological analysis, providing only one single time point information and lacking the intra-individual time profile of stem cell differentiation. In a previous study, we could already follow the differentiation of human NSCs into early and mature neurons non-invasively based on *in vivo* bioluminescence imaging (BLI) [2]. Here we show, for the first time the differentiation of a human induced pluripotent stem cell (iPSC)-based NSC line into all three neural lineages *in vitro* and *in vivo* (Fig. 1A).

Material and Methods

Human NSCs generated from iPSCs [3] were lentivirally transduced to express the imaging reporters Luc2 for BLI and EGFP for histological validation under constitutive (EF1) or cell specific promoters to image NSC differentiation into neurons (DCX, MAP2, eSyn, GAD67), astrocytes (GFAP), or oligodendrocytes (PLP)(Fig. 1B). The iPSC-NSCs were selectively stimulated to differentiate into the respective neural lineage [3]. Imaging reporter expression was validated *in vitro* using immunocytochemistry (ICC), qRT-PCR, and Western Blot. Transduced iPSC-NPCs will be implanted into the cortex of NMRI-Nude mice to follow the stem cell differentiation longitudinally *in vivo*. Histological and functional analyses will provide further data about the fate of the engrafted stem cells and the interaction with endogenous cell populations.

Results

The transduced iPSC-NPCs showed a lineage-dependent increase in EGFP expression during *in vitro* differentiation experiments (Fig. 1C). iPSC-NPCs differentiated into neuronal lineage showed EGFP expression for early neuronal markers (DCX) from day 7 post stimulation, whereas expression for mature neuronal markers (MAP2, eSyn, GAD67) started from day 15 post stimulation. For glial lineage development, EGFP expression was observed at later time points from day 21 for astrocytes (GFAP) and from day 28 for oligodendrocytes (PLP).

Discussion

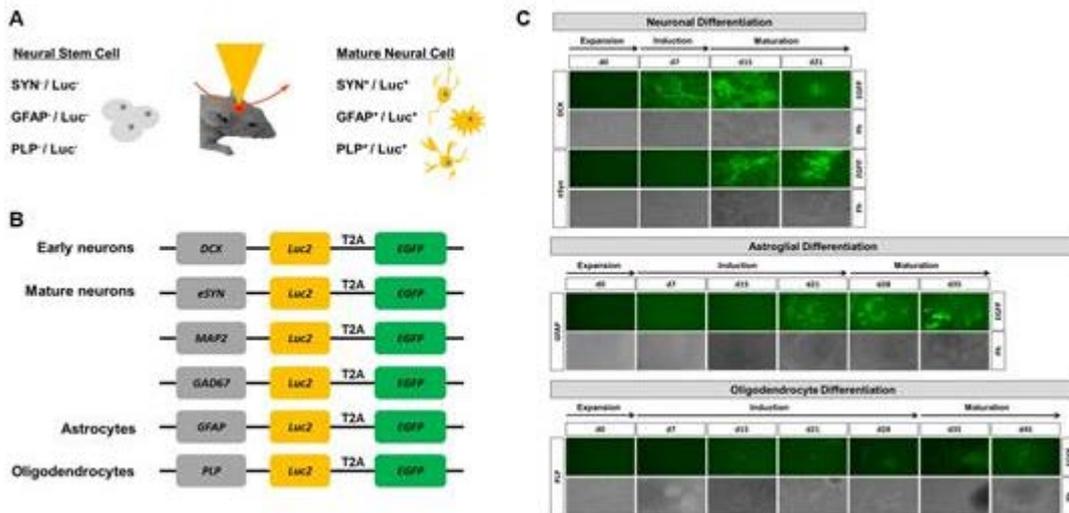
With this study, we will monitor for the first time the differentiation of implanted iPSC-derived NSCs into either of all three neural lineages in the intact living organism. For future studies, our fate mapping tool will allow proving the functional integration of the stem cell graft in combination with the DREADD technology or optogenetic tools. The implantation of our genetically modified iPSC-NSCs into neurodegenerative disease models will provide further information about possible mechanisms for

improvement by discriminating between paracrine effects and tissue integration.

References

- [1] Cefalo MG et al. *Stem Cells Int.* 2016; 2016:4869071.
 [2] Tennstaedt A et al. *Biomaterials.* 2015 Mar; 44:143-54.
 [3] Hargus G et al. *Cell Rep.* Sep 25;8(6):1697-703.

Figure 1: A) In vivo BLI to monitor NSCs differentiation into neurons (SYN), astrocytes (GFAP), and oligodendrocytes (PLP) by a promoter-dependent expression of luciferase (Luc). B) Lentiviral expression vectors to monitor differentiation into neurons (DCX, eSYN, MAP2, GAD67), astrocytes (GFAP), and oligodendrocytes (PLP). C) Neural differentiation of iPSC-NSCs transduced with cell specific promoters for neuronal, astroglial, and oligodendrocyte lineage development, detected by GFP-fluorescence.



Loss of entire multi-subunit BAF (mSWI/SNF) complexes impairs global epigenetic programs in forebrain development

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BAF (Brg/Brm-associated factors) complexes play important roles in development and are linked to chromatin plasticity at selected genomic loci. Nevertheless, a full understanding of their role in development and chromatin remodeling has been hindered by the absence of mutants completely lacking BAF complexes. Here, we report that the loss of BAF155/BAF170 in double-conditional knock-out (dcKO) mice eliminates all known BAF subunits, resulting in an overall reduction in active chromatin marks (H3K9Ac), a global increase in repressive marks (H3K27Me2/3), and down-regulation of gene expression. We demonstrate that BAF complexes interact with H3K27 demethylases (Jmjd3, Utx) and potentiate their activity. Importantly, BAF complexes are indispensable for forebrain development. Our findings reveal a molecular mechanism mediated by BAF complexes that controls global epigenetic programs and development.

Molecular profiling of peripheral glial subtypes

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Glial cells of the peripheral nervous system (PNS) are a neural crest-derived population specified during early embryogenesis. Peripheral glia can be classified into three broad categories in regard to their location; those associated with autonomic ganglia, those residing in dorsal root ganglia and those found in association with the enteric nervous system. Moreover, one can discriminate between several morphological subtypes of glia in the periphery; myelinating Schwann cells, non-myelinating Schwann cells and satellite glia. To date, researchers have only partially unravelled the mechanisms that govern the specification of peripheral glia lineages. However, there is no clear discrimination *in vivo* between all the described subtypes and their discrete roles, even though it is becoming increasingly clear that peripheral glial cells are characterized by a high degree of heterogeneity.

Here, we assess the heterogeneity of single peripheral glial cells on the transcriptional level at different developmental stages. To meet this aim we used transgenic mouse lines with inducible reporters (the fluorescent proteins TOMATO and YFP) under the control of the tamoxifen-responsive peripheral glial promoters of the genes *Pip1* and *Sox10*. Thus, we traced and isolated single cells of the glial lineage from embryonic to adult stages using Fluorescence Activated Cell Sorting (FACS). Next, we performed RNA extraction, cDNA library generation and next generation deep sequencing. Finally, the data were clustered using one of the most advanced algorithms (pathway and gene set overdispersion analysis - PAGODA) to reliably discriminate between the individual populations and reveal the true differences between cells of the same population.

In the near future, we aim to elucidate the molecular profile of each subclass of glial progenitors and glial cells at various developmental stages and during early maturation, as well as the induced glial changes that follow peripheral nerve injury or peripheral neuropathy development in a mouse model of a congenital human disease. Ultimately, we will make new hypotheses regarding the specification and discrete roles of glial cells, as well as their role in pathology and nerve recovery. Following this analysis, validation experiments will be performed *in vivo* and *ex vivo* in order to examine the functional output of each subtype.

The translational significance of this work is highlighted by the peripheral glia-related syndromes that have been described in human pathology (peripheral neuropathies). Our approach will shed light on the mechanisms that specify and maintain PNS glial populations *in vivo*. This project will potentially provide useful information that could be used for new treatments and therapies in such incurable conditions (i.e. gene therapy approaches).

Neural stem cells of rat hippocampus lack the expression of KV10.1 channels: implications for a safe neurogenesis.

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Under physiological conditions, the detection of KV10.1 potassium channels is restricted to the central nervous system (CNS) of the adult mammals investigated so far. In these species, the expression of KV10.1 potassium channels in peripheral tissues induced progression of the cell cycle, cell proliferation and transformation. The aim of the present work is to investigate KV10.1 expression and function in the neural stem cells (NSC) from the hippocampus of adult rats. In this study, NSCs isolated from hippocampus of adult rat (Cat. No. SCR022, Chemicon) were grown in media containing FGF-2 and allowed to proliferate. Alternatively, NSCs were cultivated in media with fetal calf serum (FCS) or retinoic acid (RA) to differentiate towards specific cell lineages (glia or neurons, respectively). Glia or neurons from hippocampus of rats in embryonic day 18 were controls. Every assay was performed in triplicates. The NSC populations were immunostained for the stem cell marker Nestin. The expression of lineage markers, KV10.1 channel and its paralog (KV10.2) or the splice variant (E65) were quantified in the NSC using RT2-PCR or RT-PCR. Proliferating NSC cultures were rich in Nestin and GFAP expression but poor in myelin (MBP) and neural (Tubb3) markers. FCS and RA enriched NSC cultures with GFAP and Tubb3 while FCS also promoted the expression of MBP. The expression of KV10.2 augmented with the time in culture except in the presence of RA. The expression of KV10.1 increased slightly with time in FGF-2 cultures while decreased with the time in culture with FCS or RA. The expression of the short splice variant E65 was abundant as compared to the KV10.1. Together, these data indicate that KV10.1 is expressed in very low amounts in the proliferating NSCs and in immature neurons of the adult hippocampus. We speculate that expression of E65 may keep low amounts of KV10.1 in the hippocampal NSC maintaining neurogenesis under control. Financial support: Alexander von Humboldt foundation, MPleM, Capes.

Patient-derived Pluripotent Stem Cells for the Analysis of Schizophrenia in 3D Cerebral Organoids

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Objectives

Neurodevelopmental psychiatric diseases such as Schizophrenia are associated with genetic variations. Specific single nucleotide polymorphisms (SNPs) and copy number variations (CNVs) presumably affect the neural development, resulting in modified gene expression patterns up to dysfunctions of neural cells. Reprogramming of human somatic cells enables the generation of induced pluripotent stem cells (iPS cells) representing disease- and patient-specific disease models for the analysis of DNA variations. The differentiation of iPS cells is tool for the analysis of early and late neural development enabling the functional description of genetic variations. The functional investigation of genetic variations requires the combined analysis of cellular 2D and tissue-based 3D disease models.

Material and Methods

Well-established human iPS cells and patient-specific iPS cells from Schizophrenia patients were differentiated into permanent neural stem cells (NSCs). Transcript and protein analysis confirmed the differentiation status of iPS cells and NSCs. NSCs were characterized in cellular 2D differentiation models. NSCs were further differentiated as free-floating neurospheres to improve cell proliferation and 3D growth. In order to acquire functional 3D organoids from neurospheres, we used culture conditions suitable for the generation of cortical neurons. Transcript and protein analysis confirmed the differentiation status of mature and immature cells.

Results

Protein and transcript analysis of specific markers including OCT4 and the activity of alkaline phosphatases verified pluripotency in well-established and patient-derived iPS cells as well. The analysis of several NSC markers such as SOX2 and PAX6 indicated stable generation of permanent NSCs from patient-specific iPS cells. Patient-specific iPS cells were successfully differentiated into progenitor cells expressing a variety of neural lineage markers including neural makers such as TUBB3 and STX as wells as glia cell markers such as GFAP and O4. Immunostaining and whole-cell patch-clamp recordings of mature neurons showed the presence of different neuron subtypes including inhibitory GABAergic neurons indicated by GABA and GABRA1 expression. NSCs were also successfully applied for the generation of neurospheres, which were successfully differentiated into 3D cerebral organoids. Post mitotic neural cells were identified through absent immunostaining of Ki67 recommending the maturation of neural precursors into functional neural and glial cells. The expression pattern of cortical markers such as TBR1 revealed the induction of cortical layers mimicking the developing human cortex. Organoids showed 3D patterning of cell populations visualized by immunostainings. The 3D organoid differentiation model showed the RNA and protein expression of neural lineage markers and the induction of GABAergic neurons similar to neurons obtained from cellular 2D disease models. The regional brain identity was confirmed through the expression of telencephalic and hippocampal markers.

Conclusions

We confirmed the efficient and functional differentiation of healthy and patient-derived iPS cells using cellular 2D and tissue-based 3D in vitro models. These culture systems enable functional studies of healthy and diseased human cortical development for the analysis of psychiatric diseases including schizophrenia.

Regulation of Aberrant Adult Hippocampal Neurogenesis by microRNAs After Mild Kainic Acid-Induced Status Epilepticus: Effect on Gliogenesis and Reactive Neural Stem Cells.

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Background: Under normal conditions only a small percentage of activated hippocampal Neural Stem Cells (NSCs) give rise to astrocytes. However, after KA-induced status epilepticus (KA-SE) a strong shift in cell fate takes (Sierra et al., Cell Stem Cell, 2015). Previously, we have shown that miR-124 and miR-137 are upregulated in the Dentate Gyrus 3 days after KA-SE. Additional intrahippocampal administration of miR-124 and -137 aggravated the effect of KA-SE on NSC. In this study, the role of microRNA antagonists (antagomirs) targeting miR-124 and -137 are further investigated, focusing on the astrocytic conversion of NSCs and the induction of reactive NSCs.

Methods: 7 week-old Nestin-GFP transgenic male mice were injected intrahippocampally with antagomirs targeting miR-124 and -137 or non-targeting antagomirs and mice received intrahippocampal KA injections 48 hours after antagomir administration. In a second set of experiments mice received intrahippocampal antagomir injections 2 hours after KA-SE onset. In all cases mice were sacrificed 72 hours after KA-SE. Using immunohistochemistry and confocal microscopy we studied astrocytic conversion of NSC and gliogenesis.

Results: Our data indicate an effect of antagomiR-124 and -137 administration on the rate of astrocytic conversion of NSCs, providing a first indication of a possible reversal of aberrant AHN associated with epileptogenesis.

Implications: Our results suggest that antagonizing miR-124 and -137 may contribute to control the neurogenic response to epileptic seizures in the dentate gyrus.

This work is supported by grants NWO VIDI H64.09.016, ISAO 14533 and Alzheimer Nederland to CPF

Spontaneous calcium oscillations modulated by P2Y2 receptor and L type calcium voltage gated channel activity in neurogenesis: a novel approach for studying cell fate determination

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Oscillations of intracellular calcium concentration participate in many cellular processes. Voltage-gated calcium channels and purinergic ATP-activated receptors are expressed at early steps of development, and in vitro and in vivo data provided evidence for their participation in intracellular calcium transient signaling and control of neural differentiation. Using mouse embryonic stem (ES) cells as in vitro model for neural differentiation, we tracked by real time fluorescence and luminescence imaging intracellular calcium transient activity, detected by calcium-sensitive fluorescent probe, combined with rhythmic pro-neural transcription factor expression of stable transfected cells with Mash-1 or Neurogenin-2 promoter-protein fused to the luciferase reporter. Activity modulation of purinergic receptors and L-type voltage gated calcium channels by pharmacological tools let us to conclude that neural stem cells showing augmented spike-like calcium oscillation frequency induced Mash-1 stable expression and consequently determination to GABAergic cell fate as a result of P2Y2 purinergic receptor activity. Furthermore, neuronal phenotype determination is characterized by stable expression of Mash-1 that depends on voltage-gated calcium channel activity. Overall, our studies provide novel insights into mechanisms of neurogenesis. Noteworthy, the direct correlation between spontaneous calcium signaling and Mash-1 oscillatory expression in real time is pioneering, open new applications in diverse fields of science. Funded by FAPESP and CNPq, Brazil.

The abnormal communication between neuron and oligodendrocyte disrupts myelination in NPC1 deficient mice

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Myelination, a process relies on signal conduction between neurons and oligodendrocytes, has been found abnormal in NPC1 deficient mice. However, the mechanisms of hypomyelination induced by deficient NPC1 protein are mostly unknown. In this study, we show that myelination starts in the corpus callosum from P9 in WT mice, as revealed by an increase of transcription and translation of myelin proteins, e.g., MBP and MOG, and the upregulated HMG-CoA reductase (HMG-CR), a rate limiting enzyme for cholesterol synthesis, but not in NPC1 mice. The migration of OPCs is relatively normal and Olig2 was found to be downregulated in the NPC1 corpus callosum, indicating a delay of OPC differentiation and resulting in disruption of upregulation of myelin proteins. Furthermore, both PLP accumulation in lysosome/late endosome and the low level of free cholesterol results in hypomyelination. Moreover, NPC1 primary oligodendrocytes responding to factors secreted from neurons decreases strongly. The translocation of p57 kip2 from nuclei to cytosol, an important process for cell exit from cell cycle, is suppressed in NPC1 oligodendrocytes. Therefore, we conclude that NPC1 deficiency in oligodendrocytes blocks the signal conduction between neuron and oligodendrocyte, traps oligodendrocytes in the cell cycle, and decreases cholesterol supply, resulting in hypomyelination.

The Chondroitin Sulfate Code Hypothesis and FGF Signaling in the Neural Stem Cell Niche of the Developing Mouse Forebrain

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Chondroitin sulfate proteoglycans carry glycosaminoglycan chains that are the name giving chondroitin sulfates (CS). Chondroitin sulfates are complex linear sugars and their selective sulfation pattern is elicited by eight chondroitinsulfotransferases (Chsts). This CS sulfation pattern might represent a molecular code in the cellular environment, hence the CS code hypothesis. CS removal by intraventricular injection of chondroitinase ABC has revealed their crucial role for the behaviour of neural stem cells. Here, we wanted to test the chondroitin sulfate code hypothesis in the opposite direction by overexpression of one specific Chst - uronyl-2-sulfotransferase UST - in cortical neural stem cells to achieve increased sulfation of the neural stem cell niche. We performed in utero electroporation experiments in E14.5 embryos to analyse the impact of a modified sulfation pattern by forced Ust expression on cortical neural stem cell fate in vivo. For this purpose we analyzed the proliferation and cell cycle progression of radial glia cells after EdU injections. Also, the number of newborn neurons and glia cells was determined. At E16.5, two days after Ust overexpression, we observed a change in the thickness of the cortical plate (CP), but no obvious morphological alterations of the ventricular zone (VZ). Ust overexpression increased the ratio of precursor cells to neurons without increased proliferation rates suggesting an effect on cell cycle length and/or progression. Our findings are in line with the interpretation of an enhanced FGF-signalling due to an efficient and functional Ust overexpression that leads to a specific modification of the sulfation pattern of defined chondroitin sulfate units in the neural stem cell niche of the developing cortex.

The contribution of Dgcr8 to mouse corticogenesis and neocortex expansion

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Introduction: Corticogenesis is the neurodevelopmental process leading to the formation of the cerebral cortex. The neocortex is the brain region that has undergone greatest enlargement during evolution in primates. The evolutionary expansion of the neocortex is based on differences in Neural Progenitor Cell (NPC) biology. Of note the basal (or intermediate) progenitor (bIP) hypothesis proposes that evolutionary neocortical expansion may be due to an increase in the genesis of bIPs, mostly owing to a substantial change in their mode of division and control in their proliferation-differentiation fate. The molecular mechanisms at the base of the precise coordination of cell proliferation-differentiation process of bIPs in evolution are still largely unknown.

MicroRNAs (miRNA) are small, single-stranded, regulatory non-coding RNAs that play a key role in post-transcriptional control of gene expression in cortical development. The biogenesis pathways of miRNAs, and their core components are well characterized. Recent evidence indicates that some of the components of miRNA biogenesis machinery, especially the microprocessor moieties Drosha and Dgcr8 (Di George Critical Region 8), exert also miRNA-independent gene silencing functions (Marinaro et al., Manuscript submitted).

Methods: Sustained expression of dgcr8 in the dorsolateral telencephalon of embryonic day E12.5 WT mouse embryos was obtained by In utero electroporation of dgcr8 expressing plasmid and mCherry expressing plasmid (control). Fate of the electroporated cells was analyzed at E14.5.

Results: Preliminary data of sustained expression of dgcr8 leads to a slight but significant expansion of bIPs during corticogenesis and a significant two-fold decreased number of Tbr1 positive neurons at E14.5. Identification of downstream targets of Dgcr8 is in progress.

Conclusion: Dgcr8 might have played a role in the evolutionary expansion of the mammalian neocortex.

The role of foxQ2 in insect central complex development

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The central complex (CX) is a higher order structure in the insect brain that is involved in sky compass orientation, flight control, locomotor behavior, courtship and memory. It consists of neuropils including protocerebral bridge (PB), central body (CB) with upper (CBU) and lower unit (CBL), also called fan-shaped body (FB) and ellipsoid body (EB). Both CX function and development are highly studied in *Drosophila melanogaster*. However, in *Drosophila*, the CX develops during late larval stages which prohibits to study its embryonic development. As consequence, the genetic signals specifying the identity of the neuroblasts arising in the anterior region remain poorly studied.

Therefore, we use *Tribolium castaneum* to study CX development. In *Tribolium* it is partially formed during embryogenesis and this model system offers a number of experimental possibilities. (Efficient systemic RNAi, transgenic approaches, tools for gene misexpression).

The aim of this project is to identify the neuroblasts and their lineages that contribute to central complex development and understand the genes that are required for their spatial specification. Ultimately, we would like to understand the cellular and molecular differences that lead to the different timing of CX development in *Tribolium* versus *Drosophila*. We have shown by RNAi that Tc-FoxQ2 is required for CX development. We are developing tools for analyzing neural development in *Tribolium* (e.g. generation of an antibody against FoxQ2 and of an enhancer trap line with CRISPR/Cas9 strategy to mark FoxQ2 expression in vivo) with which we will study the phenotype.

The serine protease inhibitor neuroserpin regulates developmental neurogenesis, synaptic plasticity, learning and social behaviour

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The serine protease inhibitor neuroserpin regulates the activity of tissue-type plasminogen activator (tPA) in the nervous system. Neuroserpin expression is particularly prominent at late stages of neuronal development in most regions of the central nervous system, whereas it is restricted to regions related to learning and memory in the adult brain.

The physiological expression pattern of neuroserpin, its high degree of colocalization with tPA within the central nervous system, together with its dysregulation in neuropsychiatric disorders, suggest a role in development and maintenance of the nervous system. In order to elucidate the physiological role of neuroserpin, we analysed neuroserpin-deficient mice. In particular, we investigated neuroserpin's function in neurogenesis, synaptic plasticity, and the behaviour of neuroserpin-knock out mice.

At early developmental stages, absence of neuroserpin resulted in reduced proliferation of neuronal precursor cells and in premature neuronal differentiation in the neurogenic subgranular zone of the hippocampus. Moreover, in hippocampus and amygdala we detected altered spine morphology. In the adult, morphological and functional analysis of the hippocampus showed decreased spine-synapse density and impaired long-term potentiation in neuroserpin-deficient mice. Behavioural testing indicated impairment in social behaviour, spatial learning and memory and contextual memory in mice lacking neuroserpin. Finally, we observed increased neuroserpin expression in fusiform gyrus of autistic patients.

In conclusion, we found that absence of neuroserpin impairs developmental neurogenesis and alters synaptic morphology and plasticity in vivo. Neuroserpin's role in neurodevelopment may account for the behavioural changes observed in neuroserpin-deficient mice, pointing to a role for neuroserpin in development of neuropsychiatric diseases.

Functional analysis of Lin41 in the adult stem cell niche: repurposing of a pluripotency factor in ependymal cells?

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Lin41/Trim71 is a well-known heterochronic gene encoding a member of the Trim-NHL protein family. It is primarily and ubiquitously expressed during early embryonic development where it plays a crucial role in embryonic viability and development of the neural tube. Lin41 deficient mice display embryonic lethality at E9.5 with a highly penetrant cranial neural tube closure defect. Lin41 expression starts to decline at E8.5 and it is progressively lost by E13.5. Recently, it has been published by our group that Lin41 expression is reestablished in adult mice and it is specifically restricted to the ependymal layer lining the wall of the four brain ventricles where its role is still not defined.

Poster Topic

T2: Axon and Dendrite Development, Synaptogenesis

- [T2-1A](#) Abnormal spine morphology in Niemann-Pick Type C 1 mutant mouse
Xiao Feng
- [T2-2A](#) An intact insect embryo as assay for developmental neurotoxicity testing
Michael Stern, Sarah Frömbing, Gregor Bergmann, Gerd Bicker
- [T2-3A](#) Assembling a dopaminergic synapse: The role of cell adhesion and scaffolding molecules
Rebecca Wallrafen, Thomas Dresbach
- [T2-4A](#) Branch-specific microtubule destabilization mediates axon branch loss during neuromuscular synapse elimination
Monika S. Brill, Tatjana Kleele, Laura Ruschkies, Mengzhe Wang, Natalia A. Marahori, Torben Hausrat, Derron L. Bishop, Matthias Kneussel, Thomas Misgeld
- [T2-5A](#) Circuit development and morphological phenotype analysis in primary olfactory cortex
Laura Moreno Velasquez, Stephen C. Lenzi, Dietmar Schmitz, Friedrich W. Johanning
- [T2-1B](#) Promotion of axonal collateral branching and thalamocortical connections as potential mechanism underlying erythropoietin-induced poststroke plasticity.
Eduardo Humberto Sanchez-Mendoza, David Oguama, Dirk M. Hermann
- [T2-2B](#) -Aminoisobutyric induces Neurite Outgrowth in Primary Cortical Neurons
Daniel Claude Morris, Wing Lee Cheung, Talan Zhang, Michael Chopp, Zheng G Zhang
- [T2-3B](#) Dendritic Conservation
Carsten Duch, Stefanie Ryglewski
- [T2-4B](#) Development of connectivity in a fly motor circuit
Aaron Ostrovsky, Tatjana Kovacevic, Jan Felix Evers
- [T2-5B](#) DUAL EFFECT OF EXOGENOUS GLUCOCORTICOIDS ON DENDRITES AND AXONS DURING HIPPOCAMPAL NEURONS MORPHOGENESIS
Helena Alexandra Ribeiro de Carvalho Pinheiro, Filipa I. Baptista, António F. Ambrósio, Catarina A. Gomes
- [T2-1C](#) Jelly Belly – Anaplastic lymphoma kinase signaling is an activity independent regulator of dendritic growth
Phil-Alan Gärtig, Aaron Ostrovsky, Steffen Schmelzeisen, Barbara Chwalla, Michael Landgraf, Jan Felix Evers

- [T2-2C](#) Neuroligins and BDNF: Trassynaptic Teamwork
Andoniya Petkova, Nina Gödecke, Martin Korte, Thomas Dresbach
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Abnormal spine morphology in Niemann-Pick Type C 1 mutant mouse

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Niemann–Pick type-C 1 (NPC1) disease is characterized by a progressive loss of neurons and an accumulation of unesterified cholesterol within the endocytic pathway. NPC disease shares several similarities, including late endosomal and lysosomal abnormalities, neurofibrillary tangles and cognitive impairment, with other neurodegenerative disorders, such as Alzheimer’s disease. The aim of this work was to assess whether the accumulation of unesterified cholesterol in endosome and lysosome observed in NPC disease disrupt normal function of membrane, and then affect the signal pathways which control the morphology of spine, thus finally influence the memory and learning ability in NPC1 patient. We use npc1 ko mouse as model to evaluate the morphology of spine in cortical pyramidal neuron with software analysis, and in vitro we cultivate neuron to figure out the related signal molecules, then detect special medicine to restore the defects. At last through making sure the targeted signal pathway, we want to find out agonist or antagonist to recover this defects and improve the performance of NPC1 ko mouse.

An intact insect embryo as assay for developmental neurotoxicity testing

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Developmental neurotoxicity (DNT) of environmental chemicals poses a serious threat to human health worldwide, and the resulting neurological deficits, in particular in children, negatively affect families and society. However, far too few chemicals have yet been tested, mainly because current *in vivo* test methods for assessing DNT require the use of high numbers of laboratory animals. Alternative *in vitro* testing methods monitor mainly rather simple toxicological endpoints, such as cell viability, proliferation, and neurochemical differentiation. The formation of a functional brain requires, however, the precisely timed navigation of axons within the complex neuronal tissue environment. The aim of our research is to address this complexity by monitoring defects in axonal navigation of pioneer axons of intact locust embryos after exposure to chemicals. Mechanisms of axonal guidance, such as for example growth cone navigation along molecular semaphorin gradients, are conserved between invertebrates and vertebrates. Thus, assays monitoring axonal navigation in insects will be indicative for the DNT potential of industrial chemicals in humans.

Locust embryos are kept *ex ovo* in culture overnight in the presence of test chemicals, followed by immunolabeling of leg bud pioneer neurons using an antibody against a cell surface marker (HRP). Defects in axonal outgrowth and navigation of pioneer axons are detected via conventional fluorescence microscopy and by the rapid 3D Scanning Laser Optical Tomography* (SLOT) after overnight exposure of embryos to chemicals.

As positive controls, we employed cytoskeletal inhibitors such as cytochalasin D or colchicine, which inhibit outgrowth with an IC₅₀ of 35 nM and 46 nM, respectively. As a first test compound, we measured the effect of the mitochondrial respiratory chain inhibitor, rotenone. This compound is known for its adverse effect on dopaminergic neurons in both mammals and *Drosophila* and is thus often used in Parkinson disease research, but it is also a selective inhibitor of axonal outgrowth of human neurons *in vitro*. In the locust embryonic leg assay it inhibited both pioneer neuron growth and correct pathfinding with an IC₅₀ of 28 nM and 45 nM, respectively, which is in the same range as found in human neurons. General viability of the embryo (measured by resazurin reduction assay) was significantly less affected at these concentrations, (IC₅₀ 79 nM), which identifies rotenone as a specific developmental neurotoxicant also in our insect embryo assay. Currently, the system 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.

This insect assay will serve as complementary test system to other alternative DNT testing methods. As part of a test battery, the insect embryo assay should contribute to positive identification of DNT compounds.

(Funded by BMBF 031L0062A)

* Eickhoff R, Lorbeer RA, Scheiblich H, Heisterkamp A, Meyer H, Stern M, Bicker G (2012) Scanning Laser Optical Tomography resolves structural plasticity during regeneration in an insect brain. *PlosOne* 7:e41236

Assembling a dopaminergic synapse: The role of cell adhesion and scaffolding molecules

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Three themes have been the hallmark of our understanding of neurotransmitter release and synaptic transmission: (1) neurotransmitter is released in a quantal fashion from synaptic vesicles, (2) molecular scaffolds organize transmitter release at the active zone and receptor localization in the postsynaptic density and (3) cell adhesion molecules signal across the synaptic cleft to mediate the assembly of synapses and endow synaptic junctions with specific properties. Surprisingly, very little is known about the role of synaptic scaffolds and cell adhesion molecules at dopaminergic (DA) synapses.

One of the best studied cell adhesion systems consists of postsynaptic Neuroligins (NL) and presynaptic Neurexins. NL mutations have been connected with many different, disease-associated phenotypes, including autism and Alzheimer's disease. It has been hypothesized that these phenotypes are due to an imbalance in the ratio of excitation versus inhibition. With our work, we want to test the hypothesis that - in addition to a direct impairment of the glutamatergic transmission apparatus - dysregulation of DA synapse assembly and function might contribute to these phenotypes.

Employing a novel, self-developed co-culture system that allows for the simultaneous culture of dissociated DA midbrain and hippocampal neurons, we started the characterization of DA axon terminals projecting onto hippocampal cells. We used several well-established synaptic markers (Bassoon, Synaptophysin, Synapsin, vGAT, vGluT1-3, vMAT2) and found that these are heterogeneously distributed in the axon and that the distribution changes throughout the development. This co-culture system also enables us to test the effect of NL knock down and overexpression on DA synapses.

The findings of our study give insight into the molecular composition of DA varicosities projecting onto hippocampal neurons and will introduce the possibility that cell adhesion and scaffolding molecules regulate DA synapse formation and function.

Branch-specific microtubule destabilization mediates axon branch loss during neuromuscular synapse elimination

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Developmental axon remodeling is characterized by the selective removal of branches from axon arbors. The mechanisms that underlie such branch loss are largely unknown. Additionally, how neuronal resources are specifically assigned to the branches of remodeling arbors is not understood. Here we show that axon branch loss at the developing mouse neuromuscular junction is mediated by branch-specific microtubule severing, which results in local disassembly of the microtubule cytoskeleton and loss of axonal transport in branches that will subsequently dismantle. Accordingly, pharmacological microtubule stabilization delays neuromuscular synapse elimination. This branch-specific disassembly of the cytoskeleton appears to be mediated by the microtubule-severing enzyme spastin, which is dysfunctional in some forms of upper motor neuron disease. Our results demonstrate a physiological role for a neurodegeneration-associated modulator of the cytoskeleton, reveal unexpected cell biology of branch-specific axon plasticity and underscore the mechanistic similarities of axon loss in development and disease.

Circuit development and morphological phenotype analysis in primary olfactory cortex

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The piriform (or primary olfactory) cortex is a three-layered paleocortex and the first cortical relay for olfactory processing, receiving monosynaptic input from the olfactory bulb via the lateral olfactory tract (LOT) terminating in layer Ia. In addition to receiving sensory input in layer Ia, principal neurons in the piriform cortex (PC) receive both local and long-range intracortical connections in layers Ib, II, and III. Current descriptions of PC imply a convergence of sensory and intracortical inputs onto principal cells in layers II, the main input layer of the PC. Layer II is dominated by two morphologically distinctive glutamatergic cell types: superficial pyramidal and semilunar cells, with strikingly different properties.

Pyramidal cell dendrites establish synaptic connections with LOT axons in the superficial portion of layer I (layer Ia) and with intracortical association fibers in the deeper aspect of layer I (layer Ib). Semilunar cells are a specific population of neurons located in the most superficial aspect of layer II and, in contrast to pyramidal cells, they lack basal dendrites and do not form associative synapses.

While the structure of the adult PC is well characterized, postnatal development and maturation of this cortex is less well understood. In this study, electrophysiological and imaging techniques were combined in order to identify developmental emergence of these differences between semilunar and pyramidal cells. We found that elongation of dendritic branches occurs continuously during the first two postnatal weeks whilst the determination of branching complexity occurs mostly during the first postnatal week for both types of cells. However, morphological differences between them are noticeable since the very first days after birth, mainly in the basal dendritic tree.

Promotion of axonal collateral branching and thalamocortical connections as potential mechanism underlying erythropoietin-induced poststroke plasticity.

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Background: We have previously demonstrated that Recombinant human Erythropoietin (rhEpo) induces contralesional pyramidal plasticity after transient middle cerebral artery occlusion (t-MCAO), thereby enhancing neurological recovery. To elucidate mechanisms underlying this process, we again submitted mice to t-MCAO as before (1). Thereafter, Golgi staining and retrograde tract tracing were performed at 14 or 40 days after the insult (dpi) to determine local branching of dendritic spines and changes in afferences to the motor cortex. To determine the importance of cells other than neurons in the effects found, primary cortical cell cultures were prepared to study the neuronal response to rhEpo in isolation. We found significant increases in the density of dendritic spines both in vivo at 14 dpi, and of axonal length and axonal collateral density as well as an increase in the number of primary dendrites and dendritic collaterals in vitro after 24 h of Epo exposure. Importantly, we found a significant increase of contralesional thalamocortical connections in Epo-treated mice at 40 dpi.

Results: Contrary to hippocampal neurons, which show a remarkable sprouting response to rhEpo stimulation (3), cortical neurons present a mild extension of the axon and increase in primary dendrite number. rhEpo induces an increase in the density, but not the length, of dendritic spines both in vitro and in vivo. Importantly, and in agreement with our previous findings (1), rhEpo induced an increase of midline crossing thalamocortical fibers. That layer V motor cortical neurons present the same response as isolated neurons in vitro proves that the response of these cells is direct and specific to rhEpo stimulation and not entirely influenced by the response of other cells (i.e. glial cells). The enhanced sprouting of thalamocortical projections 30 days after stroke, and increased dendritic spine density on layer V cortical neurons at 14dpi, may complementarily underlie the previously described behavioral improvements found after rhEpo therapy. Our findings demonstrate that Epo may promote an overall behavioral recovery by promoting not only corticospinal tract plasticity but also promoting thalamocortical and local cortical connectivity. Nevertheless, functional studies are necessary to characterize the functional implications on cortical excitability derived from enhanced local and thalamocortical connections.

References:

- (1)Reitmeir et al. Brain 2011 Jan;134(Pt 1):84-99.
- (2)Sanchez-Mendoza, et al. J Vis Exp.2016 Jan 18;(107).
- (3)Ransome and Turnley. Mol Cell Neurosci. 2008 Aug;38(4):537-47.

β -Aminoisobutyric induces Neurite Outgrowth in Primary Cortical Neurons

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Objectives: β -Aminoisobutyric acid (BAIBA) is a small molecule β -amino acid that is released from contracting muscle during exercise. BAIBA increases the expression of brown adipocyte-specific genes in white adipocytes in both in-vivo and in-vitro models and improves glucose homeostasis. BAIBA mediates tissue crosstalk between muscle and white fat adipocytes in subcutaneous tissue and the liver suggesting that BAIBA may be a mediator of exercise induced health benefits. Since exercise is known to promote neurogenesis, we hypothesized that BAIBA may promote neurite outgrowth in our cell culture model of primary cortical neurons (PCNs).

Methods: PCNs were isolated from day 17 rat embryos (n=3) and plated at a low cell density with DMEM containing 20% FBS in poly-D-lysine coated plates for 5 hours. The media was then changed to Neurobasal medium containing 2% B27, 1% Glutamax, 1% antibiotics, and 10 μ M 5-fluoro-2-deoxyuridine. Cultures were incubated with 0, 5 μ M and 10 μ M BAIBA, respectively, for four days at 37°C. Microtubule associated protein (MAP2) immunostaining was performed to identify and measure neurite branch lengths and number of branches using Matlab 6.5 program and ImageJ. Neurite branching was quantified by primary branching from the cell body (level 1) and secondary branching (level 2) from Level 1 branching.

Results: Total length, number of neurite branches and branch lengths at levels 1 and 2, were increased in both the 5 μ M and 10 μ M BAIBA treatment groups when compared to control. The total lengths of neurites were 95.2 \pm 53.7 μ m, 158 \pm 70.3 μ m, and 171.3 \pm 71.6 μ m while the number of branches were 4.50 \pm 1.9, 6.26 \pm 2.5, and 7.50 \pm 3.3, respectively, in control, 5 μ M and 10 μ M BAIBA treatment groups (mean \pm std) (p<0.01). The lengths at levels 1 were 87.3 \pm 46.7 μ m, 134.3 \pm 55.6 μ m and 143.0 \pm 61.1 μ m while the lengths at level 2 were 7.8 \pm 12.4 μ m, 23.6 \pm 25.7 μ m and 27.4 \pm 25.2 μ m respectively in control, 5 μ M and 10 μ M BAIBA treatment groups (mean \pm std) (p<0.01).

Conclusions: BAIBA (5 μ M and 10 μ M) treated PCNs increased both the lengths and branches of neurites when compared to control. These results demonstrate that BAIBA could potentially act as an agent to promote neurite outgrowth.

Dendritic Conservation

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Brain development requires correct targeting of multiple thousand synaptic terminals onto staggeringly complex dendritic arbors. As a possible mechanism to match input synapse number to dendrite size, synapse formation has been suggested to locally direct dendrite growth in a synaptotropic manner. However, it is unknown to what extent such 'synaptotropic' mechanisms shape the entire dendritic arbor, whether inputs with different neurotransmitters co-direct dendrite growth, and how local synaptic mechanisms operate together with global growth control.

We show in *Drosophila* that the relative amounts of GABAergic and cholinergic synaptic drive shift dendrites between different input domains of one postsynaptic neuron without affecting total arbor size. Therefore, dendrites become redistributed intra-neuronally by synaptic competition while conserving total wire length. Mechanistically, this requires local dendritic calcium influx through D 7nAChRs or through LVA channels following GABAARs mediated depolarizations. Intraneuronal dendrite redistributions counterbalance local arbor changes and reduce morphological variability, a phenomenon also described for cortical neuron dendrites.

Development of connectivity in a fly motor circuit

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Correct development of the motor circuit during embryogenesis requires a careful balance of excitatory and inhibitory input; incorrect input leaves the nervous system prone to epileptic-like seizures. This sensitivity results from changes in synaptic drive, though the molecular mechanism behind these changes is unclear. To understand these changes, we will examine the role of activity in driving changes in synapse numbers and locations, as well as the relative composition of pre-synaptic proteins at individual release sites.

As such, we have developed the dFLEX technique to enable the conditional labeling of proteins at their endogenous genomic locus. Combining dFLEX labeling of synaptic proteins with Expansion Microscopy, we are able to observe neurotransmitter specific release sites as well as protein composition at individual dendrites, allowing us to map the full set of inputs to individually identified *Drosophila* motorneurons at ~100nm resolution. By carefully staging and dissecting embryos and larvae at specific developmental time-points, we will model how synaptic input to motorneurons changes during development and how it is tuned through synaptic activity itself. This data gives novel mechanistic insight into how activity is tuning synaptic input into an identified neuron.

DUAL EFFECT OF EXOGENOUS GLUCOCORTICOIDS ON DENDRITES AND AXONS DURING HIPPOCAMPAL NEURONS MORPHOGENESIS

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The exposure to high levels of glucocorticoids during brain development, either due to pharmacological therapies or stress, can have a long term impact in brain cytoarchitecture and behaviour, increasing the susceptibility to neuropsychiatric disorders¹. In rats, alterations in hippocampal structure were already reported, such as an atrophy of mossy fiber density² and dendrite morphology³. However, it is essential to understand the mechanisms involved in such effects.

In this work, we characterized the effect of the exposure to dexamethasone, a synthetic glucocorticoid used in clinics, in hippocampal neurons, at early stages of polarization and dendritic development (two and five days in culture, respectively). We report that the exposure to dexamethasone induces dimorphic alterations in axon and dendrites, characterized by a hypertrophy in axon length (dependent on the activation of adenosine A_{2A} receptors) and atrophy in dendrites (dependent on the activation of glucocorticoids receptors). Further analysis also revealed that the tonic activation of adenosine A_{2A} receptors plays an important role in neuronal polarization.

Thus, we unravel a differential effect of glucocorticoid exposure upon dendrites and axon, and a new role for adenosine A_{2A} receptors in mediating neuronal polarization under physiological conditions. Further elucidation of these mechanisms can lead to new pharmacological tools to minimize the effects of the overexposure to glucocorticoids in critical stages of development.

Financial support: Foundation for Science and Technology, Portugal (Strategic Projects: Pest C/SAU/UI3282/2013 and Pest UID/NEU/04539/2013), COMPETE-FEDER.

1: Moisiadis VG, Matthews SG. (2014) Glucocorticoids and fetal programming part 1: Outcomes. *Nat Rev Endocrinol* 10(7):391-402.

2: Huot RL, Plotsky PM, Lenox RH, McNamara RK (2002) Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Res* 950:52-63.

3: Batalha VL, Pego JM, Fontinha BM, Costenla AR, Valadas JS, Baqi Y, Radjainia H, Muller CE, Sebastiao AM, Lopes LV (2013) Adenosine A_{2A} receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. *Mol Psychiatry* 18:320-331.

Jelly Belly – Anaplastic lymphoma kinase signaling is an activity independent regulator of dendritic growth

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Normal nervous system function depends on the formation and maintenance of appropriate synaptic connections. Mis-regulation of neuronal growth and synaptogenesis has been identified as a cause for neurological disorders.

In the *Drosophila* larval motor system we identified the secreted protein jelly belly (Jeb) and its postsynaptic receptor anaplastic lymphoma kinase (Alk) as a trans-synaptic regulator of dendritic growth: downregulation and loss of Alk lead to an increased formation of filopodia-like protrusions, but reduced overall growth of the dendritic arbor; constitutive Alk activation arrests expansion and causes network dysfunction. Unexpectedly, Alk signaling in dendrites is independent of neuro-transmission.

Using intra-vital time lapse imaging we find that normal dendritic growth is characterized by exploratory branch extension and retraction in both embryonic and postembryonic development. Loss of Alk signaling results in reduced formation of new dendritic protrusions, and an increased branch stability.

Immuno-labeling confirmed that Jeb localizes to presynaptic release sites also in the central nervous system; genomically tagged Alk (dFLEx) revealed its specific enrichment in the dendritic arbor.

In summary, our work demonstrates Jeb/Alk signaling as a trans-synaptic, activity-independent regulator of neuronal growth, integrating synaptic maturation and morphological development of both pre- and postsynaptic terminals. We are now investigating whether jelly belly might regulate dendritic growth locally to the synaptic contact, and as such would constitute an activity-independent synaptotrophic factor.

Neuroligins and BDNF: Trassynaptic Teamwork

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Synaptic maturation is a process that allows synapses to acquire their full functionality, and failures in synaptic maturation are thought to contribute to psychiatric disorders such as autism. Neuroligins are postsynaptic cell-adhesion molecules essential for postsynaptic and presynaptic maturation. But what are the transsynaptic pathways by which Neuroligins act to regulate presynaptic maturation?

Here, we show that Neuroligins and brain-derived neurotrophic factor (BDNF) cooperate to mediate presynaptic maturation. Applying BDNF to neuronal cultures mimicked the maturation-promoting effect of overexpressing the Neuroligin isoforms NL1 and NL2.

Reducing the levels of BDNF by applying a BDNF scavenger (TrkB-Fc) or depleting BDNF by Cre-lox virus transduction blocked the action of NL1 and NL2. In particular, inhibiting endogenous BDNF signaling reduced the positive effects of NL1 on presynaptic maturation and of NL2 on synapse formation. Applying BDNF to cultures from NL1-knockout mice rescued impaired presynaptic maturation both in early (DIV6) and late (DIV15) culture stages, indicating that BDNF acts downstream of NL1-mediated cell adhesion.

Our data introduce BDNF as a novel and necessary component in a transsynaptic pathway linking NL-mediated cell adhesion, neurotrophin action and presynaptic maturation.

Oxytocin induces neurite outgrowth through calcium pathways

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The research of neurodevelopmental disorders focuses on identification of molecular and genetic determinants of growth of neuronal cones and formation of synaptic connections. Causes of abnormal development of central nervous system and its neural circuits at the cell and molecular level include disturbances in growth of neuronal cones, changes of shape of cones, alterations in synaptic connections with significant impact on memory, learning and behavior. Reorganization of neuronal cytoskeleton is calcium dependent. Consequently localization, concentration, and temporal aspects of the cellular calcium signal have a complex role in regulating neurite growth. It is known, that intracellular calcium is an important secondary signaling messenger in neurodevelopmental signaling pathways; including gene transcription, axonal and dendritic outgrowth and neuronal migration. Calcium concentration can be increased from extracellular and intracellular sources. Signaling cascades of many different neuropeptides include increase of intracellular calcium concentration. The aim of the present study was to determine the role of different calcium channels in oxytocin induced neurite outgrowth. Oxytocin treatment has induced intracellular calcium increase in SH-SY5Y cells measured by fluorescent indicator FURA 2/AM. This increase was abolished by administration of intracellular chelator BAPTA/AM. Incubation of cells for 48 hours with oxytocin induced significant elongation of neurites stained with phalloidin. All tested calcium channels blockers (isradipine, nifedipine, agatoxin, mibefradil) suppressed oxytocin induced elongation of neurites. Decrease of intracellular calcium by BAPTA/AM and blocking of P-type channels by agatoxin disabled neurite elongation. Additionally, a modulatory effect of oxytocin on expression of proteins associated with exocytosis has been observed. In conclusion, intracellular calcium plays an important role in oxytocin induced neurite outgrowth.

Supported by the project 2/0119/15 of the Grant Agency of ministry of Education and Slovak Academy of Sciences (VEGA), and Projects APVV-15-0205 and APVV-15-0045 of the Slovak Research and Development Agency.

Oxytocin modulates neurite length and levels of cytoskeletal proteins associated with neuronal growth

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Over the last decade of research, it has become clear, that changes in the regulation of neurite outgrowth, alterations of neurite direction together with abnormal development of synaptic connections are manifested in neurodevelopmental disorders. Moreover, there is accumulating evidence for oxytocin is playing a role in neurodevelopmental disorders particularly autism. Recent studies have suggested that various neuropeptides may modulate neurite outgrowth, among them oxytocin. Therefore, the aim of the present studies was to describe how oxytocin influences growth and shape of neuronal processes and to explain mechanisms of oxytocin effect on neuronal cytoskeleton in SH-SY5Y cells and primary rat cortico-hippocampal neurons. Oxytocin treatment resulted in dose-dependent stimulatory effect on the morphology of the SH-SY5Y cells and time-dependent effect on neurite outgrowth of SH-SY5Y cells and primary corticohippocampal neurons. Stimulatory effect of retinoic acid and/or oxytocin has been inhibited by oxytocin receptor antagonist. Transient silencing of oxytocin receptor prevented elongation of neurites. Growth of neurites has been accompanied by increased expression of actin and drebrin suggesting changes of the actin cytoskeleton in the growth cone. Furthermore, expression changes of microtubule associated proteins and increase of intermediate filament vimentin and GTPase Cdc42 mRNA has been observed. A modulatory effect of oxytocin on synapse-associated proteins has been evidenced. It can be concluded that oxytocin contributes to the regulation of cytoskeletal proteins related to neurite outgrowth and induces neurite elongation at least at certain type of neuronal cells. Supported by the project 2/0119/15 of the Grant Agency of ministry of Education and Slovak Academy of Sciences (VEGA), and projects APVV-15-0205 and APVV-15-0045 of the Slovak Research and Development Agency.

RNA binding proteins in neuronal stress granules studied by single-molecule tracking

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Maintenance of cellular polarity as well as fast response upon extracellular cues requires fast and tightly regulated expression of proteins, especially in morphologically complex cells like neurons. Ribonucleoprotein (RNP) granules contribute to the regulation of gene expression via posttranscriptional coordination of mRNA translation, localization and degradation. These self-assembling structures lack a membrane and can be considered as dynamic microcompartments.

The Ras GTPase activating protein SH3 domain binding protein 1 (G3BP1) and the Insulin like growth factor II mRNA binding protein 1 (IMP1) are present in stress granules (SGs), i.e., RNP granules that are induced upon cellular stress. Our previous studies based on confocal laser scanning microscopy (cLSM) have shown that these two proteins colocalize in SGs and that overexpression of either of them is sufficient to induce the SG formation (Moschner et al., 2014). Furthermore, fluorescence decay after photoactivation (FDAP) analysis demonstrated that IMP1 exhibits rather slow SG-cytosol exchange with SGs being its preferred location, whereas G3BP1 readily fluctuates between the SG and cytosolic phase. Induction of stress by sodium-arsenite treatment increased the fraction of G3BP1 in SGs. Nevertheless, the dynamic organization of SGs and their components at the super-resolution level and, hence, their behavior remain unclear. Currently, there exist two concepts trying to explain the behavior of mRNPs, the liquid droplet model and the scaffolding assembly. In the former model, the SGs are formed via liquid-liquid phase separation (LLPS), remain amorphous and are held together by surface tension. In the latter model, the SGs grow upon binding of cytosolic proteins to a relatively robust scaffold presumably made up of proteins of the same type.

In order to shed light on the internal arrangement of G3BP1 and IMP1 inside SGs, we monitored the mobility of Halo-tagged G3BP1 and IMP1 constructs within granules induced upon sodium-arsenite treatment in neuronally differentiated PC12 cells using total internal reflection microscopy (TIRF) followed by single-molecule tracking (SMT) analysis. Our data reveals a lifetime of 700 ms if both G3BP1 and IMP1 are expressed, while expression of only one of these proteins leads to a significantly decreased binding time of 400 ms. This might indicate an interaction of those proteins inside SGs, thus favoring the scaffolding assembly model. On the contrary, 2D cluster analysis revealed only a weak overlap between the binding hotspots of G3BP1 and IMP1, suggesting a widespread distribution in an amorphous phase. Currently, we are analyzing the mobility of G3BP1 and IMP1 to gain insight into the diffusion properties of these proteins in SGs.

Reference:

Moschner K*, Sündermann F*, Meyer H, Pereira da Graca A, Appel N, Paululat A, Bakota L, and Brandt R (2014) *RNA-protein granules modulate tau isoform expression and induce neuronal sprouting*. (*joint first authors) J. Biol. Chem. 289:16814-16825

TrkB-dependent EphrinA reverse signaling guides callosal axon growth downstream of Neurod2/6

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Abnormal development of the corpus callosum (CC) is relatively common and can cause a wide spectrum of cognitive impairments in humans. Here, our work in our lab shows that basic helix loop helix transcription factors Neurod2 and Neurod6 are essential regulators for the fasciculation and guidance of callosal axons. In Neurod2/6-deficient mice, the formation of CC are severely defected. We use acallosal Neurod2/6-deficient mice to study callosal axon guidance within the ipsilateral cerebral cortex in vivo. Without Neurod2 and Neurod6, callosal axons fail to traverse the cingulum and do not reach the cortical midline. We identify EphrinA4 (EfnA4) as transcriptional target of Neurod2/6, and In utero electroporation of EfnA4 (or EfnA5) into neocortical pyramidal neurons of Neurod2/6 deficient embryos is sufficient to cell-autonomously rescue callosal axon fasciculation and migration along the normal callosal path towards the midsagittal plane. Mechanistically, EfnA4 forms a co-receptor complex with TrkB in reverse signaling, and hence regulate AKT cascades in vitro and in vivo via Ntrk2's SHC-binding tyrosine. Co-electroporation of dominant negative TrkB K571N or TrkB Y515F, but not TrkC K572N or TrkB Y816F, completely abolishes the ability of EfnA4 to rescue callosal axon guidance in Neurod2/6 deficient mice. We also show that the Eph receptors are abundantly expressed in the cortical plate and ventricular zone, but minimally expressed in the intermediated zone (IZ) of the cortex, while ephrinA ligands are largely present on the callosal axons in the IZ. In addition, reverse signaling from extracellular domain of EphA receptors to EfnA4 leads to active axonal retraction in vivo. The complementary expression and repulsive interaction of EphA receptors and ephrinA ligands suggest a permissive channel for callosal axon navigation before midline crossing. Thus, ephrinA ligands coordinate the guidance of callosal axons via interaction with Ntrk2 in cis and with EphA receptors in trans.

Role of Dscam1 in dendritic branch growth of central neurons

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Neurons are composed of two major domains axon and dendrites. Dendrites receive synaptic input and are highly branched. In order to maintain their territory and reach out to their proper synaptic partners, dendrite needs to space out evenly and avoid overlapping within their sister branches. The process of non-overlapping in sister-branches is achieved by repelling each other, and this phenomenon is known as self-avoidance of dendrites. Down syndrome cell adhesion 1 (Dscam1) is widely accepted as an important player in self-recognition and sister-branch avoidance in neurons, thus realizing even spacing of dendrites in their receptive fields. In *Drosophila*, a vast diversity of Dscam1 splice isoforms has been suggested to provide a nervous system wide code for self-avoidance and subsequent spacing¹.

However, recent studies in *Drosophila* have shown additional roles of Dscam1 in both dendritic branch growth² and axonal collateral branching³. It is not known in which context Dscam1 regulates self-avoidance versus branch growth, and the mechanisms underlying Dscam1 mediated new branch formation and growth remain unknown. To address these questions, we first compare the role of Dscam1 in different types of central neurons in *Drosophila*. In a second step, we aim to undertake the molecular mechanism by which Dscam1 regulates dendrite growth.

Despite the known role of Dscam1 in antennal lobe interneuron dendrite spacing, we find no effects of Dscam1 in local ventral nerve cord or in descending interneurons, neither with respect to spacing nor growth. By contrast, we find that Dscam1 is critically required for dendrite growth in all types of efferent neurons that we have tested, including larval and adult motoneurons and aminergic neurons innervating muscles. Similarities and differences between different types of neurons will now help to address the decision point of controlling self-avoidance versus growth and to address the mechanisms underlying Dscam1 mediated growth control.

References :

- (1) Schmucker D, Flanagan JG. Neuron. 2004 Oct 14;44(2):219-22. Review.
- (2) Hutchinson KM, Vonhoff F, Duch C. J. Neurosci. 2014 Jan 29;34(5):1924-31.
- (3) He H, Kise Y, Izadifar A, Urwyler O, Ayaz D, Parthasarathy A, Yan B, Erfurth ML, Dascenco D, Schmucker D. Science. 2014 Jun 6;344(6188):1182-6.

Serum Response Factor (SRF) regulates dendritic spines' maturation

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Dendritic spines' shape changes from thin elongated filopodia-like structures to stable mushroom dendritic spines during brain development. Re-arrangement of neuronal connections plays an important role in proper circuitry formation. Serum Response Factor (SRF), one of the major transcription factors in the brain, plays a prominent role in regulating various programs of gene expression in the adult brain in response to increased activity. The aim of our study was to investigate the functions of SRF in the regulation of structural plasticity during brain development. We found that lack of SRF in the hippocampal neurons during development resulted in increased number of filopodia-like protrusions and decreased number of mushroom spines with the general lack of changes in the overall density of dendritic spines in vitro. Moreover, spines of SRF knocked down neurons exhibited altered morphology, highlighted by increased length and area of filopodia-like and long spines. Furthermore, SRF-depleted neurons had lower level of surface AMPAR GluR1 and GluR2 subunits. We showed that the number of functional synapses and their activity was lower in SRF-depleted cells as shown by a reduction in the frequency and amplitude of mEPSC. These findings indicate that SRF regulates transcription of genes essential for synapse formation and maturation during hippocampal development.

Supported by Polish National Science Center grant (SONATA BIS 2) DEC-2012/07/E/NZ3/01814.

The role of glia in the development of GABAergic and glutamatergic neuronal networks in vitro in a novel culture system

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Cortical neurons can be divided into two distinct classes based on transmitter phenotype. Glutamatergic neurons mediate excitation and promote activity, while GABAergic interneurons (INs) mediate inhibition, which counterbalances and shapes this activity. Only through a delicate balance of excitation and inhibition can cortical networks function. Necessary for this function are glial cells, which are known to secrete substances that promote neuronal growth and survival, and excitatory synapse formation. Exactly how glia influence the development of INs is not fully understood. Therefore, our aim was to study the effects of glial derived factors on the development of IN networks and how this compares to the development of glutamatergic networks. To achieve this, we obtained purified IN or glutamatergic neuronal cell populations, by FAC sorting fluorescent neurons harvested from transgenic rodent lines. These cells were then cultured alone or grown in non-contact co-cultures with glial cells for up to 6 weeks. Our results indicate that both purified INs and glutamatergic neurons depend strongly on glial support for their long-term survival and morphological development. Surprisingly, despite extensive morphological deficits, purified INs maintain a typical resting membrane potential, can repetitively fire action potentials and establish synaptic transmission, albeit with reduced connectivity. In contrast, purified glutamatergic networks develop poorly in the absence of glia; although capable of maintaining trains of action potentials, glutamatergic neurons possess depolarized resting membrane potentials and fail to establish synaptic transmission. In summary, both GABAergic and glutamatergic neurons show reduced survival, connectivity and altered morphology that are all collectively dependent on support from glial cells. Interestingly however, only glutamatergic neurons seem to depend strongly on glia for the establishment of synaptic transmission. GABAergic neurons, on the other hand, can establish synaptic transmission in the absence of glia.

VEGFR2-ephrinB2 cooperative signaling controls dendritic arborization and synapse formation

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Nervous and vascular systems build up a dense and highly branched network using similar cellular structures (growth cones and endothelial tip cells). The vascular endothelial growth factor (VEGF), originally described as key regulator of angiogenesis, is now known to play important roles in the nervous system although it is still unclear which receptors transduce those signals in neurons. Additionally, in the hippocampus, multiple cell types express VEGF, thus it is also unclear which cellular sources of VEGF contribute to proper neuronal development and function. To address these unknowns, we first identified the spatial-temporal expression of VEGF in vessels, neurons, and astrocytes. We show that *in vivo*, cell-type specific VEGF deletion results in a compensatory increase of VEGF secretion by other cell types. Next, we show that the specific deletion of VEGFR2 in neurons results in defects in dendritic arborization and spine morphogenesis in the CA3 region of the hippocampus. These structural defects are accompanied by a reduced long-term potentiation at the associational-commissural – CA3 synapses. Mechanistically, in agreement with an evolutionary conservation of molecular mechanisms involved in developmental programs of both nervous and vascular systems, we identify a conserved function of ephrinB2 in regulating neuronal VEGFR2 internalization and activation in neurons. EphrinB2 and VEGFR2 co-localize in dendritic spines of mature neurons and the VEGF-mediated effect on spine maturation is abolished in ephrinB2 knockout neurons. *In vivo*, compound genetics demonstrate the physiological crosstalk of VEGFR2 and ephrinB2 to control dendritic arborization, spine morphogenesis and synaptic plasticity during hippocampal development. In totality, the results presented here show that VEGF exerts a direct effect on neurons via signaling through neuronal expressing VEGFR2.

Visualisation of endogenous protein expression, localisation and turnover in single neurons

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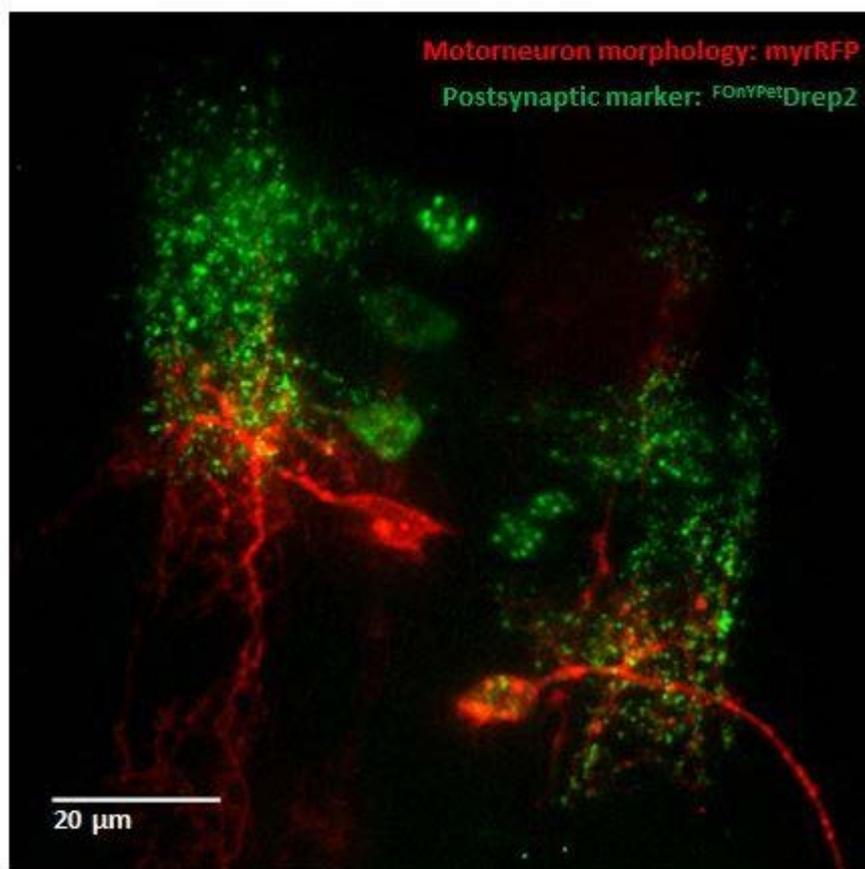
Fluorescently tagged synaptic proteins have been instrumental in unravelling the molecular mechanisms of synapse formation in living animals. Unfortunately, overexpression has been shown to induce artefacts in protein trafficking and the development of normal synapses; recent work therefore targets fluorescent tags to endogenous loci, or employs tagged exogenous alleles with intact UTRs.

However, these techniques do not allow the study of synaptogenesis between individual neurons in the dense synaptic neuropil of the central nervous system. We therefore developed the dFLEX cassette to conditionally label endogenous proteins in single cells. Upon insertion into the genome, the dFLEX cassette is initially spliced out of transcribed mRNA; upon inversion of the cassette by a recombinase the dFLEX cassette becomes an artificial exon that is included in mRNA transcripts. It can be adapted for many biological questions by placing different motifs into the cassette, including fluorescent proteins, non-fluorescent tags, mutated exons, or stop cassettes.

We used the dFLEX label to investigate protein synthesis and turnover of the presynaptic protein Bruchpilot (Brp) in the intact nervous system. We show that the amount of Brp at individual presynaptic sites increases in the first 2 days of larval development until it reaches steady state. Through pulse-chase experiments we determined the lifetime of Brp as approximately three days. In the same vein we estimate the lifetime of Brp transcripts to around one day. Recent data hint that Brp proteins might be synthesized locally at the presynaptic site; we are now testing this hypothesis by 1) localizing Brp mRNA transcript with fluorescent in situ hybridization, and 2) reveal ribosome localization by expression of GFP-tagged RpL10a.

Motorneuron morphology: myrRFP

Postsynaptic marker: F_{OnyPet} Drep2



Role of PTEN phosphorylation in brain development

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Phosphatase and tensin homolog located on chromosome 10 (PTEN) was originally characterized as a tumor suppressor that can inhibit proliferation, migration, cell growth and apoptosis in a number of different cells. PTEN is also highly expressed in neurons and recent work indicates that de-regulation of PTEN affects important neuronal functions in the developing nervous system, which have been attributed to its role in controlling neurogenesis, neurite outgrowth, synaptogenesis, and synaptic plasticity. Human germline PTEN mutations or conditional deletions of PTEN in mice have provided insights into fundamental links between PTEN deregulation and neurological disorders such as macrocephaly, ataxia, seizures, mental retardation and autism.

The regulation of PTEN activity and function crucially involves phosphorylation of a cluster of serine and threonine residues in the PTEN C-terminus. Within this cluster, PTEN can be phosphorylated at T366 by Glycogen Synthase Kinase 3 (GSK3), which has been suggested to regulate activity and PTEN protein turnover. Since PTEN antagonises and functions as a key upstream regulator of PI3K/GSK3 signalling, the fact that it is phosphorylated and regulated also by GSK3 itself, could indicate feedback regulation within this signalling cascade. Currently, little is known concerning neuronal functions of PTEN T366 phosphorylation.

To determine the contribution of PTEN^{T366} to neuronal development and/or function, we analyzed mice in which this phosphorylation site was inactivated as a result of the introduction of a germline point mutation (PTEN^{T366A}). Whilst homozygous PTEN^{T366A/T366A} mice, hereafter referred to as PTEN^{T366A} mice, are viable and fertile, subtle differences in cortical lamination were observed. Thus, we will present our data that investigates cortical neurogenesis and post-neurogenesis processes in different cortical layers of PTEN^{T366A} mice, with an emphasis on the cell-type specificity and fine organization of dendritic arbors. Our results highlight specific roles of PTEN-T366 phosphorylation in generating anatomical and functional synaptic connections, possibly affecting sensory processing and higher cognitive function.

Poster Topic

T3: Developmental Cell Death, Regeneration and Transplantation

- [T3-1A](#) Absolute reduction of olfactory bulb layer volume during absolute growth of the olfactory bulb – a sign for developmental changes of information processing ?
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- [T3-2A](#) Analysis of regeneration and myelination associated proteins in human neuroma
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- [T3-1C](#) Localization of reelin signaling pathway components in murine midbrain and striatum
Belal Mahmoud Rahhal, Björn Björn Spittau, Ahmad Sharaf, Kerstin Kriegelstein
- [T3-2C](#) Long-term cultivation of organotypic nigrostriatal slice cultures
Sarah Maria Elisabeth Joost, Andreas Wree, Stefan Jean-Pierre Haas
- [T3-1D](#) MicroRNA-132 improves regeneration in primary dopaminergic midbrain neurons
Lucas A. Caldi Gomes, Anna-Elisa Roser, Mathias Bähr, Paul Lingor
- [T3-2D](#) Walking of the stick insect *Sipyloidea sipyilus* with a regenerated leg
Reinhard Lakes-Harlan

Absolute reduction of olfactory bulb layer volume during absolute growth of the olfactory bulb – a sign for developmental changes of information processing ?

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Information processing requires morphological structures, which develop in mammals not only pre- but also postnatally. Especially in altricial species, the brain growth continues far beyond birth. The American mink (*Neovision vison var. atratus*) is born with eyes and ears closed and depends primarily on its sense of smell for nutrition and neonatal survival. As reported before (Weiler & Bennegger, 2015) the olfactory bulb, the main station for olfactory information processing, follows its own growth pattern, different to the rest of the brain.

Information processing however depends on the composition of the neurons and neuronal layers. Taken the fact, that the olfactory sense is functional already from birth on, the question arises: Is the olfactory bulb growing uniformly or do the layers also show signs of allometric growth?

Therefore we analyzed the absolute volume of each olfactory bulb layer in a total of 36 female minks at different postnatal ages (newborns (postnatal day 0 =P0) up to 7 months = P210) using histological sections and a morphometric system. A continuous increase in volume was observed in the external plexiform layer (P0: $0.04 \pm 0.01 \text{ mm}^3$; P60: $13.62 \pm 0.38 \text{ mm}^3$; P210: $24.42 \pm 1.56 \text{ mm}^3$) and granule cell layer (P0: $0.30 \pm 0.02 \text{ mm}^3$; P60: $12.98 \pm 0.47 \text{ mm}^3$; P210: $30.85 \pm 1.43 \text{ mm}^3$) following the continuous increase of the whole olfactory bulb volume. In contrast, volume maxima at P60 with subsequent significant reduction ($p < 0.01$) were reached in the internal plexiform layer (P60: $3.70 \pm 0.30 \text{ mm}^3$; P90-120: $2.02 \pm 0.47 \text{ mm}^3$), the internal medullar layer (stratum album: P60: $13.95 \pm 0.54 \text{ mm}^3$; P90-120: $11.32 \pm 0.79 \text{ mm}^3$; P210: $9.71 \pm 2.27 \text{ mm}^3$), and the subependymal layer (P60: $4.69 \pm 0.13 \text{ mm}^3$; P90-120: $2.64 \pm 0.14 \text{ mm}^3$; P210: $1.12 \pm 0.34 \text{ mm}^3$). The mitral cell layer showed a significant maximum at P90-150 ($4.50 \pm 0.27 \text{ mm}^3$; P210: $3.78 \pm 0.37 \text{ mm}^3$). These results indicate that layer specific growth pattern exist in the olfactory bulb, even with overshoot phenomena. Absolute layer volume reduction in a continuously growing olfactory bulb even maximizes differences in the composition and indicates differential olfactory information processing during specific developmental phases.

The specific absolute volume reductions can be caused by different factors. While the overshoot and concomitantly following reduction in the mitral cell layer might be a result of passing cells on their way from the center of the bulb to the outer layers, the absolute reduction of the subependymal layer might be related to the retraction of the ventricle to a more posterior region, out of the bulb, leaving the migratory stream for renewal of neurons. Reduction in the stratum album indicates a retraction of centrifugal fibers from the brain during juvenile brain reduction (reported earlier, Weiler & Bennegger, 2015). All of this concentrates information-processing neurons within the bulb.

These results indicate a rearrangement of neurons and underlying networks, by establishing a filtering system, according to the necessity of increasing olfactory challenges during biological phases: as

neonates to identify milk and social cues; as juveniles detect family members, enemies, prey, predators; as adults additionally identify sexual partners; suggesting that, although the olfactory system is functional at birth, the information processing changes during postnatal life.

Weiler & Bennegger, 2015; Are olfactory bulb and brain volume growing proportionally? 11th German Neurosci Soc.

Analysis of regeneration and myelination associated proteins in human neuroma

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Neuromas are pathologic nerve distensions caused by a nerve's response to trauma. After a traumatic lesion to a peripheral nerve, axonal regeneration at the lesion site is possible in case the soma of the axon remains intact, and the nerve as such is still in continuity. Sprouting axons attempt to cross the injury site, as long as scar tissue or a gap do not counteract sprouting. If target-oriented sprouting is prevented by an exaggerated connective tissue response a spindle or bulb shaped neuroma will form. It consists of disorganized unmyelinated mini-axons intermingled within connective tissue. The nerve will be dysfunctional to non-functional, and at times can be severely painful due to ectopic excitation phenomena. So far, rat models have shown that regeneration associated genes (RAGs) play an important role for axonal regeneration and for the establishment of a growth-supporting environment by Schwann cells. On a molecular scale, however the precise sequence has not been resolved for human nerve. Therefore human neuroma formation, its influencing factors and differences in neuroma composition are of interest.

To analyze the axonal distribution in six human neuromas, three stump neuromas and three neuromas in-continuity have been histologically and biochemically characterized. Immunostaining of neurofilament-protein (NF) and the growth-associated protein 43 (Gap43) showed a significant reduction of axons from the proximal to the distal segment of each neuroma. No axonal structures could be found in the distal nerve stump.

Beside NF-containing nerve fibers, axons expressing large amounts of Gap43 could be detected. Axonal growth cones labeled with Gap43 antibodies sprout alongside NF-positive fibers in a piggyback-like manner, underlining the remaining axonal plasticity within neuroma tissue. Myelin sheath labeling revealed mature NF-expression as well as Gap43-expression of axons, which become myelinated by Schwann cells. This was validated by the Schwann cell marker S100. Results suggest that the capability of Schwann cells to myelinate regenerating axons is preserved in neuroma tissue. In this regard there seems to be no difference between stump neuroma and neuroma in continuity. No structural discrepancies could be depicted. Correlation of results to corresponding clinical findings (e.g. age, latency between trauma and reconstructive surgery, functional result of grafting) will help to improve surgical strategy including timing. In the long run further work-up of molecular coherences might prompt pharmacotherapeutic approaches to support regeneration after reconstruction.

Grant support: The project was generously supported by an intramural fund of faculty VI of the Carl-von-Ossietzky University Oldenburg to Thomas Kretschmer (Forschungspoolförderung für Projekt "Molekularbiologische Untersuchungen an humanen Neuromen")

Cell-free artificial implants of electrospun fibers in a three-dimensional gelatin matrix support sciatic nerve regeneration *in vivo*

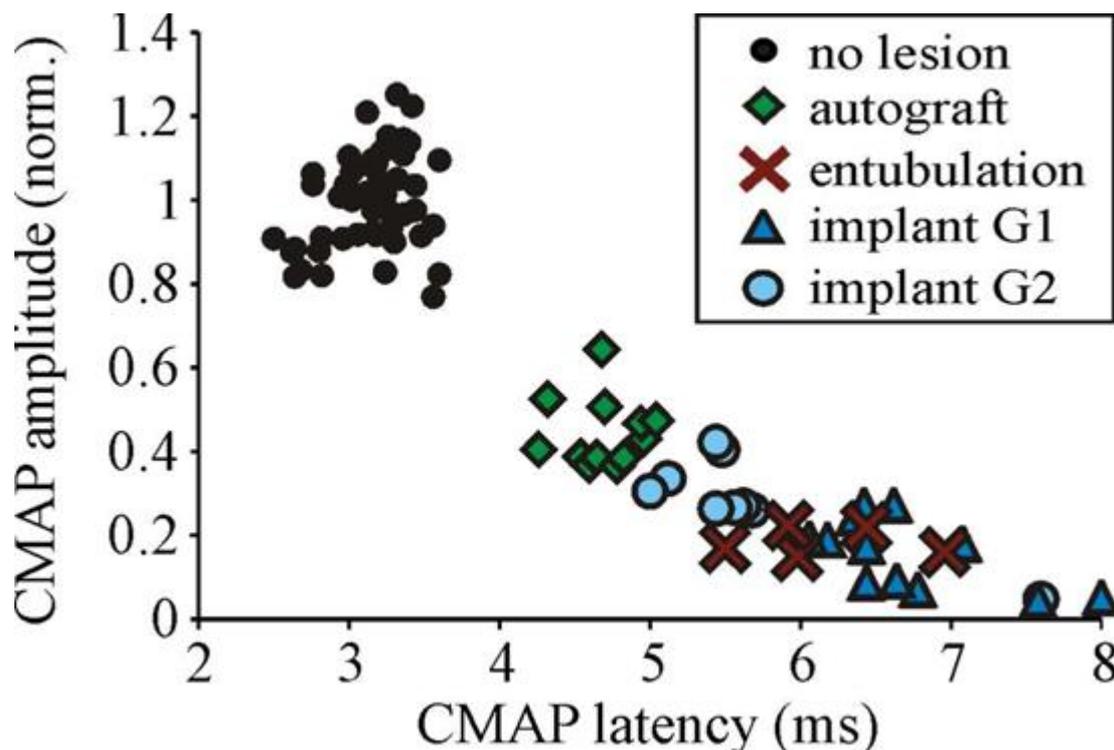
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Surgical repair of larger peripheral nerve (PN) lesions requires the use of autologous nerve grafts. At present, clinical alternatives to avoid nerve transplantation consist of empty tubes, which are only suitable for the repair over short distances and have limited success. We developed a cell-free, three-dimensional scaffold for axonal guidance in long distance nerve repair. Sub-micron scale fibers of biodegradable poly-ε-caprolactone (PCL) and collagen/PCL (c/PCL) blends were incorporated in a gelatin matrix and inserted in collagen tubes. The conduits were tested by replacing 15 mm long segments of rat sciatic nerves *in vivo*. Biocompatibility of the implants and nerve regeneration were assessed histologically, with electromyography and with behavioral tests for motor functions. Functional repair was achieved in all animals with autologous transplants, in 12 of 13 rats that received artificial implants with an internal structure and in half of the animals with empty nerve conduits. In rats with implants containing c/PCL fibers, the extent of recovery (compound muscle action potentials, motor functions of the hind limbs) was superior to animals that had received empty implants but not as good as with autologous nerve transplantation. Schwann cell migration and axonal regeneration were observed in all artificial implants, and muscular atrophy was reduced in comparison with animals that had received no implants. The present design represents a significant step towards cell-free, artificial nerve bridges that can replace autologous nerve transplants in the clinic.



Extensive elongation and branching characterize repair Schwann cells that form post injury and support efficient nerve regeneration

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The Schwann cell injury response has been viewed by us and many others as dedifferentiation, generating denervated Schwann cells in the distal stump of injured nerves that resemble developing Schwann cells, namely immature Schwann cells or Schwann cell precursors. This now requires revision, because it has become apparent that developing and denervated cells show distinct molecular expression, have different functions, and are under distinct transcriptional control. Because denervated cells are specialized for repair and differ from other cells in the Schwann cell lineage, we refer to them as repair Schwann cells. The generation of repair cells from myelin and Remak cells, which combines both loss and gain of phenotypes, resembles transformations termed direct (or lineage) reprogramming in other systems (Jessen and Mirsky *J. Physiol.* 2016 doi: 10.1113/JP270874). In the present work we have asked: do the differences between developing and repair Schwann cells also extend to cellular morphology? Using RosaYFP/PLP-CreERT2 mice to label Remak and myelin Schwann cells in uninjured nerves, we have examined the descendants of these cells in injured nerves 1,4,10 weeks and 6 months after transection without innervation. Remarkably, we find that myelin and Remak cells elongate up to 3 fold as they transform to repair Schwann cells, achieving an average length of 820 \pm 29 micrometers. About half the cells also branch forming slender parallel processes. While myelin and Remak cells are 404 \pm 19 micrometers and 246 \pm 7 micrometers on average, respectively, immature Schwann cells measure only 113 \pm 5 micrometers. Repair cells are therefore 7-8 times longer than developing Schwann cells. Their extreme length and possession of parallel processes enables these cells to carry out one of their main functions, the generation of continuous regeneration tracks (Bungner bands) along denervated distal nerves. This morphological specialization establishes an additional clear-cut distinction between developing and repair Schwann cells.

Human Spinal Cord Neural Progenitors and neurotrophic factor mimetic-loaded mesoporous silica particles Assist Regeneration of Sensory Fibers into the Spinal Cord after Dorsal Root Avulsion

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Spinal nerve root injuries are characterized by the tearing of spinal roots away from the spinal cord. This leads to a longitudinal spinal cord injury and results in the interruption of motor (ventral root) and sensory (dorsal root) nerve fibers (Carlstedt 2016). Reimplantation of ventral roots is performed routinely in patients and shows good recovery of motor functions (Kachramanoglou et al. 2011). In contrast, sensory axons are unable to overcome the growth inhibitory environment at the spinal cord lesion site (Fraher 2000). Neurotrophic factors stimulate axonal outgrowth of transected dorsal roots and in less severe injuries induce reinnervation of spinal cord neurons (Wang et al. 2008; Ramer et al. 2000). Alternatively, olfactory ensheathing cells transplanted to the transected ends of dorsal roots can form a growth permissive tissue bridge and allow functional reconnection of sensory fibres with spinal cord targets (Ramón-Cueto & Nieto-Sampedro 1994; Li et al. 2004). We recently showed that human spinal cord neural progenitor cells (hspNP) from either embryonic (hESC, (Hoeber et al. 2015) or fetal (hfNSC, Hoeber et al., 2016) sources as well as neurotrophic factor mimetic-loaded mesoporous silica particles (MesoMIM, Hoeber et al., 2016) transplanted to the site of avulsed dorsal roots assist regeneration of sensory fibers into the adult mouse spinal cord. All three approaches supported regeneration of sensory axons when they were applied separately, whereas when hspNP were implanted together with MesoMIM sensory regeneration failed. MesoMIM diminished the ability of hspNP to form a tissue bridge that allowed ingrowth of sensory axons. In contrast to MesoMIM, hspNP treatments resulted also in a strong reduction of the growth inhibitory glial scar. In long term behavioral experiments, animals undergoing avulsion of the dorsal root showed a severe loss of hind limb sensitivity and grip strength. In contrast, hspNP treated animals showed both improved sensitivity and strength.

In conclusion, engrafted hspNP can form growth permissive tissue bridges that allow sensory regeneration across the scarred spinal cord interface. Mimetics of neurotrophic factors locally delivered by mesoporous silica particles stimulate axonal outgrowth similar to classical studies of locally or systemically administered neurotrophic factors, but fail to improve hspNP depended regeneration.

Carlstedt, T., 2016. New Treatments for Spinal Nerve Root Avulsion Injury. *Frontiers in Neurology*, 7(August), pp.23–26. Available at: <http://journal.frontiersin.org/Article/10.3389/fneur.2016.00135/abstract>.

Fraher, J.P., 2000. The transitional zone and CNS regeneration. *Journal of anatomy*, 196 (Pt 1(2), pp.137–158. Available at: <http://doi.wiley.com/10.1046/j.1469-7580.1999.19420161.x>.

Hoeber, J. et al., 2015. Human Embryonic Stem Cell-Derived Progenitors Assist Functional Sensory Axon Regeneration after Dorsal Root Avulsion Injury. *Scientific Reports*, 5(8), p.10666. Available at: <http://www.nature.com/doi/10.1038/srep10666>.

Hoeber, J. et al., 2016. A Combinatorial Approach to Induce Sensory Axon Regeneration into the Dorsal Root avulsed Spinal Cord. *Stem Cells and Translational Medicine*, (under revision)

Kachramanoglou, C. et al., 2011. Novel strategies in brachial plexus repair after traumatic avulsion. *British journal of neurosurgery*, 25(1), pp.16–27.

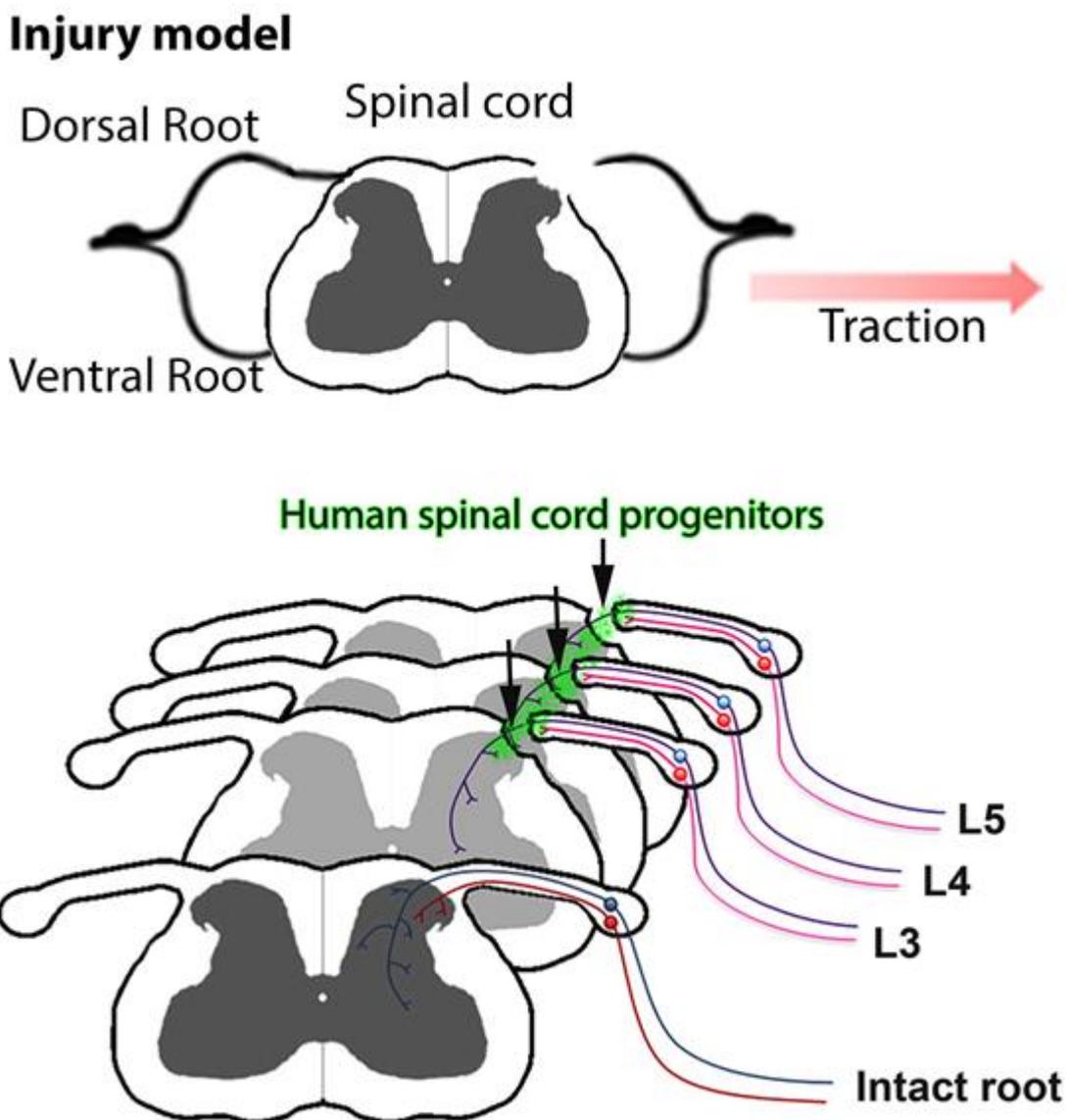
Li, Y. et al., 2004. Interaction of transplanted olfactory-ensheathing cells and host astrocytic processes provides a bridge for axons to regenerate across the dorsal root entry zone. *Experimental Neurology*, 188(2), pp.300–308.

Ramer, M.S., Priestley, J. V & McMahon, S.B., 2000. Functional regeneration of sensory axons into the adult spinal cord. *Nature*, 403(6767), pp.312–316.

Ramón-Cueto, a & Nieto-Sampedro, M., 1994. Regeneration into the spinal cord of transected dorsal root axons is promoted by ensheathing glia transplants. *Experimental neurology*, 127(2), pp.232–244.

Wang, R. et al., 2008. Persistent restoration of sensory function by immediate or delayed systemic artemin after dorsal root injury. *Nature neuroscience*, 11(4), pp.488–496.

This work was supported by the Swedish Research Council, proj no. 5420 and 20716, Stiftelsen Olle Engkvist Byggmastare, Signhild Engkvist's Stiftelse and the Swedish Institute's Visby program Dnr 00613/2011



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Localization of reelin signaling pathway components in murine midbrain and striatum

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We investigated the distribution patterns of the extracellular matrix protein Reelin and of crucial Reelin signaling components in murine midbrain and striatum. The cellular distribution of the Reelin receptors VLDLr and ApoER2, the intracellular downstream mediator Dab1, and the alternative Reelin receptor APP were analyzed at embryonic day 16, at postnatal stage 15 (P15), and in 3-month-old mice. Reelin was expressed intracellularly and extracellularly in midbrain mesencephalic dopaminergic (mDA) neurons of newborns. In the striatum, Calbindin D-28k+ neurons exhibited Reelin intracellularly at E16 and extracellularly at P15 and 3 months. ApoER2 and VLDLr were expressed in mDA neurons at E16 and P15 and in oligodendrocytes at 3 months, whereas Dab1 and APP immunoreactivity was observed in mDA at all stages analyzed. In the striatum, Calbindin D-28k+/GAD67+ inhibitory neurons expressed VLDLr, ApoER2, and Dab1 at P15, but only Dab1 at E16 and 3 months. APP was always expressed in mouse striatum in which it colocalized with Calbindin D-28k. Our data underline the importance of Reelin signalling during embryonic development and early postnatal maturation of the mesostriatal and mesocorticolimbic system, and suggest that the striatum and not the midbrain is the primary source of Reelin for midbrain neurons. The loss of ApoER2 and VLDLr expression in the mature midbrain and striatum implies that Reelin functions are restricted to migratory events and early postnatal maturation and are dispensable for the maintenance of dopaminergic neurons.

Long-term cultivation of organotypic nigrostriatal slice cultures

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The nigrostriatal pathway is of vital importance for motor function and plays a crucial role in Parkinson's disease (PD). This pathway consists of dopaminergic neurons located in the substantia nigra of the midbrain projecting towards the striatum, thereby serving as dopaminergic input to the basal ganglia circuit. Since the degeneration of these dopaminergic neurons leads to PD, the nigrostriatal pathway is of great interest for medical research.

Manipulating the nigrostriatal pathway in model systems *in vivo* is complex and time-consuming, so the application of organotypical slice cultures for modelling the nigrostriatal pathway could be a valuable alternative. While minimizing the amount of animal experiments, slice cultures offer a convenient model system easily accessible for manipulation and conserving the three-dimensional environment of the brain. However, organotypical slice culture systems of the nigrostriatal system are not extensively characterized yet and further work is needed to make this model system applicable for research.

For establishing a nigrostriatal slice culture, we used mice expressing EGFP under the tyrosine hydroxylase promoter, allowing to specifically observe the development of dopaminergic neurons during culture under sterile conditions. The brains of five day old mouse pups were rapidly dissected, embedded in agarose and cut sagittally into 350 μm thick slices with a McIlwain Tissue Chopper. Slices containing substantia nigra and striatal tissue were selected and incubated at the air-liquid interface on semi-porous membrane inserts. The initial medium, containing 25% of horse serum, was changed to serum-free medium after five days. After one to two weeks of cultivation, slices were fixed and immunohistochemistry was performed on wholemount slices.

Immunohistochemical stainings against GFAP, Iba1, ChAT and other neuronal markers revealed the conservation of morphologically unimpaired neuronal, glial and microglial cell populations in cultivated slices. Substantia nigra, the striatum as well as other structures like the hippocampal formation or the olfactory bulb showed a high degree of tissue preservation and a well preserved morphology. However, although numerous EGFP-containing dopaminergic neurons were detected in the substantia nigra, EGFP-containing fibers towards the striatum were not observed.

To address this issue, a co-culture system of frontal sections containing substantia nigra and striatum in direct contact is currently established to enhance and observe outgrowth of dopaminergic fibers towards the striatum. Further experiments aim at the optimization of the culture conditions, e.g. by adding growth factors to stimulate dopaminergic outgrowth.

In total, current results emphasize the potential of organotypical slice cultures for the investigation of the nigrostriatal pathway. This rather simple methodology offers a neuronal model with preserved three-dimensional brain environment, easily accessible for manipulations like pharmacological compound application, cell transplantation or electrophysiological assessment. Our studies will take part in the characterization and development of this promising model system allowing deeper insight in the nigrostriatal pathway under physiological or diseased conditions.

MicroRNA-132 improves regeneration in primary dopaminergic midbrain neurons

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Parkinson's Disease (PD) is an age-related neurodegenerative disease, prevalent in up to 2% of individuals aged over 60 years. PD is characterized by marked cell loss in the substantia nigra and consequent dopamine depletion in the striatum, resulting in serious deficits of motor control. The limited regenerative capability of neurons in the central nervous system and the only incomplete understanding of PD pathophysiology complicate the development of disease-modifying treatments. Recent studies indicate that a dysfunction of gene expression regulators might contribute to the pathophysiology of brain diseases. MicroRNAs (miRs), a class of small non-coding RNAs, act as post-transcriptional regulatory factors of gene expression, targeting mRNAs and eliciting either translational repression or mRNA degradation. By manipulation of microRNA expression levels, neuroprotective and neuroregenerative strategies might be explored. Previously, we identified changes in microRNA expression levels in primary midbrain neurons (PMNs) upon development, which are closely involved in maturation of dopaminergic neurons. miR-132 was found highly regulated during development and thus might contribute potentially to neuroregenerative/neuroprotective mechanisms. Here we investigated the effects of increased levels of miR-132 in dopaminergic PMNs. For that, cultured cells were transfected with a synthetic mimic for this microRNA. After TH-immunostaining, average neurite length and regeneration in dopaminergic neurons were measured by neurite tracing. High levels of miR-132 significantly increased neurite outgrowth and regeneration in dopaminergic PMNs. We hypothesized that these effects are mediated by repression of P250GAP, a miR-132 target protein. This GTPase-activating protein regulates actin dynamics suppressing the RAC1-PAK actin remodelling pathway and thus, directly influences neurite morphogenesis. Our further results show that increased miR-132 levels result in reduced P250GAP levels in PMNs. In addition, levels of the activated form of RAC1 were found increased upon higher levels of miR-132. We demonstrate that miR-132 plays an important role in dopaminergic morphogenesis and regeneration, suggesting that it might be a valuable tool for the development of novel curative treatments for this disease.

Walking of the stick insect *Sipyloidea sipylos* with a regenerated leg

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Insects can regenerate lost body appendages to an amazing degree. Especially stick insects are known for a good regeneration capacity. Here, we investigated functional regeneration in adult stick insects. How do they use a regenerated midleg for walking and stepping over an obstacle? Therefore the walking and stepping has been videotaped and analysed in respect to different parameters. The data were compared to intact controls and to adults without a regenerated midleg.

Nymphs of stages 3 to 6 had the left midleg ablated. Morphological regeneration occurred within two moults resulting in regenerated legs in the adult. Regeneration occurred in all adults when the leg was ablated in stages 3 and 4, in 6 out of 8 cases after ablation in stage 5, but no regeneration occurred after ablation in stage 6. The regenerated leg in adults has about the same dimensions as the intact leg. Movements of the regenerated leg indicate a functional regeneration. Furthermore, the walking pattern between intact adults and those with a regenerated leg does not differ. The walking velocity (about 6cm/s) on a flat arena is similar under all conditions. In intact adults and those with a regenerated leg the stance width is the same for all legs and the percentage of stance duration during movement is similar. However, a difference can be detected in animals without a regenerated leg. Here, the stance widths are leg specific, with the left hindleg showing larger steps, perhaps to compensate for the missing midleg. Movements across an obstacle show the same patterns for animals with regenerated leg as intact animals.

Thus, the data show that after morphological regeneration the normal function is restored. It might be assumed that the underlying neuronal circuits are the same. However, during the whole regeneration process neuronal plasticity might occur, because the animals with a missing midleg have different movement patterns. To analyse this further, neurophysiological studies and studies on the behaviour of nymphs during the regeneration process are needed.

Poster Topic

T4: Neurotransmitters, Retrograde messengers and Cytokines

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Autocrine endocannabinoid signaling in cortical neurons

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The endocannabinoid system consists of lipid messenger molecules and their G protein-coupled receptors, cannabinoid type 1 and type 2 receptor. It is involved in a variety of physiological processes including development, food intake, nociceptive processing, immunomodulation as well as synaptic transmission and plasticity. The two major endocannabinoids N-arachidonoyl-ethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are metabolized in and released from postsynaptic neurons before acting on the presynaptic receptors to modulate transmitter release. 2-AG also induces autocrine self-inhibition in some hippocampal and cortical pyramidal cells as well as cortical interneurons. In these cells several high-frequency bursts of action potentials or direct activation of cannabinoid receptors lead to a long-lasting hyperpolarization, which is called slow self-inhibition (SSI). Interestingly, different mechanisms were described on how hippocampal and cortical neurons implement this long-lasting hyperpolarization. In our project we carry out a detailed investigation of SSI in cortical pyramidal cells and interneurons in order to better understand the role and mechanism of this phenomenon.

Distribution of cholinergic fibers in the visual cortex in p75NTR knockout mice

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The cholinergic system plays critical roles in cortical plasticity, attention and learning.

Within the visual cortex the cholinergic system seems to play a central role in visual perception. The cholinergic neurons which project to the visual cortex are located in the basal forebrain. It has been shown that mice deficient for the low-affinity neurotrophin receptor p75NTR display increased numbers of cholinergic neurons in the basal forebrain and a denser cholinergic innervation of the hippocampus. This prompted us to analyze whether the cholinergic system in the visual cortex of adult p75NTR deficient mice is altered. By analyzing the densities of cholinergic fibers within layer IV as well as within layer V of the visual cortex, we found that adult p75NTR deficient mice display increased cholinergic fiber densities. However, this increase was not accompanied by an increase in the density of local cholinergic neurons within the visual cortex. This indicates that the enhanced cholinergic innervation of the visual cortex is due to alteration of the cholinergic neurons located in the basal forebrain, projecting to the visual cortex. The increased cholinergic innervation of the visual cortex makes the p75NTR deficient mice to an attractive model to study the necessity of the cholinergic system for the visual cortex.

Elucidating the mode of action of the neonicotinoid imidacloprid on honey bee kenyon cells using Ca^{2+} - imaging

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Neonicotinoids currently represent the most widely used class of insecticides worldwide. Besides the lack of reasonable alternatives the initial success of neonicotinoids is mainly due to their selective efficacy on insects. However, side effects on humans have been observed. Specific human disease patterns are believed to be due to the commercial expansion of neonicotinoids in plant protection. The European Food Safety Authority (EFSA) concluded developmental neurotoxicity potential for imidacloprid and acetamiprid, two main representatives of neonicotinoid insecticides. According to this, the two neonicotinoids may impair brain functions such as learning and memory.

To allow an accurate risk assessment of these substances a profound understanding of their mode of action in neurons is inevitable. Therefore, we use kenyon cells (KCs) from the honey bee mushroom bodies to analyze short term effects of imidacloprid on their main target – nicotinic Acetylcholinreceptors – and compare them to effects of similar agonists such as nicotine and acetylcholin. We succeeded in establishing the Ca^{2+} - imaging as the method of choice to conduct this analysis. It represents an alternative to the widely used Patch-Clamp method and enables the monitoring of a high number of KCs at the same time. Using this method, we observed imidacloprid as exerting nicotine-like effects and partial acetylcholin-like effects on KCs. By applying a size criterion we could distinguish between large and small KCs. Large KCs showed a better Ca^{2+} - response than the smaller ones.

Additionally, biochemical methods like antibody staining will be applied, aiming at the evaluation of chromatin status in the treated cells. Using this combination of imaging and biochemical methods will enable us to elucidate the possible correlation between the agricultural use of neonicotinoids and the impairment of neuronal processes.

H₂S evoked NMDA-dependent inhibition network activity of neonatal rat hippocampal slices

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Hydrogen sulfide (H₂S) is an endogenous gasotransmitter with neuroprotective properties that participates in the regulation of transmitter release and neuronal excitability in various brain structures. H₂S is synthesized by three enzymes: cystathionine γ -synthase, cystathionine β -lyase and 3-mercaptopyruvate sulftransferase/cysteine aminotransferase. In nerve systems the main source of synthesis H₂S is cystathionine γ -synthase and high level enzyme expression observed in the embryonic and early postnatal period of organism development that is apparently necessary for the growth and maturation of neural networks for the protection of neurons and astrocytes in the conditions of oxidative stress. During the first two postnatal weeks - a period of intense neuronal growth and synaptogenesis, - the hippocampal network generates periodic synchronized neuronal discharges, known as giant depolarizing potentials (GDPs) (Ben-Ari et al., 1989). It was shown that the expression of CBS in the nervous system during the embryonic period is generally low and increased from late embryonic to early postnatal period (Bruintjes et al., 2014). The role and mechanisms of H₂S actions in the maturation of neural networks however remains unclear. The goal of study is investigate the mechanisms of exogenous and endogenous H₂S actions on neuronal spontaneous activity in hippocampal slices of neonatal rats.

Experiments were performed on slices of neonatal Wistar rats (postnatal days P3–P7) using extracellular field wire electrodes or patch-clamp techniques in whole cell configuration in the CA3 pyramidal cell layer of hippocampus. Pneumatic picopump was used to puff-apply NMDA (50 μ M+50 μ M glycine for activation of NMDA-receptors), glutamate (1 mM, for AMPA/kainate-receptors) or GABA (100 μ M, for GABA-receptors) from a glass pipette at a distance of about 50–250 μ m from the body of neuron.

At first we tested the actions of exogenous donor of H₂S - NaHS and the substrate of its synthesis L-cysteine on the activity of immature hippocampal neurons. The application of NaHS (100 μ M) and L-cysteine (1mM) caused initial increasing and subsequent suppression of the frequency of GDPs and action potentials in hippocampus in a reversible manner during first postnatal week. The neonatal hippocampal network is organized in recurrent excitatory loops, pyramidal cells and interneurons being excited by the depolarizing effects of GABA and glutamate, which provide for synchronous neuronal discharges (GDPs), largely mediated by GABA(A) and NMDA receptor activation. Application of NaHS (100 μ M) did not significantly change the area and the amplitude of GABA_A-currents and AMPA/kainate-currents. In addition, we tested the effect of NaHS on NMDA-receptor mediated currents at holding potentials of +30 mV. In the presence of GABA (A/B) and AMPA/kainate receptors inhibitors administration of NaHS induced decreasing of amplitude outward currents which were completely blocked by the NMDA-receptor antagonist d-APV. These effects did not fully recover after NaHS washout.

So, main finding of our study is that H₂S induced suppression of neuronal network activity of rat neonatal hippocampus by reducing NMDA receptor-mediated currents in the immature brain.

The work was supported by Russian Science Foundation [grant No. 14-15-00618].

Homocysteine and its derivatives increase the activity of maxi calcium-activated potassium (BK) channel and decrease exocytosis of secretory granules in rat GH3 cells

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Homocysteine (Hcy) is a nonproteinogenic, sulfur-containing amino acid, biosynthesized via the methionine metabolism and is a homolog of cysteine. Under normal circumstances, most of the Hcy formed in transmethylation reactions is remethylated back to methionine or converted into cysteine in transsulfuration reactions. Genetic alterations of enzymes involved in Hcy metabolism, such as cystathionine γ -synthase (CBS), methionine synthase (MS), or methylenetetrahydrofolate reductase (MTHFR) or by inadequate supply of folate, vitamin B-12, or vitamin B6 leads to impaired of remethylation or transsulfuration reactions and Hcy is metabolized to the cyclic thioester Hcy-thiolactone, metabolic conversion catalyzed by methionyl-tRNA synthetase. Hcy is also easily oxidized to homocystine. Abnormalities of Hcy metabolism lead to increase of Hcy level - hyperhomocysteinemia (HHcy). HHcy is associated with increased risk for many disorders, including pathology of nervous system. The molecular mechanisms by which Hcy and its derivatives cause cellular pathology are still unclear.

Maxi calcium-activated potassium (BK) channels are present in numerous cells being involved in controlling electrical activity, such as action- or synaptic potentials, in hormone secretion. In this study, we investigated the effects of Hcy and its derivatives on BK channels and on the exocytosis of secretory granules in GH3 pituitary tumor cells using electrophysiological and fluorescent methods.

GH3 pituitary cells were obtained from the European Collection of Cell cultures (ECACC). The activity of BK channels was investigated using patch-clamp technique in whole-cell and exercised patch modes. The processes of exocytosis of secretory granules were studied using FM 1-43. L-homocysteine (Hcy), DL-Homocystine, DL-Homocysteine thiolactone hydrochloride, EGTA (ethylene glycol-bis(b-aminoethyl ether)-N,N',N''',N''''-tetraacetic acid) and FM1-43 were obtained from Sigma Aldrich.

The whole-cell patch clamp technique was used to recording outward K⁺ currents of GH3 cells. Hcy and its derivatives did not alter the amplitude of outward K⁺ currents in short-term application. But we observed the increasing the total K⁺ currents after long-term incubation (24 h). Single BK channels were recorded at a holding potential of +30 mV. Hcy and its derivatives had no effect on single BK channel activity in outside mode however increased the activity of BK-channel in inside-out mode which can be explained by the modest permeability of these amino acids which need transport systems to penetrate the cell membrane. Fluorescent studies have shown that Hcy and its derivatives decreased the basal and evoked exocytosis of secretory granules, containing growth hormone (GH) and prolactine.

We suggest that elevated cellular level of Hcy and its derivatives during HHcy increase the activity of BK channels by acting from the internal side of cell membrane and decrease exocytosis of secretory granules containing growth hormone. These findings implicate adverse effects of Hcy on developmental impairments and neurotoxicity during HHcy.

Acknowledgements. This project was supported by the Russian Science Foundation (Grant No. 14-15-00618)

Hydrogen sulfide activates TRPV1 receptors in rat trigeminal neurons and increases the activity of trigeminal nerve

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Hydrogen sulfide (H₂S) a member of gasotransmitters family, is involved in regulation of great variety of physiological functions, including nociception and inflammation. H₂S produced endogenously from L-cysteine by the enzymes cystathionine γ -synthase (CBS), cystathionine γ -lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST) along with additional contribution of cysteine aminotransferase (CAT) or D-amino acid oxidase (DAO). It was shown that CBS is abundantly expressed in rat TG neurons and H₂S donor - NaHS increases the excitability of trigeminal neurons by suppression of potassium conductance. In pathological inflammatory conditions, the increase of CBS expression in trigeminal neurons was demonstrated suggesting a role for H₂S in inflammation-induced hyperalgesia. Controversial data existed about the activating effects of H₂S on the family of TRP receptors, obtained in in vivo and vitro experiments.

The aim of our study was to reveal the effect of NaHS on the firing of trigeminal (TG) nerve using suction electrode recordings in peripheral branches of the TG nerve in isolated rat meninges. We also studied the effects of NaHS on TRPV1 currents by patch clamp recordings in isolated trigeminal neurons.

All animal experiments were performed in accordance with the European Community Council Directive of September 22, 2010 (2010/63/EEC) and approved by the Animal Care and Use Committee of University of Eastern Finland and the Ethics Committee of Kazan Federal University. Recordings of electrical activity of trigeminal nerve were performed using isolated rat hemiskull preparations obtained from adult (P35–36) rats at room temperature.

The TRPV1 currents were recorded in isolated trigeminal neurons from P9-P12 rats. TRPV1 currents were evoked by focal application of capsaicine in concentration 1 μ M. Sodium hydrogen sulfide (NaHS) was used as donor of H₂S.

Bath application of NaHS (100 μ M) increased the action potential frequency of trigeminal nerve and this effect was prevented by the inhibitor of TRPV1 receptors capsazepine (10 μ M). At the same time NaHS increased the amplitude of capsaicine induced currents in isolated trigeminal neurons. Moreover the focal application of NaHS (100 μ M) on trigeminal neurons induces inward currents which was inhibited by capsazepine. The obtained data suggest that NaHS directly activates TRPV1 receptors and induces the inward currents, which may increase the firing rate of trigeminal neurons.

We propose that activation of TRPV1 receptors by H₂S during chronic inflammation process is contributes to the increased excitability of the trigeminal system and may be implicated in the generation of nociceptive firing underlying migraine pain.

The work was supported by Russian Science Foundation 14-15-000618

Modulation of Locus Coeruleus Neurons by 5-Hydroxytryptamine

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About 50% of all noradrenergic projections arise from the Locus Coeruleus (LC) reaching throughout the entire brain. Due to its widespread and complex network the LC has been associated with the control of many different behavioral and cognitive functions such as arousal, stress, attention and memory processes (J. Psychopharmacol., 2013, 27(8):659-93). As an important prerequisite to unravel the physiological role of the LC, it is crucial to understand the intrinsic electrophysiological properties of the LC neurons. In previous work, certain electrophysiological properties of LC neurons have been characterized. However, since the experiments have been performed in various organisms using different recording techniques the current data might not be quite comparable. Here we used perforated patch-clamp recordings, which do not disturb intracellular signaling, to comprehensively characterize LC neurons intrinsic electrophysiological properties in mice. Overall we found a homogeneous population of neurons with similar intrinsic electrophysiological properties including input resistance, excitability and action potential waveform. All LC neurons generated very regular pacemaker-like activity. Synaptic isolation did not disrupt pacemaking but significantly increased the action potential frequency in all experiments. All recorded neurons showed delayed excitation after hyperpolarization and prolonged hyperpolarization after sustained excitation.

In rodents the LC receives serotonergic projections from the dorsal raphe nucleus and LC neurons are inhibited by serotonin (5-hydroxytryptamine, 5-HT) application. The physiological relevance of this connection is still not fully understood, hence we tested the effect of 5-HT on the electrophysiological properties of LC neurons. Here we found that LC neurons in mice showed a complex response to 5-HT. On the one hand 5-HT caused a concentration dependent hyperpolarization accompanied by a reduction in action potential frequency, and a decrease in cell input resistance. On the other hand, the excitability in presence of 5-HT was drastically increased. Together this might improve the signal to noise ratio in the LC, sharpening the noradrenergic signaling deriving from LC neurons. Our results confirm that 5-HT modulates LC neurons, but also suggest that the modulatory effects are more complex than previously thought.

Acknowledgements

We thank H. Wratil for outstanding technical assistance. This work was supported by the Max-Dellbrück-Award and grants from the Deutsche Forschungsgemeinschaft to PK.

Spatial analysis of putative peptide release sites in the ventral lateral neurons of the fruit fly *Drosophila melanogaster*

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Neuropeptide release apparently is independent of synaptic contacts: neuropeptide containing dense-core vesicle (DCVs) exocytosis has been shown to occur in pre-terminal axons, dendrites and cell bodies. Yet, quantitative data are largely missing, and it is unclear whether wide-spread non-synaptic peptide release is a rule or rather an exception.

To provide more data here, our aim is to comprehensively characterize release sites for DCVs in peptidergic neurons in the fruit fly *Drosophila*, using pigment dispersing factor (PDF) as a model. PDF is a neuropeptide specifically expressed by a subset of circadian clock neurons called ventral lateral neurons (LN_vs). The LN_vs also express further neuropeptides, including short neuropeptide F (sNPF).

As a first step, we currently investigate the spatial relationship between active zones and putative DCV release sites in the LN_vs by genetically labeling active zones and a combination of high resolution light and electron microscopy. Furthermore, we are characterizing the colocalization of PDF and the short neuropeptide F (sNPF) in these DCVs. First results will be reported.

Tyramine functions as a neuromodulator of *Drosophila* larval motoneurons

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In many species motor behavior is modulated by biogenic amines. In spinal cord for example, the gain of motor output is strongly enhanced by aminergic drive from brainstem. Similarly, invertebrate motor behaviors, such as insect flight and walking, or crawling in *C.elegans* and *Drosophila* larvae are under modulatory control of biogenic amines. In multiple invertebrates, interactions of the amines octopamine and tyramine have been demonstrated to be essential for both initiation and maintenance of locomotor behaviors. The cellular targets and molecular mechanisms of tyramine and octopamine interaction remain largely unknown. This study combines the genetic power of *Drosophila* with patch clamp recordings, immunocytochemistry, and calcium imaging of identified motoneurons to pinpoint possible effects of tyramine on crawling motoneurons.

Crawling motoneurons projecting to larval *Drosophila* body wall muscles can be distinguished by morphological and physiological criteria into those with small boutons (Is motoneurons), that innervate multiple muscles of one segment, and those with big boutons (Ib motoneurons), that innervate only one muscle. Due to immunocytochemistry we could show that axons of tyraminergergic neurons are in close proximity to Is motoneuron dendrites, indicating that paracrine release may directly affect motoneuron properties. Our opto- and electrophysiological data confirm direct tyraminergergic action on motoneuron excitability and synaptic integration.

In-situ current clamp recordings show that bath application of tyramine reversibly decrease the firing frequencies and increase the delay to the first action potential of each burst of Is motoneurons, as induced by somatic ramp or square pulse current injections in a dose dependent manner (10^{-4} M to 10^{-6} M in standard saline). This decrease in Is motoneuron excitability is in accord with decreased locomotor activity of larvae with genetically increased tyramine levels, as it could be shown with behavioral experiments in *Drosophila* larvae. Targeted RNAi knock-down of each tyramine receptor show that these effects are mediated exclusively via the Oct-TyrR. In somatic voltage clamp recordings potassium and calcium currents appear unchanged following tyraminergergic modulation. By contrast, dendritic calcium imaging clearly shows decreased postsynaptic calcium influx upon activation of cholinergic receptors. We now test whether tyramine modulates dendritically localized L-type calcium channels or the nicotinic AChR.

In summary, this study reveals direct effects of the biogenic amine tyramine on *Drosophila* central neurons. We could also identify the receptor mediating the modulatory effects of tyramine and have first clues about the ion channels involved. The apparent similarities to aminergic modulation of spinal motoneurons promised *Drosophila* as a useful model to dissect the underlying mechanisms and behavioral consequences.

Poster Topic

T5: G Protein-linked and other Receptors

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- [T5-2D](#) Tonic inhibition in the basal amygdala is under control of modulatory transmitter systems
Susanne Meis, Thomas Endres, Thomas Munsch, Volkmar Lessmann

Activin A reduces GIRK current to excite dentate gyrus granule cells

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In the nervous system, the TGF-beta family member activin A was originally identified as a neurotrophic and neuroprotective factor. More recent evidence from our and other laboratories showed that activin A also tunes excitatory and inhibitory neurotransmission in the brain in a fashion that impacts on cognitive functions and affective behavior. In addition to its canonical signaling through SMAD proteins, activin might also act on other pathways including ERK-MAPK signaling. Both physiological and pathological stimuli were found to induce activin signaling. On the physiological side, activin levels are significantly up-regulated by brief trains of stimuli that induce long-term potentiation as well as by behavioral stimulation such as environmental enrichment (EE). More pronounced up-regulation is observed after epileptic activity or acute brain injury, as well as after electroconvulsive seizure (ECS) which represents a rodent model of electroconvulsive therapy in pharmacoresistant depression.

Since up-regulation of activin levels after EE and, more so, ECS was most prominent in the dentate gyrus of the hippocampus, we became interested to determine the electrophysiological effects of enhanced activin signaling in dentate gyrus granule cells (DGGCs). We performed whole-cell recordings from DGGCs in mouse brain slices, which were prepared after 12 h of EE or 12 h after a single ECS-inducing stimulation (25 mA for 0.5 s at 50 Hz). We report here that EE and ECS strongly enhanced the intrinsic excitability of DGGCs. When we examined DGGCs from transgenic mice which over-express a dominant-negative activin receptor IB mutant (dnActRIB), EE failed to increase their excitability, and the effect of ECS was mitigated, indicating the involvement of activin signaling in the electrophysiological effects of EE and ECS. We then mimicked the up-regulation of activin that was induced by these two treatments by incubating control slices with activin A (25-100 ng/ml) for 3-10 h. Activin incubation reproduced the effects of EE and ECS on granule cell firing. Interestingly, activin A was capable of increasing DGGC firing, even when superfused only for few minutes. This almost immediate effect of activin was not abrogated by SB 421532 (10 μ M), which blocks SMAD-dependent signaling, suggesting that it was mediated by non-canonical signaling.

In voltage-clamped DGGCs, acute activin A superfusion produced an apparent inward current which reversed near EK. This effect of activin A was suppressed by tertiapin Q, which blocks G protein-gated inwardly rectifying potassium channels (GIRK), or by BaCl₂, which non-specifically blocks inward rectifier K⁺ currents. Furthermore, activin A inhibited GIRK currents evoked by activation of GABAB or adenosine receptors. Finally, tertiapin Q produced similar excitatory effects on DGGC firing and occluded the effect of activin after both acute and chronic incubation.

In summary, our study links the increased excitability of DGGCs after EE and ECS to an activin A-mediated reduction of a standing GIRK current, most likely involving a SMAD-independent signaling pathway.

Crosstalk between metabotropic receptors and Ca_v1.2 channels in somatostatin-expressing hippocampal interneurons

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Influx of Ca²⁺ ions into dendrites through high-voltage activated Ca²⁺ (Ca_v) channels triggers diverse modulatory processes suggesting that the intracellular concentration of Ca²⁺ is a critical parameter for synaptic plasticity. The Ca_v1.2 which conduct L-type currents, have been shown to have an impact on dendritic Ca²⁺ signaling and are potentiated by metabotropic G protein-coupled receptors (GPCRs) e.g. GABA_BR and mGluR1. To obtain morphological and functional evidences for the interaction and dynamics of GABA_BR, mGluR1 and Ca_v1.2 channels we examined the subcellular organization of these proteins and their spatial relationship on dendritic shafts of somatostatin-expressing CA1 stratum oriens/alveus interneurons (SOMIs) using a combination of pharmacological and SDS-FRL-based quantitative immunoelectron microscopic approaches. Activation of GABA_BR through bath application of the agonist baclofen led to a decreased surface expression of both mGluR1 and Ca_v1.2, while the activation of mGluR1 had no impact on the density of these proteins. In contrast, when GPCRs are antagonized with CGP54626 and LY367385, respectively, we observed no alteration in the density of Ca_v1.2 channels. To quantify the spatial relationship of the Ca²⁺ channels and receptors, the distances between particles for the channel subunits and both mGluR1 and GABA_BR were measured. This analysis revealed that the majority of the immunoparticles for Ca_v1.2 were located within a distance of 100 nm from the closest GPCR. These data show that GABA_BR can dynamically regulate the surface expression of both mGluR1 and Ca_v1.2 on dendritic shafts of SOMIs in the CA1 area of the hippocampus suggesting a crosstalk between GABA_BR, mGluR1 and Ca_v1.2.

Serotonin-mediated function of cell adhesion molecule L1 in neuronal morphology

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Stress-related disorders are known to be associated with the functional disturbance of neuronal adhesion molecule L1, which intra- and extracellular signaling pathways are critically involved in axonal development, neuronal survival and growth. Here we have shown that the matrix metalloproteinase-9 (MMP-9) can cleave the neuronal L1 thereby regulating homophilic/heterophilic interaction and paracrine functions of L1. We have also demonstrated that L1 fragments generated by MMP-9 cleavage are involved in spine formation in cultured hippocampal neurons. Together with the observation that the L1 can initiate neurite outgrowth via phosphorylation of actin-binding protein cofilin, this data suggests that MMP-9-mediated proteolysis of L1 might be involved in L1-initiated signaling pathway regulating actin rearrangement in neurons. Furthermore, we investigated a possible interplay between L1 and serotonergic system with the focus on serotonin receptor 5-HT₄ (5-HT₄R). In the mammalian brain this receptor contributes to regulation of learning and long term memory and is involved in various central and peripheral disorders, including neurodegenerative disease and depression. We have shown that stimulation of 5-HT₄R induces the release of enzymatically active MMP-9 in hippocampal neurons, where 5-HT₄R and L1 are tightly co-localized at the synapses. Moreover, we observed a direct interaction between 5-HT₄R and L1 on the cell membrane of HEK293 cells by using co-immunoprecipitation analysis.

We have previously shown that 5-HT₄R stimulation results in activation of the small GTPase RhoA, leading to cell rounding and neurite retraction. Here we demonstrate that this effect can be mediated by phosphorylation of cofilin. Thus, cofilin may represent a common downstream effector for both 5-HT₄R and L1 suggesting that 5-HT₄R, MMP-9 and L1 belong to the same signaling module involved in regulation of neuronal morphology. Taken together, our results demonstrate that serotonin might regulate the function of adhesion molecule L1, on the one side, in 5-HT₄R/MMP-9-dependent manner, and, on the other side, via direct interaction between 5-HT₄R and L1, and can thus represent a novel molecular mechanism by which serotonin can regulate the formation and plasticity of neuronal networks and serve as a target for pharmacological intervention into stress-related disorders.

Modulation of medial prefrontal cortex (mPFC) pyramidal neurons by noradrenaline.

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Aims: Noradrenaline (NA) is an important factor in the regulation of cognitive brain functions and affective processes. The medial prefrontal cortex (mPFC) receives dense noradrenergic projections from locus coeruleus. Impaired modulation of PFC by NA has been implicated in widespread neuropsychiatric diseases such as posttraumatic stress disorder, attention deficit hyperactivity disorder, and depression. The aim of this study was to investigate which adrenergic receptor subtypes are the main target of NA in the layer V mPFC pyramidal neurons and what are the cellular mechanisms underpinning the effects of NA in these neurons.

Methods: The resting membrane potential and holding currents were recorded in the layer V mPFC pyramidal neurons. Gramicidin perforated-patch and classical whole-cell recordings were made in brain slices of 3-week-old rats. Tested compounds were applied to the bath and/or to the solution in the recording pipette.

Results: α_1 -receptor agonists either did not evoke any changes in the membrane potential (phenylephrine, 100 μ M) or evoked hyperpolarization (cirazoline, 100 μ M) which was reduced in the presence of the I_1 -imidazoline receptor antagonist (efaroxan, 100 μ M) and not affected by the α_1 -receptor antagonist (prazosin, 100 μ M). α_2 -receptor agonists either did not affect the membrane potential (medetomidine, 100 μ M) or caused hyperpolarization (clonidine, 100 μ M) which was abolished only by the selective blocker of HCN channels (ZD7288, 50 μ M) and not affected by α_2 -receptor antagonist yohimbine (60 μ M). Application of NA evoked dose-dependent depolarization of membrane potential and inward currents. The effects were considerably reduced by the nonselective α -receptor antagonist propranolol (60 μ M) and the selective α_1 -receptor antagonist metoprolol (60 μ M), but not by the selective α_2 -receptor antagonist ICI 118,551 (50 μ M). The nonselective α -receptor agonist isoproterenol, the α_1 -receptor agonist dobutamine and the selective α_3 -receptor agonist BRL37344 mimicked the inward currents caused by NA. The effects of BRL37344 on holding currents were decreased in the presence of cesium ions (3mM) and the selective blocker of HCN channels (ZD7288, 50 μ M). Pretreatment with the phospholipase C inhibitor (U73122, 10 μ M) reduced the inward current caused by the selective α_3 -receptor agonist BRL37344. Application of adenylyl cyclase inhibitor SQ22536, both extracellularly (100 μ M) and intracellularly (1mM), did not affect the amplitude of the current.

Conclusions: We conclude that NA modulates mPFC pyramidal neurons via α_1 - and α_3 -receptors, causing membrane depolarization and inward currents. α_3 -receptor activation evokes inward current due to HCN channel activation. The effect is probably mediated by the phospholipase C signalling pathway and does not involve adenylyl cyclase.

Supported by National Science Centre, Poland, grant 2014/15/N/NZ4/04760 and FW5/PM2/16.

Palmitoylation of hyaluronan receptor CD44 influences its function in hippocampal neurons

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The extracellular matrix (ECM) and its modifiers arose as important regulators of neuronal morphology and synaptic plasticity contributing to physiological processes such as learning and memory. Moreover, the ECM has been implicated in the onset and progression of various diseases of the central nervous system, including Alzheimer's Disease, epilepsy and schizophrenia. 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. Localisation and signalling properties of CD44 can be modified by post-translational modifications. Palmitoylation is the most common post-translational lipid modification of proteins which represents the reversible attachment of the C16 saturated fatty acid palmitate to cysteine residue(s). Even though it is known that CD44 can be palmitoylated, the functional consequences of CD44 palmitoylation in the brain have not been studied yet.

Here we investigated the impact of this lipid modification on the function of neuronal CD44. We demonstrated that CD44 undergoes palmitoylation in different regions of the rodent brain. In rat hippocampal neurons, we found two populations of CD44: non-palmitoylated CD44 and CD44 that was mono-palmitoylated at its cytoplasmic cysteine residue. We showed that palmitoylation is not necessary for CD44 homo-dimerisation, but still impacts the downstream signalling in neuroblastoma cells. Furthermore, by silencing endogenously expressed CD44 and over-expressing palmitoylation-deficient CD44 mutants we studied the effects of this lipid modification on CD44-mediated regulation of neuronal morphology.

The Adhesion GPCR Latrophilin/CIRL acts as a putative metabotropic mechanosensor

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The mechanical habitat holds critical information for the development and daily function of cells. Adhesion-type G protein-coupled receptors (aGPCRs) have emerged as a large molecule family with potential mechanosensory properties, however their mechanotransduction mechanism remained unclear. The *Drosophila* receptor Latrophilin/CIRL provides insights into the molecular and physiological rationale of the aGPCR mechanosensing paradigm. dCIRL acts in chordotonal mechanosensory neurons by modulating ionotropic receptor currents, the initiating step of cellular mechanosensation. This process depends on the length of the extended ectodomain and the tethered agonist of the receptor, but not its autoproteolysis, a characteristic biochemical feature of the aGPCR family. Intracellularly, the sensitivity of mechanosensory neurons inversely correlates with cAMP concentration. dCIRL contributes to this process by quenching cAMP levels upon mechanical activation. Collectively, these results provide direct evidence that the aGPCR dCIRL acts as a molecular sensor and signal transducer that detects and converts mechanical stimuli into a metabotropic response.

Tonic inhibition in the basal amygdala is under control of modulatory transmitter systems

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Tonic inhibition (I_{tonic}), mediated by extrasynaptically located GABA_A receptors, emerges as an essential factor that shapes neuronal excitability and was only recently described for the amygdala. So far, the role of modulatory transmitter systems in the brain on I_{tonic} is not well known. We therefore aimed at analysing the impact of neuromodulators on I_{tonic} in the amygdala. Using patch clamp recordings in slice preparations, we quantified I_{tonic} as the inward shift in holding potential upon addition of the GABA_A receptor antagonist bicuculline. We first showed that the current amplitude of I_{tonic} in projection neurons of the mouse basal amygdala is modified by preincubation with the neurosteroid THDOC (100 nM), while the benzodiazepine diazepam (1 μ M) is ineffective, suggesting an involvement of the α -subunit of GABA_A receptors. In addition, we demonstrate that the monoamines noradrenaline and serotonin, as well as acetylcholine, strongly enhance frequency and amplitude of spontaneous IPSCs. These effects were mediated by the α_1 noradrenergic receptor, the 5HT₂ receptor and predominantly by the non- α_7 nicotinic ACh receptor, respectively. Upon addition of the respective agonists, I_{tonic} was strongly activated. As the increase in frequency, amplitude and charge of sIPSCs strongly correlated with the amplitude of I_{tonic} , we conclude that spill-over of synaptic GABA leads to activation of I_{tonic} and may thereby dampen amygdala excitability. As thus, phasic and in particular the subsequent activation of tonic inhibition may be an important constraint of amygdala function under physiological and pathological conditions. This work was supported by the DFG (SFB 779/B6).

Poster Topic

T6: Ligand-gated, Voltage-dependent Ion Channels and Transporters

T6-1A Retracted

[T6-2A](#) Activation of renal ClC-K chloride channels is dependent on an intact N-terminus of their accessory subunit barttin
Martin Fischer, Stefan Thiemann, Christina Schaal, Alina Rahtz, Jeanne de la Roche, Daniel Wojciechowski

[T6-3A](#) Alternative splicing as a mechanism to increase ion channel diversity
Lukas Kilo, Stefanie Ryglewski

[T6-4A](#) Assessing the role of HCN channels in mouse hippocampal neurons using virus delivered gene-interfering tools
Matthias Deutsch, Anne Günther, Arnd Baumann

[T6-5A](#) Chloroform is a potent activator of cardiac and neuronal Kir3 channels
Sina Kollert, Frank Döring, Erhard Wischmeyer

[T6-6A](#) Closing in on bimodal action of the anticonvulsant Topiramate by employing the honeybee (*Apis mellifera*)
Marie-Luise Kümmel, Uli Müller

[T6-7A](#) Deletion of auxiliary Ca²⁺ channel subunit $\alpha_2\text{-3}$ specifically reduces P/Q (Ca_v2.1) but not L-type Ca²⁺ currents of spiral ganglion neurons
Friederike Stephani, Stefan Münkner, Jutta Engel

[T6-1B](#) Enigma of rebound depolarization (RD) in the medial prefrontal cortex (mPFC) pyramidal neurons
Przemyslaw Norbert Kurowski, Pawel Szulczyk

[T6-2B](#) Insertion of a glutamate (V166E) at the pore entrance provides an additional gating process for human hClC-Ka chloride channels
Daniel Wojciechowski, Kira Stecher, Martin Fischer

[T6-3B](#) Lactate is a potent inhibitor of the capsaicin receptor TRPV1
Jeanne de la Roche, Isabella Walther, Waleria Leonow, Axel Hage, Mirjam Eberhardt, Peter W. Reeh, Susanne Sauer, Martin Fischer, Andreas Leffler

[T6-4B](#) Na-K-ATPase mediated neuronal adaption

- [T6-5B](#) Novel chemical and molecular tools for the identification of RNA editing-competent neurons and RNA-edited glycine receptor (GlyR) proteins
Florian Hetsch, Svenja Kankowski, Nicolai Dorka, Nicole Horn, Larissa Kraus, Jochen Meier
- [T6-6B](#) Novel forced intercalation probes for the detection of glycine receptor (GlyR) RNA editing at the single cell level
Svenja Kankowski, Andrea Knoll, Felix Hövelmann, Oliver Seitz, Jochen Meier
- [T6-1C](#) Potassium chloride co-transporter 2 expression is upregulated by potassium chloride and ampakine in chicken auditory brainstem *in vitro*
Marcus Joseph Wirth, Soeren Damgaard, Lars Roentgen, Hermann Wagner
- [T6-2C](#) Probing the channel gating of a glutamate receptor with photoactive unnatural amino acids
Anahita Poshtiban, Mette H. Poulsen, Viktoria Klippenstein, Valentina Guise, Andrew Plested
- [T6-3C](#) Probing the function of $\alpha 2$ calcium channel subunits in the genetic model system *Drosophila melanogaster*
Laurin Heinrich, Stefanie Ryglewski, Carsten Duch
- [T6-4C](#) Proton-dependent modulation of mouse HCN channels
Daniela Ricarda Drose, Simone Weyand, Alejandro Giorgetti, Paolo Carloni, Marc Spehr
- [T6-5C](#) Role of the presynaptic scaffolding proteins Bassoon and Piccolo in the regulation of voltage-gated calcium channels at the release sites and of synaptic vesicles cycling within the presynapse
Eneko Pina, Carolina Montenegro-Venegas, Maria Andres-Alonso, Claudia Marini, Eckart D. Gundelfinger, Anna Fejtova
- [T6-6C](#) Strategies to Stably Record Calcium Currents in Substantia nigra Dopaminergic Neurons
Ursel Collienne, Andreas C. Klein, Simon Heß, Stephan Bremser, Peter Kloppenburg
- [T6-7C](#) The contribution of the two binding sites to the opening of the adult nicotinic acetylcholine receptor
Dmitrij Ljaschenko, Josef Dudel, Remigijus Lape, Manfred Heckmann
- [T6-1D](#) Subcellular organization of presynaptic Ca^{2+} channels at hippocampal mossy fiber synapses
Julia Bank, Johannes Jordan, Ákos Kulik
- [T6-2D](#) TGF- β regulates NBCe1 expression and activity in mouse cortical astrocytes.
Shokoufeh Khakipoor, Christian Ophoven, Eleni Roussa
- [T6-3D](#) The Alzheimer's protease BACE1 as a subunit and non-enzymatic regulator of neuronal and cardiac KCNQ channels
Sandra Lehnert, Maren Schülke, Vanessa Linke, Stephanie Hartmann, Christian Alzheimer, Tobias Huth
- [T6-4D](#) Trpc5 cation channels contribute to hormone regulation

in the hypothalamus

Thomas Blum, Ana Moreno-Perez, Anela Arifovic, Petra Weissgerber, Veit Flockerzi, Marc Freichel, Frank Zufall, Trese Leinders-Zufall

[T6-5D](#) Variable ion channel expression in identified single neurons - homeostatic plasticity or genetic variation?

Carola Staedele, Andrés Gabriel Vidal-Gadea, Wolfgang Stein

[T6-6D](#) Vasoactivity of heme degradation products (HDPs) on cerebral arterioles *in vivo* and *in vitro*
Alexander Jörk, Milena Günther, Nicolas Witsch, Marcel Ritter, Raphael Andreas Seidel, Georg Pohnert, Matthias Westerhausen, Otto Wilhelm Witte, Knut Holthoff

Activation of renal ClC-K chloride channels is dependent on an intact N-terminus of their accessory subunit barttin

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ClC-K chloride channels are essential for sodium chloride reabsorption in the loop of Henle of the kidney and for secretion of potassium by the stria vascularis of the inner ear. Renal and inner ear chloride channels comprise of two pore-forming ClC-K subunits and the accessory subunit barttin. Barttin consists of 320 amino acids that putatively form a short cytoplasmic N-terminus (aa1-8), two transmembrane helices (aa9-54) and a long intracellular C-terminus (aa55-320). While intact helices are mandatory for incorporation of ClC-K into the surface membrane, a short stretch of the C-terminus (aa55-72) is important for ClC-K channel activation (Scholl et al., 2006). Truncation of the C-terminus (E88X) eliminates a sorting signal that inserts the channel in the basolateral membrane of epithelial cells (Janssen et al., 2009). We here investigate the role of the short N-terminus of barttin and its impact on ClC-K channel function.

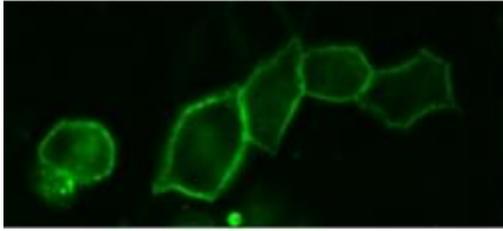
Confocal imaging was used to study subcellular distribution of WT and N-terminally mutated barttin-CFP in transiently transfected MDCK-II cells. Deletion of the complete N-terminus (del2-8) impaired trafficking of barttin to the plasma membrane, while barttin with deletion of shorter stretches (del2-7, del2-4, del5-8) inserted in the membrane like WT barttin and also promoted trafficking of co-expressed ClC-K channels to the surface membrane (chaperone function of barttin).

We next performed whole cell patch clamp recordings from transiently transfected HEK293T cells to investigate the role of barttin mutations on ClC-K channel gating. Currents of human hClC-Ka channels were nearly abolished when parts of the barttin N-terminus were removed (del2-8, del2-7, del2-6, del5-7, del7-8, del8). Milder mutations (del2-4, del2-5) reduced current amplitudes by at least 50% without affecting the single channel conductance. Substitution of the N-Terminus by poly-alanine sequences of different length also failed to efficiently activate hClC-Ka indicating that not the length of the sequence but the correct position of single amino acids is important for proper function. We therefore introduced point mutations and found prominent current reduction for E4R, K5A and T6V barttin. Disease-causing mutations R8L and R8W (bartter syndrome) have been previously reported to impair channel activation as well (Janssen et al., 2009).

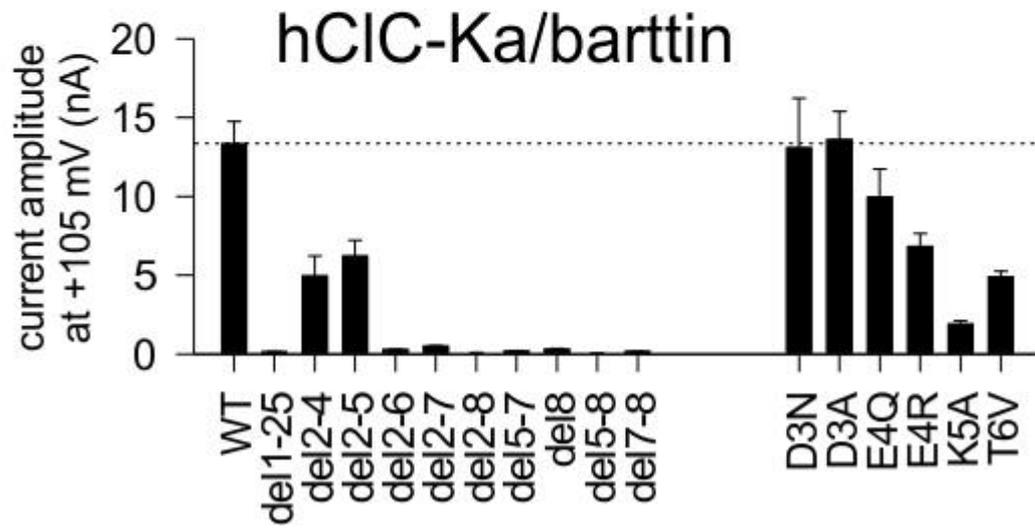
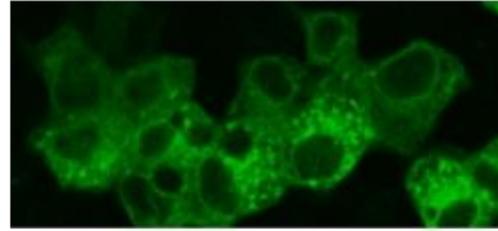
In contrast to human hClC-Ka, the rat homolog rClC-K1 was less affected by N-terminal barttin mutations. We here used the mutant V166E rClC-K1 that is well established to investigate voltage dependence of gating. While WT barttin constitutively opens the slow gate of V166E rClC-K1, the accessory subunit with completely deleted N-terminus or shorter deletions insufficiently opened that gate. The effect was partly recovered when the excess of barttin expression was considerably enhanced indicating that binding ability of N-terminal mutated barttin to the pore-forming subunit was reduced.

In conclusion, the N-terminus of barttin includes a signal for intracellular trafficking and is important for hClC-Ka channel activation. N-terminal deletions do not prevent the interaction of barttin with the ClC-K proteins, but partly reduce the binding affinity. Chaperone function of barttin is not influenced. Activation of human hClC-Ka channels essentially relies on an intact barttin N-terminus, while gating of the rat homolog rClC-K1 is mildly modulated supporting species-specific differences of ClC-K channels.

WT barttin



del2-8 barttin



Alternative splicing as a mechanism to increase ion channel diversity

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Voltage gated calcium channels (VGCCs) are an integral part of neuronal membranes. They facilitate neuronal signal transduction, gene expression, neuronal development, synaptic vesicle release, and play a crucial role in excitability. The ten vertebrate VGCC genes can be divided into 3 families, each of which corresponds to only one gene in *Drosophila*. One of those genes, the *Drosophila* Ca_v2 homolog, *DmCa1A/cacophony* is responsible for at least three distinctly different calcium channels in the same *Drosophila* wing depressor motoneuron (Ryglewski et al. 2012; Ryglewski, Kilo, Duch 2014). These currents can be electrically and/or pharmacologically isolated resulting in high voltage activated (HVA) and low voltage activated (LVA) currents. This *cacophony* calcium channel diversity may be due to various reasons such as alternative splicing or different combinations in accessory subunits.

It has been shown that specific sites within the *cacophony* gene are spliced alternatively. This leads to several isoforms resulting in different proteins for the α_1 subunit. Two of these alternative exons are of special interest in this study as they encode regions of the *cacophony* calcium channel that may account for the distinct functional differences between the various *cacophony*-based currents. Those isoforms are tested *in vitro* for function before introducing them into *DmCa1A* null mutant flies. *In vitro* electrophysiology of VGCCs co-expressed with accessory subunits as well as *in situ* patch clamp recordings have already yielded initial results for a proof of concept.

Assessing the role of HCN channels in mouse hippocampal neurons using virus delivered gene-interfering tools

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Question:

Hyperpolarization and cyclic nucleotide-gated (HCN) ion channels are expressed in a wide range of neuronal tissue. On the cellular level they contribute to the regulation of the resting membrane potential, integration of synaptic input at the dendrites, regulation of presynaptic neurotransmitter release, as well as generation of rhythmic activity. Thus, HCN dysfunction or altered HCN gene expression might lead to several pathologic conditions, like epilepsy, neuropathic pain, Parkinson's disease or age-related working memory decline. To investigate HCN channel function and/or dysfunction within single neurons as well as within neuronal networks, isoform-specific downregulation of HCN channel expression will be addressed.

Methods:

A versatile tool to study gene expression and function is to generate knockout (KO) animals with the gene of interest being genetically inactivated. Unfortunately, the generation of HCN KO mouse models often resulted in embryonic lethality. To overcome these limitations HCN channel isoforms will be specifically downregulated by utilizing mRNA and gene interfering techniques. To this end, we take advantage of a cell autonomous process called RNA interference (RNAi). This process mediates mRNA breakdown initiated by the application of short hairpin RNAs (shRNAs), delivered by e.g. recombinant adenoassociated viruses (rAAV). As an alternative approach, we will construct recombinants encoding enzymatically inactive Cas9 nuclease (dCas9) which can be directed to specifically bind to transcriptional start regions of the *hcn* genes, thereby interfering with their expression. The specificity and efficacy of *hcn* gene knockdown will be monitored immunologically and with quantitative molecular biological assays. Alterations of neuronal activity at the cellular level will be evaluated via electrophysiological techniques as well.

Conclusion:

The knowledge achieved by these experiments should help to expand the understanding of HCN channel function and their contribution to neuronal network activity.

Chloroform is a potent activator of cardiac and neuronal Kir3 channels

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Chloroform was one of the first volatile anesthetics discovered in the late 19th century and widely-used over decades in clinical practice. In the 20th century it was replaced by halothane and later by isoflurane and sevoflurane. Chloroform was abandoned because of inadmissible side effects like cardiac bradycardia up to cardiac arrest. Nowadays chloroform is still found in many consumer products like refrigerants and often used as solvent.

Chloroform has been identified as a potent activator of TRPV1 currents leading to an increased influx of cations and thereby to a depolarization of dorsal root ganglion (DRG) neurones. We have recently shown that TRPV1 activation by the inflammatory substance lysophosphatidic acid (LPA) is counterbalanced by the simultaneous activation of TRESK currents. We therefore asked the question whether chloroform as activator of TRPV1 currents is also able to augment TRESK currents which are also expressed in DRG neurones. When expressed in *Xenopus* oocytes ramp recordings display an outward rectifying TRESK current that is augmented 1.37 ± 0.02 -fold upon application of 6.2 mM chloroform. This demonstrates that in addition to LPA chloroform represents a second substance that is able to activate as well TRPV1 as TRESK currents.

In contrast to mice, DRG neurones from rats express G protein-activated Kir3.1/3.2 channels which, upon activation, could also counterbalance depolarizing TRPV1 currents. After expression in *Xenopus* oocytes, increasing concentrations of chloroform augmented Kir3.1/3.2 currents up to 1.99 ± 0.09 fold with an IC₅₀ of 3.37 ± 0.22 mM and a steep dose-response dependence. The exponent of a fitted Hill-equation is close to four, indicating a highly cooperative binding to the channel protein.

We next tested the chloroform effect on other Kir channels. When expressed in *Xenopus* oocytes the cardiac isoform Kir3.1/3.4 is massively augmented upon application of chloroform (2.74 ± 0.3 fold). In contrast, the inwardly rectifying potassium channel Kir2.1 is not affected by chloroform. This illustrates the selective enhancement of G protein-activated Kir3 channels by chloroform.

Under physiological conditions Kir3 channels are activated by seven-helix receptors coupled to Gi/o-proteins, e.g. the 5-HT_{1A} receptor. We therefore co-expressed Kir3.1/3.2 channels together with 5-HT_{1A} receptors and applied both agonists successively and simultaneously, respectively. Application of 0.3 mM 5-HT increased Kir3.1/3.2 currents 1.49 ± 0.1 fold, application of chloroform 2.04 ± 0.18 fold. When both substances were applied simultaneously current amplitude increased 2.55 ± 0.31 fold, identifying both agonists as perfectly additive.

In contrast to chloroform, other volatile anesthetics like halothane and isoflurane are able to inhibit Kir3.1/3.2 channels. Because sevoflurane is one of the most used anesthetics in clinical practice, we investigated the effect of sevoflurane on Kir3.1/3.2 channels. 10 mM sevoflurane inhibit current amplitude by 26% leading to a normalized remaining current of 0.74 ± 0.05 .

Upon activation Kir3 currents hyperpolarize excitable cells and might represent a good therapeutic strategy to counterbalance depolarizing, pain inducing TRP currents in peripheral neurones. Because Kir3 channels are also expressed in central neurones and heart cells they also have an impact on mood and heart diseases. Hence, Kir3 channels provide a new therapeutic target for these clinical settings.

Closing in on bimodal action of the anticonvulsant Topiramate by employing the honeybee (*Apis mellifera*)

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Topiramate (TPM) is a broad-spectrum antiepileptic drug with a unique chemical structure and a multifactorial mechanism of action. It is known to facilitate synaptic inhibition and to block excitatory receptors. Its special properties make TPM one of the most effective agents used for the treatment of epileptic seizures. Although much is known about the range of affected signal systems, less is understood about the mechanisms behind these impacts, such as binding or interaction sites and the crosstalk between the multiple action sites. The aim of this work was to use new approaches by establishing the honeybee as model organism to answer some of the still unsolved questions and to gain new insights and ideas about TPMs' modes of action. We could show that the effects of TPM on signal transmission in the nervous system of honeybees are directly comparable to those TPM effects occurring in human neurons. This fact suggests that TPM acts on cellular mechanisms and elements that are common between vertebrates and invertebrates. The basic mechanisms of action of Topiramate as bimodal agent therefore seem to be conserved. This clearly validates employing the honeybee as model organism in our context with the aim to evaluate the conserved mechanisms behind the effectiveness of Topiramate in animals.

Deletion of auxiliary Ca²⁺ channel subunit $\alpha_2\delta$ -3 specifically reduces P/Q (Ca_v2.1) but not L-type Ca²⁺ currents of spiral ganglion neurons

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Introduction: Spiral ganglion (SG) neurons connect hair cells with central auditory neurons and therefore are indispensable for auditory signal transmission. Myelinated type I SG neurons comprise 95% of all SG neurons and make a precise 1 to 1 connection with inner hair cells. Proper function and morphology of auditory nerve fiber synapses require the auxiliary Ca²⁺ channel subunit $\alpha_2\delta$ -3 (Pirone et al., J Neurosci 2014). To determine the role of $\alpha_2\delta$ -3 for SG neurons, we analyzed Ca²⁺ currents in cultured SG neurons isolated from $\alpha_2\delta$ -3^{+/+} and $\alpha_2\delta$ -3^{-/-} mice before (P5) and after the onset of hearing (P20).

Methods: Patch-clamp recordings of Ca²⁺ currents were performed on enzymatically dissociated SG neurons isolated from neonatal (P5) or juvenile (P20) mice (cf. Lv et al., J Neurosci 2012). Mice deficient with a targeted deletion of the CACNA2D3 gene coding for $\alpha_2\delta$ -3 ($\alpha_2\delta$ -3^{-/-}) generated by Deltagen (Neely et al., Cell 2010), were used. Cochleae were transversally cut in the middle, and SG neurons from the apical and basal halves of the cochlea were cultured separately for 2-3 days.

Results: Ca²⁺ currents of SG neurons were isolated by blocking voltage-gated K⁺ currents by TEA (30 mM), 4-AP (15 mM) and linopirdine (100 μ M) in the bath and by 110 mM Cs⁺ in the pipette solution. Large voltage-gated Na⁺ currents were fully suppressed by extracellular NMDG (110 mM). L-type Ca²⁺ currents were blocked by superfusing SGNs with 10 μ M nimodipine. The fraction of L-type currents amounted to 30 - 35 % of the total Ca²⁺ current in both $\alpha_2\delta$ -3^{+/+} and $\alpha_2\delta$ -3^{-/-} mice. P/Q-type Ca²⁺ currents were blocked by superfusion with 1 μ M ω -agatoxin IVA. Ca_v2.1-mediated currents were very small in SG neurons isolated from neonatal mice. However, in SGNs isolated from P20 $\alpha_2\delta$ -3^{+/+} mice, Ca_v2.1 currents contributed 50 – 55 % to the total Ca²⁺ current. Ca_v2.1 channels of P20 SG neurons had a clear preference for $\alpha_2\delta$ -3 because their Ca_v2.1-mediated current was reduced to 20-25% of the total Ca²⁺ current in $\alpha_2\delta$ -3^{-/-} mice.

Conclusion: Our data show that proper sizes of Ca_v2.1 Ca²⁺ currents in P20 SG neurons required the presence of $\alpha_2\delta$ -3, whereas expression of L-type Ca²⁺ currents was independent of $\alpha_2\delta$ -3. The role of $\alpha_2\delta$ -3 for other Ca²⁺ current subtypes and a potential compensatory up-regulation in $\alpha_2\delta$ -3^{-/-} SG neurons remains to be determined.

Enigma of rebound depolarization (RD) in the medial prefrontal cortex (mPFC) pyramidal neurons

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Background: The medial prefrontal cortex (mPFC) pyramidal neurons were characterized by periods of increased excitability following hyperpolarizing voltage steps. This property was known as rebound depolarization (RD). RD of mPFC pyramidal neurons can be involved in normal and pathological brain processes such as working memory, sleep, epileptic seizures. The aim of this study was to clarify the ionic basis of RD in mPFC layer V pyramidal neurons.

Material and methods: Experiments were performed on layer V mPFC pyramidal neurons in slices isolated from young (18-22-day-old), adolescent (38-42-day-old) and adult (60-65-day-old) male rats. Recordings of membrane potential were performed in whole-cell current-clamp configuration in the absence of Ca⁺⁺ ions and in the presence of tetrodotoxin (TTX, 0.5 µM) Glutamatergic and GABAergic blockers in extracellular solution. RD was evoked by current steps from -100 pA to +200 pA in 20 pA increments from the resting membrane (usually around -69 mV). The current steps were preceded by -300 pA current steps.

Results: RD was abolished when Na⁺ ions were replaced by equimolar concentration of choline chloride in the extracellular solution. RD was abolished in the presence of Ca⁺⁺ ions in the extracellular solution. RD was attenuated by TRP channel blockers (flufenamic acid, FFA, 200 µM; ruthenium red, RuR, 30 µM). RD was not affected by the following reagents: HCN channel blockers (ZD 7288, 50 µM; Cs⁺, 2 mM), NaLCN channel blockers (Gd⁺⁺⁺, 10 µM; Cd²⁺, 1 mM; verapamil, 1 mM), ionotropic receptors antagonists (nicotinic receptor antagonist, mecamylamine hydrochloride, 10 µM; P2X antagonist, iso-PPADS tetrasodium salt, 30 µM; 5-HT₃ antagonist, MDL 72222, 30 µM).

Conclusion: The obtained results suggest that TRP channels are responsible for Na⁺-dependent and Ca⁺⁺-dependent RD in layer V mPFC pyramidal neurons.

The study was supported by National Science Centre (Poland) grant no: 2015/17/N/NZ4/02889 and Medical University of Warsaw grant no: FW5/PM1/16.

Insertion of a glutamate (V166E) at the pore entrance provides an additional gating process for human hCIC-Ka chloride channels

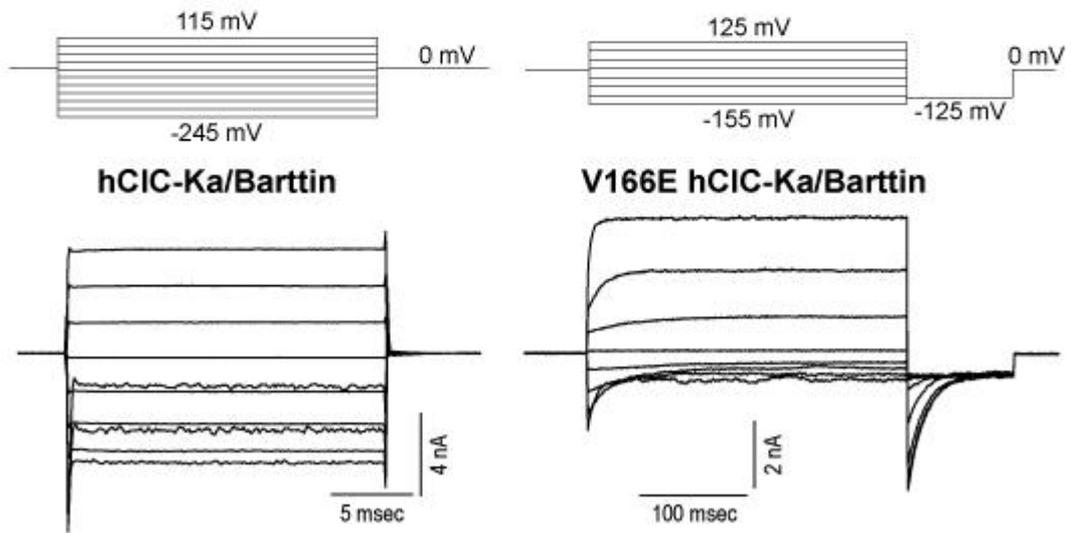
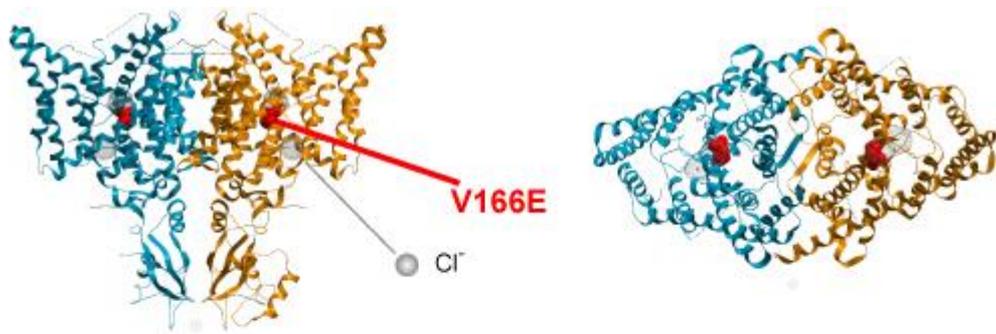
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CIC chloride channels are organized as dimers. Each subunit forms a conduction pathway (so-called protopore) that is gated individually by a fast protopore gate. Both protopores are also regulated by a common slow gate. While the mechanism of common gating is unknown, crystal structure analyses identified a conserved glutamate side chain as molecular determinant for fast protopore gating (Dutzler et al. 2003, Feng et al. 2009). All members of the CIC channel family express this gating glutamate except the renal CIC-K channels. Nevertheless, CIC-K chloride channels exhibit both, common and protopore gating. Earlier studies showed that the insertion of a gating glutamate by the substitution V166E in the rat rCIC-K1 channel does not introduce an additional gating process, but inverts voltage dependence of protopore and common gating; and also alters single channel conductance (Fischer et al. 2010).

Here, we introduced the gating glutamate (mutation V166E) into the human homolog hCIC-Ka. Confocal imaging of transfected MDCKII cells illustrates unaltered subcellular distribution of V166E hCIC-Ka/barttin complexes. Whole cell patch clamp analyses of transiently transfected HEK293T cells reveal an additional gating process for V166E hCIC-Ka. Whereas WT hCIC-Ka channels are fully opened at potentials between -100 mV and +100 mV and do not display any time-dependent current relaxation upon voltage steps, V166E hCIC-Ka is activated at positive potentials and deactivated upon hyperpolarization. Responses to voltage steps are very similar to the time-dependent current relaxations of V166E rCIC-K1. We furthermore investigated gating properties at very negative voltages below -155 mV, since WT hCIC-Ka channels are known to close on a very fast time scale upon such a strong hyperpolarization. This gating property remains unaffected by the insertion of the gating glutamate. Moreover, non-stationary noise analysis revealed an unchanged unitary conductance of 20 pS for WT and mutant V166E hCIC-Ka channels.

Our findings thus unravel that the insertion of a gating glutamate in hCIC-Ka introduces an additional voltage dependent gate, while the same mutation in rCIC-K1 inverts voltage dependence of pre-existing gates. In conclusion, protopore gating of other CIC channel subtypes might also depend on more functional determinants aside from the gating glutamate.



Lactate is a potent inhibitor of the capsaicin receptor TRPV1

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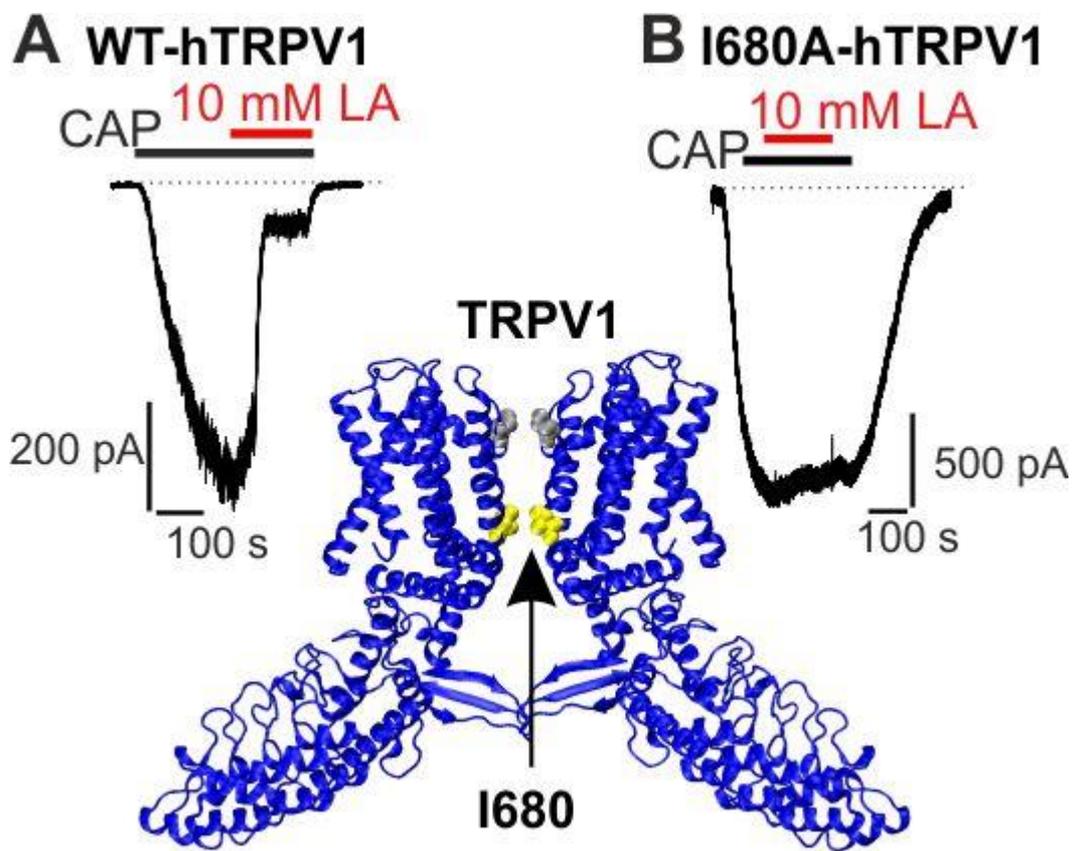
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Lactate (LA) is known to accumulate in ischemic tissue, in particular during excessive muscle work and in pain states of myocardial infarction. Sensory neurons are equipped with numerous nociceptors, of which ASIC3 and TRPA1 have been demonstrated to be sensitized and activated by LA. However, it is unclear how LA acts on TRPV1, a main receptor for acidosis in sensory neurons. A current study on rat spinal cord slices displays an increase of pain relevant neuropeptide CGRP while challenged with LA. By contrast, intracellular acidosis was shown to inhibit TRPV1. Consequently, this apparent controversial data needs further clarification, especially regarding the functional effects of LA targeting TRPV1.

Our study focused on patch clamp, calcium imaging and CGRP release experiments to unravel the LA-mediated effects on TRPV1. Membrane currents of TRPV1 evoked by capsaicin, protons, heat and proalgesic agents in HEK293T cells were clearly inhibited by physiological as well as pathophysiological concentrations of LA. Additionally, LA reduced the TRPV1-dependent Ca^{2+} influx in HEK293T cells and native DRG neurons, and inhibited CGRP release from sciatic nerves containing TRPV1. LA shifts the open probability of TRPV1 towards more positive voltages and forces the channel to its closed state at physiological potentials, which fully explains the inhibition by LA. Site directed mutagenesis revealed an effect of LA on the lower gate of TRPV1, since mutation I680A constitutively opened the lower gate and simultaneously abolished LA inhibition. Approaches on excised patches (inside-out and outside-out) and cell-attached recordings revealed that LA inhibits TRPV1 from the extracellular side and independently of intracellular acidosis.

Taken together, LA is a potent endogenous inhibitor of TRPV1 and seems to prevent the multimodal nerve terminal from further depolarization under ischemic conditions. We here show for the first time that TRPV1 distinguishes between acidosis and lactic acidosis what might be important for the regulation of pain perception during ischemic states.



Na-K-ATPase mediated neuronal adaption

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Na-K-ATPases regulate cellular homeostasis by maintaining ionic concentrations on both sides of neuronal membranes within physiological ranges. Besides this regulatory role, it has also been suggested that they contribute to neural computation, in particular to mechanisms of adaptation [1,2].

Even though evidence for such pump-related neural adaptation has been increasing, the prerequisites for the pump to actually mediate adaptation are not well understood. In particular, experimental results on pump-based adaptation seem contradictory and are complicated by the fact that experimental protocols differ and were performed at different temperatures [1, 3-5].

Here, we take a mathematical modeling approach to shed further light on the relevance of the Na-K-ATPase for neural adaptation. We implemented a conductance-based neuronal point model and coupled it with previously described dynamics of the Na-K-ATPase [Luo and Rudy, 1994], allowing intracellular and extracellular ionic concentrations to vary. Temperature dependence of the Na-K-ATPase was included in the model, in order to disentangle the temperature-induced switch of adaptation, observed by Gullidge and co-workers [2]. In principle, the net current produced by the pump can exert similar effects as other, voltage-gated adaptation currents on a cell's excitability, see also [6,7]. In this study, we aim to identify and disentangle contributions of the pump current to adaptation with a focus on adaptation to mean and/or variance of input statistics.

This work was supported by the German Federal Ministry of Education and Research (01GQ0901, 01GQ1403) and the DFG (GRK 1589/2).

[1] Arganda, S., Guantes, R., and de Polavieja, G. G. (2007). Sodium pumps adapt spike bursting to stimulus statistics. *Nature Neuroscience*, 10(11):1467–1473.

[2] Gullidge, A. T., Dasari, S., Onoue, K., Stephens, E. K., Hasse, J. M., and Avesar, D. (2013). A sodium-pump-mediated afterhyperpolarization in pyramidal neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 33(32):13025–13041.

[3] Sanchez-Vives, M. V., Nowak, L. G., and McCormick, D. A. (2000). Cellular Mechanisms of Long-Lasting Adaptation in Visual Cortical Neurons In Vitro. *The Journal of Neuroscience*, 20(11):4286–4299.

[4] Schwindt, P. C., Spain, W. J., Foehring, R. C., Chubb, M. C., and Crill, W. E. (1988). Slow conductances in neurons from cat sensorimotor cortex in vitro and their role in slow excitability changes. *Journal of Neurophysiology*, 59(2):450–467.

[5] Schwindt, P. C., Spain, W. J., and Crill, W. E. (1989). Long-lasting reduction of excitability by a sodium-dependent potassium current in cat neocortical neurons. *Journal of Neurophysiology*, 61(2):233–244.

[6] Wang, Y.-C., Yang, J.-J., and Huang, R.-C. (2012). Intracellular Na(+) and metabolic modulation of Na/K pump and excitability in the rat suprachiasmatic nucleus neurons. *Journal of Neurophysiology*, 108(7):2024–2032.

[7] McCarren, M. and Alger, B. E. (1987). Sodium-potassium pump inhibitors increase neuronal excitability in the rat hippocampal slice: role of a Ca²⁺-dependent conductance. *Journal of Neurophysiology*, 57(2):496–509.

Novel chemical and molecular tools for the identification of RNA editing-competent neurons and RNA-edited glycine receptor (GlyR) proteins

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C-to-U RNA editing of glycine receptors can play an important role in disease progression of epilepsy as it may contribute to neuropsychiatric symptoms of the disease. However, so far only bulk material from patients was used for the detection of RNA-edited GlyR-coding mRNA. We present agonist and antagonists that are specific for RNA-edited GlyRs and reveal for the first time neuronal endogenous expression of these pathogenic proteins. Furthermore, a novel fluorescence-based C-to-U RNA editing sensor will be presented that indicates RNA editing by nuclear translocation of mCherry protein. Our system may be useful not only for the identification of RNA editing-competent neurons and underlying regulatory mechanisms, which so far has remained a challenging open question in neuroscience, but also for the identification of cell types that are afflicted with increased C-to-U RNA editing of GlyR-coding mRNA and corresponding protein expression in epilepsy.

Novel forced intercalation probes for the detection of glycine receptor (GlyR) RNA editing at the single cell level

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C-to-U RNA editing of glycine receptors can play an important role in disease progression of epilepsy as it may contribute to neuropsychiatric symptoms of the disease. However, so far only bulk material from patients was used for the detection of RNA-edited GlyR-coding mRNA. We present new Forced Intercalation (FIT) probes that are able to detect the single nucleotide exchange in GlyR-coding mRNA at the single cell level by strong enhancement of fluorescence upon hybridization only with fully complementary RNA targets. These probes may be useful for the identification of RNA editing-competent neurons in epilepsy.

Potassium chloride co-transporter 2 expression is upregulated by potassium chloride and ampakine in chicken auditory brainstem *in vitro*

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-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the mature central nervous system. The reversal potential for chloride determines, whether a cell depolarizes or hyperpolarizes after opening of a GABA-A-receptor channel. Important regulators of the chloride homeostasis are the transporters of the SLC12 family. In the vertebrate brain, the inward directed sodium-potassium-chloride-cotransporter 1 (NKCC1) and the outward directed potassium-chloride-cotransporter 2 (KCC2) are the best analysed members of this family.

Neurons of the *Nucleus laminaris* (NL) and the *Nucleus magno-cellularis* (NM) receive GABAergic input from the *Nucleus olivaris superior* (SON). The physiological relevance of this input is to increase the accuracy of sound localization. Our previous studies *in vivo* showed an expression of NKCC1 and KCC2 in neurons of NM and NL. The temporal profiles of KCC2 expression suggested a correlation with synaptic activity.

To analyze the regulation of KCC2 expression, organotypic roller-tube cultures (Gähwiler, 1981) were prepared. Auditory brainstem was explanted at embryonic day 10 and harvested after 7 days *in vitro*. During the time *in vitro* either 25 mM potassium chloride or 300µM CX546 (ampakine) were added to the culture media to enhance synaptic activity. Quantitative real-time PCR and SDS PAGE and Western blot with chemiluminescent immunodetection were performed to analyze the expression of KCC2 and actin or GAPDH. Expression of KCC2 was normalized to actin or GAPDH expression.

Our data show an increase of KCC2 expression both under KCL and CX546. The mRNA expression increases in both cases 3.8-fold compared to control. Under CX546 the protein expression also increases 3.8-fold, but under KCL only 1.6-fold.

We conclude from our data, that an activity-dependent component exists in the regulation of chloride transporter expression.

Probing the channel gating of a glutamate receptor with photoactive unnatural amino acids

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Ionotropic glutamate receptors (iGluRs) are responsible for fast synaptic transmission throughout the nervous system. Despite considerable study, the conformational change of the transmembrane domain (TMD) underlying ion channel activation remains unclear. Here, we aim to explore the function and dynamics of the transmembrane region of AMPA-type glutamate receptors using unnatural amino acid (UAA) photo-cross-linkers, p-benzoyl-L-phenylalanine (BzF) and p-azido-L-phenylalanine (AzF). Using mammalian cells as expression system, AzF and BzF were individually introduced throughout the TMD of the AMPA receptor GluA2 by genetically-encoded UAA mutagenesis. Outside-out patch clamp recording of receptors activated by a fast-perfusion system was combined with synchronized exposures to UV light via epi-illumination, to characterize the functionality of the AzF and BzF containing iGluR constructs, as well as their individual photo-controllable profiles. AzF and BzF were individually inserted in 23 sites throughout the TMD. Glutamate induced currents could be measured from 18 constructs containing AzF at different sites, while only 10 constructs containing BzF resulted in a glutamate-activated current. The glutamate activated currents of mutant receptors had similar characteristics to wild type channels. Exposing channels harboring AzF to UV light had a range of effects, from inhibition to potentiation, dependent on the insertion site. Exposure of channels incorporating BzF in the TMD to UV light did not have any effect on currents. In contrast, photoactivation of BzF in the Pre-M1 linker was found to increase peak current by up to 50 %. Our results demonstrate that UAAs can be incorporated site specifically in the TMD and can trap key moving parts of the ion channel.

Probing the function of $\alpha_2\delta$ calcium channel subunits in the genetic model system *Drosophila melanogaster*

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Voltage-gated calcium channels (VGCC) are essential for normal brain function. Ca^{2+} influx through VGCCs functions as charge carrier in neuronal information processing and as ubiquitous intracellular messenger. The pore-forming proteins of VGCCs are the α_1 subunits and they comprise three different subclasses (CaV1-3). CaV1 and CaV2 channels act together with auxiliary α_2 , β , and γ subunits, which significantly increases the functional diversity of VGCCs. This study analyzes the function of α_2 subunits. In vertebrates and invertebrates there are four different α_2 genes (α_2 1-4), which are thought to modify the biophysical properties, localization and stabilization of α_1 . Functional defects of α_2 cause a number of neuronal disorders. However, at current it is largely unknown what the different functions of the numerous α_2 - α_1 combination are, whether each combination serves a different function, or whether bifunctional redundancy exists between a subset of the α_2 - α_1 combinations.

I address this question by employing a combination of molecular biological, neuroanatomical, electro- and optophysiological methods in the genetic model system *Drosophila melanogaster*.

Our data indicate that different α_2 regulate distinct biophysical properties of CaV2 channels in different compartments of neurons. In larval *Drosophila* motoneurons α_2 3 has been reported essential for neuromuscular synaptic function and development. Accordingly, RNAi knock down of α_2 3 impairs CaV2 channel function in the axon terminal and thus neuromuscular transmission in adult flight motoneurons. By contrast, our data indicate that α_2 3 has no effect on somatodendritic CaV2 channels. This indicates that one CaV2 channel interacts with different α_2 subunits to fulfill distinctly different functions in different compartments of the same neurons. Similarly, preliminary data indicate that different α_2 subunits may guide CaV1 channels to different compartments of larval *Drosophila* motoneurons. To assess the localization of all α_2 subunits in different compartments of different types of neurons we now generate fly strains in which these proteins are endogenously tagged with GFP by employing the "MiMIC protein trap" technique. We expect to unravel the functions of different α_2 - α_1 combinations, that these can be generalized between different types of *Drosophila* neurons and also hold in vertebrates. This knowledge will allow us to selectively test the functions of somatic, dendritic, and axonal CaV1 and CaV2 channels by targeted knock-down of the respective partner α_2 subunits, which is not possible with pharmacological tools or genetic manipulation of the α_1 subunits.

Proton-dependent modulation of mouse HCN channels

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels belong to the superfamily of voltage-gated pore loop channels. The corresponding hyperpolarization-activated cation currents (I_h) have been described in a variety of cardiac cells and neurons. The four mammalian HCN channel isoforms (HCN1 – HCN4) serve diverse neuronal functions, from rhythmic oscillatory activity and generation of intrinsic resonance to dendritic integration and synaptic plasticity. HCN channels are tetrameric complexes consisting of either homomeric or heteromeric subunit assemblies. Depending on subunit composition, the channels are modulated by cyclic nucleotides as well as both intra- and extracellular proton concentrations.

Protons serve a variety of important functions in cells. Under certain pathological conditions, acid-base balance is disrupted. For example, acidosis occurs commonly in a variety of neurological disorders and is a main contributing factor to neural injury. Here, we analyze the influence of changing extracellular pH values on HCN channel function. First, we investigate the basic biophysical properties of each homomeric HCN channel recombinantly expressed in HEK293T cells. In electrophysiological recordings, HCN channel expressing cells are identified using a GFP tag. Next, we study alterations of biophysical channel properties upon variation of extracellular pH, ranging from sour to basic conditions. Finally, in ongoing experiments, we aim to mutate potential pH-sensing domains based on computational models of protein interactions with extracellular protons and analyze the structure-function relationship.

This work was supported by the Deutsche Forschungsgemeinschaft SPP 1392: "Integrative Analysis of Olfaction"

Role of the presynaptic scaffolding proteins Bassoon and Piccolo in the regulation of voltage-gated calcium channels at the release sites and of synaptic vesicles cycling within the presynapse

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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 CAZ define the release sites, localize voltage-dependent calcium channels (VDCC) 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. Our recent study demonstrated the importance of Bsn as a specific factor that localizes CaV2.1 to active zones via molecular interaction with the Rim-binding proteins (RBPs). The role of Pclo in the recruitment and localization of VDCC is still unclear. By biochemical analysis we have identified RBP2 as a binding partner of Pclo and demonstrated that this two proteins can be potentially linked via this interaction. We hypothesized that Pclo acts in analogy to its paralogue Bsn and regulates channel recruitment to the presynaptic release sites. We investigated the function of both presynaptic scaffolding proteins by measurement of the presynaptic calcium influx, analysis of channel expression levels, assessment of the synaptic vesicle (SV) recycling and the size of the presynaptic vesicle pools at levels of single synapses in mouse hippocampal neurons. Unlike Bsn deletion of Pclo did not affect either the expression levels or the function of the VDCC. However deletion of both large scaffolding proteins led to changes in the SV cycle. Taken together, our study dissects the redundant and overlapping functions of the two highly related proteins of CAZ, Bsn and Pclo, and highlight their importance for the correct functioning of the presynapse.

Strategies to Stably Record Calcium Currents in Substantia nigra Dopaminergic Neurons

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The midbrain dopaminergic (DA) neurons encompass neurons in the retrorubral area, the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNpc). Morphologically, these neurons are associated with two systems: the nigrostriatal pathway, which is mainly build by projections of the SNpc neurons and the mesocorticolimbic system, which arises primarily from the VTA neurons. Historically, the nigrostriatal pathway has been associated with the modulation of motor functions, while the mesocorticolimbic system was thought to be involved in motivation, reinforcement and reward-seeking behaviors. However, it becomes increasingly clear that SNpc and VTA neurons have overlapping projections and that all three DA neuron subpopulation contribute to reward-related behaviors.

DA neurons are autonomous pacemakers: They generate action potentials with a constant, very regular frequency, even if synaptic inputs are pharmacologically blocked. *In vivo*, this pacemaker activity can be modulated by synaptic input leading to a burst firing pattern and a hyperpolarized, non-firing state. The resulting firing patterns determine the DA release in the projection areas and are correlated with the prediction and detection of rewards.

In SNpc neurons, the autonomous pacemaking depends critically on the precise control of intracellular calcium dynamics. The calcium influx that is necessary to mediate pacemaking is thought to be generated by orchestrated activation of several voltage-activated calcium channels with specific functional properties. Especially L-type calcium channels are thought to mediate a substantial part of the calcium influx present during the pacemaker activity, because these channels are known to activate at sub-threshold membrane potentials. However, direct, quantitative and stable measurements of the voltage-activated calcium currents have been challenging due to 1) imperfect voltage control due to the complex morphology of SNpc neurons, 2) the presence of large outward current and 3) a rapid wash-out of the calcium currents. While the mean peak amplitudes typically reached -2.0 ± 0.2 nA at the beginning of whole-cell patch clamp recordings in acute adult brain slice preparations, these calcium currents ran down within several minutes. Therefore, we aim to improve the recordings by optimizing the extra- and intracellular solution used for whole-cell measurements, and/or by combining several approaches including perforated patch clamp recordings and discontinuous single electrode voltage clamp.

The contribution of the two binding sites to the opening of the adult nicotinic acetylcholine receptor

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The muscle nicotinic acetylcholine receptor (nAChR) is one of the most thoroughly studied ligand gated ion channels. Channel opening is triggered by binding of one or two agonists. We have previously shown by using high resolution single channel recordings that embryonic mouse nAChRs produce three classes of openings. The short and the intermediate openings originate from receptor with only one binding site, or , occupied while longer openings occurred in bursts generated by receptor with both binding sites occupied (Hallermann et. al., 2005). When site is blocked by the specific blocker α -Conotoxin-M1 (CTx), only intermediate openings remain, suggesting that they were produced by receptor with agonist bound to site (Stock et. al., 2014).

In the present work, we applied acetylcholine (ACh) and epibatidine (Ebd) to the adult mouse muscle nAChR, in which binding site is exchanged for binding site. Interestingly, high resolution single channel recordings revealed four different open time classes in the adult receptor upon ACh application, 1: 3 μ s, 2: 40 μ s, 3: 180 μ s and 4: 800 μ s. Ebd recordings showed very similar open time distributions. At 10 nM ACh concentration, mainly 2 and 3 openings were observed, while at 100 nM all four classes were seen. At higher concentrations 1 and 4 openings dominated the open time distribution. After blocking with CTx, 2 openings remained suggesting that they were generated by mono-liganded receptor with the non-blocked . It is possible that 3 openings originated from receptor with occupied site and 4 openings stem from the di-liganded receptor. The interpretation of the 1 openings is not certain yet, since the concentration dependence of these openings is less clear compared to other open time classes.

We fitted a kinetic mechanism by maximising likelihood of the entire sequence of open and shut time intervals (Colquhoun et. al., 2003). In order to get good fit for adult mouse nAChR single channel currents in presence of CTx it was essential to include a single binding step, pre-open shut state and a single open state. The maximum likelihood fit allowed us to estimate affinity of isolated binding site and efficacy of mono-liganded openings.

Colquhoun D, Hatton CJ & Hawkes AG. (2003). The quality of maximum likelihood estimates of ion channel rate constants. *J Physiol* 547, 699-728.

Hallermann S, Heckmann S, Dudel J & Heckmann M (2005). Short openings in high resolution single channel recordings of mouse nicotinic receptors. *J Physiol* 563, 645-662.

Stock P, Ljaschenko D, Heckmann M & Dudel J (2014). Agonists binding nicotinic receptors elicit specific channel-opening patterns at α - γ and α - δ sites. *J Physiol* 592, 2501-2517.

Subcellular organization of presynaptic Ca²⁺ channels at hippocampal mossy fiber synapses

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By connecting the gyrus dentatus with the hippocampus proper, the axons of granule cells, the mossy fibers (MFs), represent an important element of the main excitatory, trisynaptic pathway of the hippocampal proper. The axons form anatomically specialized synapses depending on the nature of their postsynaptic targets. Large MF boutons innervate thorny excrescences of CA3 pyramidal cells (PCs), dendrites of basket cells, and hilar mossy cells, whereas their small filopodial extensions contact with dendrites of GABAergic interneurons. The highly specialized function of MF synaptic specializations critically depends on the fine control of transmitter release that requires a well-regulated local Ca²⁺ signaling initiated by the opening of high voltage-activated Ca²⁺ (Ca_v) channels.

Here, we used high-resolution SDS-digested freeze-fracture replica immunoelectron microscopy together with smoothed distance transform analysis of Ca_v2.1 (P/Q-type) and Ca_v2.2 (N-type) immunogold labeling to determine the ultrastructural organization and distribution patterns of the two types of Ca_v channels at MF synapses.

Detailed analysis showed that (i) the Ca_v2.1 as well as the Ca_v2.2 Ca²⁺ channels in MF terminals are organized in clusters, (ii) the active zones (AZs) of MFs showed a higher density of Ca_v2.1 channels than that of Ca_v2.2, (iii) the clusters of Ca_v2.1 are scattered over the AZs of MFs, (iv) clusters of Ca_v2.2 distributed over the AZs of MF-interneuron dendrite synapses but they preferentially localized to the periphery of presynaptic membrane specializations of MF-PC spine synapses. Our data demonstrate similar nano-architecture but distinct density and distribution patterns of Ca_v2.1 and Ca_v2.2 channels at MF synapses.

TGF- β regulates NBCe1 expression and activity in mouse cortical astrocytes.

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pH is essential for cellular function and survival, and its homeostasis is mainly regulated by H⁺ and HCO₃⁻ concentrations. Thereby, the solute carrier (SLC) 4 protein family plays a crucial role. Cortical astrocytes express high levels of SLC4A4, the electrogenic Na⁺/HCO₃⁻ cotransporter (NBCe1). Depending on HCO₃⁻ gradient, NBCe1 may transport HCO₃⁻ in both directions. We have previously shown that the expression and activity of the NBCe1 are significantly up-regulated after blocking K⁺ channels (4AP treatment) in mouse cortical astrocytes (Schrödl-Häußel et al., 2015). Another Slc family member, the SLC12A5, requires TGF- β for its trafficking and activity (Roussa et al., 2016). We have hypothesized that TGF- β regulates NBCe1 expression and activity in mouse cortical astrocytes as well. Cortical astrocytes from mouse postnatal day 2 are treated at *in vitro* day 21 with 100 μ M 4AP for 20 minutes in the presence or absence of 10 μ M SB431542 (TGFBR1 blocker) or with 2 ng/ml TGF- β with or without 3 μ M SIS3 (Smad3 blocker) and 10 μ M SP600125 (JNK inhibitor). NBCe1 protein is examined by immunoblotting, and the extracellular active TGF- β ; is measured by MLEC assay. Slc4a4 gene expression is studied by quantitative real-time PCR, and direct interaction of TGF- β with slc4a4 is detected by chromatin immunoprecipitation. NBCe1 activity is analyzed by recording intracellular H⁺ concentrations using the dual-excitation ratiometric pH indicator, BCECF AM. The results show that treatment of astrocytes with 2ng/ml TGF- β for 30 minutes significantly upregulates Slc4a4 gene expression. Activation of TGF- β signaling also upregulates NBCe1 protein, an effect that is diminished in the presence of either SP600125 or SIS3 for 30 minutes. Similarly, treatment of astrocytes with TGF- β increased NBCe1 activity, as shown by the significant increase in the rate of alkalinization and acidification. Again, this effect was not observed after blocking JNK pathway or TGF- β canonical pathway. Moreover, 4AP-dependent up-regulation of NBCe1 protein significantly decreased after pre-incubation of astrocytes with 10 μ M SB431542 for 30 minutes, compared to astrocytes treated only with 100 μ M 4AP for 20 minutes. 4-AP-dependent NBCe1 activity, reflected by increased in rate of alkalinization and acidification was also significantly reduced after perfusion of astrocytes with 10 μ M SB431542, compared to 4AP treated astrocytes. Interestingly, extracellular active TGF- β increased in the presence of 100 μ M 4AP for 20 minutes. TGF- β ; effect on Slc4a4 transcription is a direct one, as shown by increased smad3 binding to the Slc4a4 promoter following TGF- β treatment. We conclude that 4AP-dependent upregulation of NBCe1 protein and activity requires TGF- β . TGF- β directly regulates NBCe1 transcription and activity via the canonical pathway.

The Alzheimer's protease BACE1 as a subunit and non-enzymatic regulator of neuronal and cardiac KCNQ channels

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Beta-site APP-cleaving enzyme 1 (BACE1) is the rate-limiting enzyme in the generation of Aβ, a central player in the pathogenesis of Alzheimer's disease and the main component of amyloid plaques in patients' brains. Although BACE1 is a promising therapeutic target, most of the physiological functions of this ubiquitously expressed protein are still elusive.

We have previously reported that BACE1, but not BACE2, acts as a multifaceted modulator of neuronal and cardiac KCNQ channels. In the brain, BACE1 is an important regulator of the M-current (KCNQ2/3), which controls neuronal excitability. Reduced M-current likely explains the epileptic phenotype of BACE1-deficient mice. In cardiac myocytes, KCNQ1 assembles with KCNE1 subunits to generate a delayed rectifier K⁺ current, which contributes to action potential repolarization. Notably, in both systems, BACE1 acts independent of its proteolytic function by directly binding to the channels.

To determine how KCNQ forms channel complexes with BACE1, we established a number of optical interaction assays and chimera approaches. Analysis of BACE1/KCNQ at the plasma membrane by fluorescence recovery after photobleaching (FRAP) revealed their physical assembly. Bimolecular fluorescence complementation (BiFC) confirmed BACE1/KCNQ complexes. For further investigation of the interaction, we chose KCNQ1 as a model system. In terms of molecular regulation, KCNQ1 is the best characterized member of the KCNQ family and has been analyzed in complex with its known subunit KCNE1, which shares structural elements with BACE1. BiFC competition experiments on BACE1 or KCNE1 binding to KCNQ1 and KCNQ1 complex formation with BACE1-BACE2 chimera revealed their putative interaction domains. BACE/KCNQ stoichiometry was interrogated with two independent experimental approaches. First, we performed electrophysiological recordings of fusion constructs with 1 BACE1 and 1/2/4 KCNQ1 subunits. Second, we applied single molecule imaging in total internal reflection fluorescence (TIRF) microscopy.

These complementary electrophysiological and optical approaches are expected to unravel the intricacies of the non-proteolytic interactions between BACE1 and KCNQ1/KCNE1 channels.

Trpc5 cation channels contribute to hormone regulation in the hypothalamus

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We have begun to analyze the function of transient receptor potential (Trp) channels in the mammalian central nervous system, in particular the role of the Trp channel Trpc5 in hypothalamic hormone regulation. Hypothalamic dopamine levels in mice decrease during early pregnancy to maintain an increased prolactin level. Prolactin sustains the corpus luteum to produce progesterone and thus prepares the uterine endometrium for implantation (nidation). Prolactin secretion is predominantly regulated by dopaminergic neurons located in the arcuate nucleus of the hypothalamus. Excitatory responses of these neurons are proposed to depend on canonical transient receptor potential (Trpc) channels. We detected Trpc5 channel expression in these cells and reasoned that this channel could mediate prolactin regulation. We thus analyzed a novel strain of Trpc5-deficient mice and observed an elevated occurrence of infertility in these animals. Female Trpc5-deficient mice show a strong imbalance of multiple hormones. Specifically, they exhibit reduced prolactin levels (hypoprolactinemia), which results in an enhanced rate of infertility. Trpc5-deficient males also show decreased prolactin levels, together with a diminished sperm motility. We are currently performing recordings in dopaminergic neurons of the arcuate nucleus to understand the cellular and molecular mechanisms underlying this effect. These experiments should enable us to discern the role of Trpc5 in the regulation of prolactin levels of the body, and they could provide a genetic basis for understanding hypoprolactinemia in humans.

Supported by Deutsche Forschungsgemeinschaft grants SFB-Transregio 152, SFB 894, Schwerpunktprogramm 1392 and the Volkswagen Foundation.

Variable ion channel expression in identified single neurons - homeostatic plasticity or genetic variation?

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The activity of individual neurons is achieved through the combined action of ion channels in the cell membrane. However, ion channel conductance levels vary substantially between animals of the same species, and between neurons of the same type (Schulz et al. 2006 Nat Neurosci 9:359-362; Golowasch et al. 1999 J Neurosci 19:RC33-1; Golowasch et al. 2002 J Neurophys 87:1129-31; Liss et al. 2001 EMBO J 20:5715-24). This is despite the fact that neural network performance is virtually identical. One predication from these studies is that this variability is an important factor in maintaining robust neural function in evolution and within the life span of an animal. While all neurons have mechanisms that homeostatically regulate intrinsic excitability and responses to synaptic input, it is unclear whether differences between animals are due to genetic variability between individuals or to experience-dependent homeostatic plasticity. Addressing this question has been challenging in existing animal models because of insufficient knowledge about neural circuit connectivity, weak access to cellular and circuit dynamics, or the availability of genetic tools. We are studying conductance level variability using single-cell electrophysiology and molecular tools in identified neurons of the marbled crayfish, *Procambarus virginalis*. Marbled crayfish are an all-female species with genetically homogenous animals that reproduce parthenogenetically (summary: Vogt 2010 Biogerontology 11:643-69). To determine conductance diversity, we are measuring ion conductance levels in the same identified neurons of the stomatogastric ganglion and compare them across animals. In addition, we are measuring ion channel expression levels using single-cell qRT-PCR to confirm the results of our electrophysiological measurements. For comparison, we carry out similar experiments in the crab, *Cancer borealis*. These animals are wild-caught and have previously been shown to possess large variability of ion channel conductances levels and correlated mRNA levels (Schulz et al. 2006 Nat Neurosci 9:359-362; Schulz et al. 2007 PNAS 104:13187-91). We hypothesize that the variability between identified neurons is reduced in marbled crayfish as a result of their genetic homogeneity. To test this, we raise animals under identical environmental conditions, minimizing differences in life-history, before conductance and expression levels are compared. An additional test group consists of a genetically non-homogenous species of crayfish (*Procambarus clarkii*) that are kept at similar environmental conditions. We are also considering the possibility to manipulate gene expression using transgenic animals by introducing genetic constructs into the germ line of marbled crayfish.

Vasoactivity of heme degradation products (HDPs) on cerebral arterioles *in vivo* and *in vitro*

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Delayed vasospasm of cerebral arterioles and arteries are supposed to be the most common cause of morbidity and unfavorable prognosis in patients who initially survived subarachnoid hemorrhage (SAH). According to the recent guidelines concerning vasospasm treatment, therapeutic options are restricted to the application of the calcium channel antagonist nimodipine or hemodynamic interventions without improving clinical outcome. A growing body of evidence has demonstrated that heme and bilirubin oxidation end products (BOXes), originating from degraded hemoglobin around ruptured aneurysms, are involved in inhibiting large conductance BK_{Ca} potassium channels in vascular smooth muscle cells, whereby blocked BK_{Ca} channels fail to hyperpolarize cell membrane and increase the myogenic tone. Using two-photon laser scanning microscopy of the brain vasculature in mouse visual cortex *in vivo*, we demonstrate that BK_{Ca} channel inhibitors as well as synthetic isomers of BOXes cause a long-lasting diameter decrease of pial arterioles in living mice. This vasoconstrictive effect depends on BK_{Ca} channel activity, because it was absent in mice, expressing dysfunctional BK_{Ca} channels through a conventional knockout of the Slo1 gene. Further investigations on acute brain slices suggest that recently isolated intermediates of oxidative bilirubin degradation, also known as propentdyopents (PDPs), are also able to induce arteriolar vasoconstriction. In conclusion, our study confirms the contribution of heme degradation products to impaired vascular diameter regulation after SAH and highlights the importance of early cerebral microvessel vasospasm.

Poster Topic

T7: Synaptic Transmission, Pre- and Postsynaptic organization

- [T7-1A](#) Characterizing synaptic sound encoding in near physiological conditions
Lina Jaime, Chao-Hua Huang, Tobias Moser
- [T7-2A](#) Complexin 1 regulates synaptic vesicle release at glycinergic synapses in the mammalian auditory brainstem
Francisco José López-Murcia, Kerstin Reim, Nils Brose, Holger Taschenberger
- [T7-3A](#) Contribution of somatostatin interneurons to network activity in the developing hippocampus *in vitro*
Tom Floßmann, Knut Kirmse, Otto W Witte, Knut Holthoff
- [T7-4A](#) Developmental changes in the vesicular content at an inhibitory synapse in the cochlear nucleus
Jana Nerlich, Rudolf Rübsamen, Ivan Milenkovic
- [T7-5A](#) Differential distribution of synaptosomal associated protein 47 kDa isoform (SNAP47) in the mouse and rat hippocampus.
Agnieszka Muenster-Wandowski, Jie Song, Heike Heilmann, Imre Vida
- [T7-6A](#) Distinct functions of Piccolo and Bassoon at the calyx of Held
Christoph Koerber, Thomas Kuner, Daniel Parthier
- [T7-7A](#) Distinct roles of auxiliary α_2 subunits of voltage-gated calcium channels in excitatory/inhibitory neurotransmission and network activity.
Anna Maria Ciuraszkiewicz, Arthur Bikbaev, Jennifer Heck, Romy Schneider, Martin Heine
- [T7-8A](#) Foundations of high-fidelity synaptic transmission at intra-cortical synapses between layer 5 pyramidal neurons
Hartmut Schmidt, Grit Bornschein, Jens Eilers, Hartmut Schmidt
- [T7-9A](#) Functional role of Bassoon at mouse cone photoreceptor ribbon synapses
Norbert Babai, Johann Helmut Brandstätter, Andreas Feigenspan
- [T7-10A](#) Functional spine analysis with Activity-based Automatic Region of interest Generation (AARG)
Charlie Jonathan Gilbride, Camin Dean
- [T7-11A](#) Glutamate dynamics in the cleft of Schaffer collateral synapses
Christian Schulze, Céline D. Dürst, J. Simon Wiegert, Thomas G. Oertner

- [T7-1B](#) Hippocampal output to the medial entorhinal cortex: functional monosynaptic projections to layer Va neurons
Franziska S. Lorenz, Andreas Draguhn, Alexei V. Egorov
- [T7-2B](#) Imaging glutamate release at individual Schaffer collateral synapses
Céline D. Dürst, Christian Schulze, J. Simon Wiegert, Thomas G. Oertner
- [T7-3B](#) Impaired sound encoding in PSD-95 knockout mice
Gulnara Yamanbaeva, Sang Yong Jung, Man Ho Wong, Nicola Strenzke
- [T7-4B](#) *In vivo* STED imaging of PDS-95 and Gephyrin in the visual cortex of the living mouse
Waja Wegner, Heinz Steffens, Katrin I. Willig
- [T7-5B](#) *In vivo* time lapse imaging of axonal dense core vesicle trafficking in anaesthetized and awake mice
Johannes Knabbe, Joris Nassal, Heinz Horstmann, Matthijs Verhage, Thomas Kuner
- [T7-6B](#) Interaction of Piccolino and RIBEYE at the photoreceptor ribbon synapse
Tanja Müller, Kaspar Gierke, Johann Helmut Brandstätter, Hanna Regus-Leidig
- [T7-7B](#) Intracellular sodium loading under ischemic conditions *in situ* and *in vivo*
Niklas Jonny Gerkau, Cordula Rakers, Gabor C. Petzold, Christine Rosemarie Rose
- [T7-8B](#) Multivariate Analysis of Synaptic Parameters at the *Drosophila* Neuromuscular Junction
Sebastian Sydlik, Martin Müller
- [T7-9B](#) Nanoscopic Organization of Bassoon and Piccolo at the Active Zone of the Calyx of Held
Maja Klevanski, Frank Herrmannsdörfer, Thomas Kuner
- [T7-10B](#) Nrg3 is a major Bace1 substrate and controls synaptogenesis.
Thomas Müller, Maria Sheean, Birgit Voigt, Stephanie Braud, Rene Jüttner, James Poulet, Jörg Geiger, Carmen Birchmeier
- [T7-11B](#) Orientation and organization of Bassoon: from the Golgi to the synapse
Tina Ghelani, Fabian Göttfert, Rene Ebrecht, Fred Wouters, Thomas Dresbach
- [T7-12B](#) Tight distribution of synaptic vesicle release sites generated by Unc13A limits and synchronizes neurotransmission
Alexander Matthias Walter, Suneel Reddy-Alla, Mathias Böhme, Eric Reynolds, Christina Beis, Malou Mampell, Ulises Rey, Tanja Matkovic, Meida Jusyte, Husam Babikir, Fabian Göttfert, Stephan Hell, Stephan Sigrist
- [T7-1C](#) Periodic F-actin structures shape the neck of dendritic spines
Julia Bär, Oliver Kobler, Bas van Bommel, Anja Konietzny, Marina Mikhaylova
- [T7-2C](#) Persistent sodium current modulates distal axonal excitability in CA1 neurons
Peter Müller, Andreas Draguhn, Alexei V. Egorov
- [T7-3C](#) Presynaptic mitochondrial calcium release enhances short-term facilitation during brief high-

frequency stimulation
Che Ho Yang, Won-Kyoung Ho, Suk-Ho Lee

[T7-4C](#) Regulation of activity-dependent compensatory endocytosis at central synapses by N-cadherin
Sushma Dagar, Bernd Van Stegen, Rebekka Ochs, Hermann Aberle, Kurt Gottmann

[T7-5C](#) Role of Bassoon in the regulation of synaptic vesicle pool size
Carolina Aida Montenegro Venegas, Eneko Pina, Maria Andres-Alonso, Eckart Gundelfinger, Anna Fejtova

[T7-6C](#) Role of the TrkB receptor kinase domain in ligand-independent TrkB transactivation
Rohini Gupta

[T7-7C](#) Separate pathways for high and low frequency signals in the cerebellar cortex
Isabelle Straub, Laurens Witter, Miriam Hoidis, Abdelmoneim Eshra, Niklas Byczkowicz, Igor Delvendahl, Kevin Dorgnas, Elise Savier, Ingo Bechmann, Martin Krüger, Philippe Isope, Stefan Hallermann

[T7-8C](#) Single action potential evoked disynaptic inhibition in vivo
Jean-Sebastien Jouhanneau, Jens Kremkow, James F. A. Poulet

[T7-9C](#) Synaptic performance of lemniscal input fibers onto inferior colliculus neurons
Sina Brill, Eckhard Friauf

[T7-10C](#) Synaptic translation of neuroligin 1, 2 and 3
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Characterizing synaptic sound encoding in near physiological conditions

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Inner hair cells (IHCs) are responsible for transducing mechanical sound waves into electrical signals in the auditory system. Sound encoding relies on the synapses between IHCs and the postsynaptic boutons of afferent spiral ganglion neurons. Upon stimulation, the receptor potential triggers the opening of voltage-gated calcium 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).

The Ca²⁺ nanodomain hypothesis of exocytosis control proposes that only few Ca²⁺ channels that are positioned in nanometer proximity from the vesicular release site govern the Ca²⁺ concentration driving a synaptic vesicle's release (Moser, et al. 2006). To test whether this hypothesis holds for physiological sound encoding, we performed paired patch clamp experiments in near physiological conditions on murine IHC ribbon synapses after the onset of hearing. We altered presynaptic Ca²⁺ influx by changing the number of open Ca²⁺ channels or single channel currents and analyzed the change in the evoked excitatory post-synaptic currents.

References

Moser, T., & Vogl, C. (2016). New insights into cochlear sound encoding. *F1000Research*, 5, F1000 Faculty Rev-2081. <http://doi.org/10.12688/f1000research.8924.1>

Moser, T., Neef, A., & Khimich, D. (2006). Mechanisms underlying the temporal precision of sound coding at the inner hair cell ribbon synapse. *The Journal of Physiology*, 576(Pt 1), 55–62. <http://doi.org/10.1113/jphysiol.2006.114835>

Complexin 1 regulates synaptic vesicle release at glycinergic synapses in the mammalian auditory brainstem

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Auditory information processing relies on sustained and temporally precise high-frequency synaptic transmission in order to preserve the exact timing of sound input. Complexins (Cplx) – small presynaptic proteins that bind with high affinity to assembled SNARE complexes – have previously been implicated in the regulation of synaptic strength and the timing of synaptic vesicle (SV) release at two large glutamatergic calyceal auditory brainstem synapses, the endbulbs and the calyces of Held. However, the role of Cplx at inhibitory synapses *in-situ* has hardly been studied.

We therefore studied the role of Cplx at glycinergic MNTB-->LSO synapses, which presynaptically only contain Cplx1, by comparing spontaneous and evoked synaptic transmission in Cplx1 knock-out (KO) mice and wild-type (WT) littermates.

Analyses of acute brainstem slices obtained from mice at postnatal days 13-16, showed that Cplx1 KO reduces the amplitudes of action potential (AP)-evoked IPSCs in response to afferent fiber stimulation, indicating that Cplx1 enhances the probability of SV fusion at MNTB-->LSO synapses. Contrary to data obtained at several model invertebrate synapses, we further found that Cplx1 KO leads to reduced frequencies of spontaneous and miniature IPSCs, but leaves the kinetics of spontaneous and evoked IPSCs unaltered. Finally, we established that short-term synaptic plasticity (STP) is profoundly altered at Cplx1 KO MNTB-->LSO synapses probed with AP trains at 5-100 Hz. While WT synapses showed synaptic depression that gradually increased with higher stimulation frequencies, this STP pattern was converted to synaptic facilitation in Cplx1 KO MNTB-->LSO synapses, consistent with a reduced SV release probability in the absence of Cplx1.

Taken together, our findings show that Cplx1 is a positive regulator of AP-evoked glycine release at MNTB-->LSO synapses. The reduced rates of spontaneous release we observed in the absence of Cplx1 are inconsistent with a 'fusion clamp'-like function of Cplx1 at these synapses. Because both excitatory ipsi-lateral and inhibitory contra-lateral synaptic inputs to LSO neurons are strongly affected in Cplx1 KO mice, we speculate that integration of binaural input at the level of the LSO is severely compromised in the absence of Cplx1.

Contribution of somatostatin interneurons to network activity in the developing hippocampus *in vitro*

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A large body of evidence suggests that depolarizing GABAergic transmission promotes synchronized network activity (GDPs) in the developing hippocampus *in vitro*. However, the contribution of distinct subtypes of GABAergic interneurons remains incompletely understood. In the present study, we address the question whether somatostatin-positive (SOM) GABAergic interneurons participate in the GABAergic control of GDP generation. In agreement with previous data, confocal Ca²⁺ imaging experiments in the CA1 region of acute hippocampal slices revealed that GDPs were strongly attenuated by inhibition of the chloride importer NKCC1. Optogenetic activation of SOM interneurons using Channelrhodopsin 2 (H134R) induced GABA_A-receptor dependent postsynaptic currents in pyramidal cells already at postnatal day 1. In the presence of ionotropic glutamate receptor antagonists, photoactivation of SOM interneurons evoked action potential firing in a considerable fraction of CA1 pyramidal cells. Furthermore, preliminary Ca²⁺ imaging data show that photoactivation of SOM interneurons could induce GDP-like network events which were strongly attenuated by NKCC1 inhibition. Collectively, the present data support the idea that SOM interneurons may promote the generation of synchronized neuronal network activity in the developing hippocampus.

Developmental changes in the vesicular content at an inhibitory synapse in the cochlear nucleus

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Spherical bushy cells (SBCs) in the ventral cochlear nucleus integrate acoustically driven excitatory input from the auditory nerve (AN) with non-primary inhibitory inputs to precisely encode the temporal structure of sounds. During the first postnatal week, GABA and glycine show a depolarizing effect on SBCs thereby also triggering calcium signals. In mature rodents, a slow hyperpolarizing GABA/glycinergic signaling exhibits an activity dependent conductance build-up, thus endowing inhibition with high-pass filter properties for large and well-timed excitatory inputs.

It remained elusive, whether GABA and glycine are released from distinct presynaptic vesicles or coreleased from the same vesicle. Here, we determined the GABAergic and glycinergic contribution to the inhibitory vesicle content by means of whole-cell recordings of spontaneous vesicle release on SBCs in acute brainstem slices of gerbils from P1 up to P25.

Miniature inhibitory postsynaptic currents, reflecting the release of one vesicle, were first detectable at P2 in a small subset of neurons. The frequency, amplitude and rise time of mIPSCs showed a significant increase up to P25. The decay time constant was unchanged during maturation. In the first postnatal week, no purely glycinergic mIPSCs were detected, inferred by the lack of mIPSCs frequency change under strychnine. Thus, all mIPSCs contained a GABAergic component. The block of GABA_AR, on the contrary, reduced the mIPSCs frequency, suggesting a large portion of presynaptic vesicles containing solely GABA. The remaining mIPSCs were consistent with a corelease of glycine and GABA in a subset of vesicles. During maturation, the frequency and amplitude of glycinergic mIPSCs increased, whereas the fraction of GABAergic events decreased. In P23-25 gerbils all mIPSCs exhibit a glycinergic component, while no pure GABAergic events were observed. Here, mIPSCs with large amplitudes feature GABAergic components, again suggesting a corelease of GABA and glycine in a subset of vesicles.

The results suggest that before hearing onset inhibitory synaptic terminals predominantly release GABA, whereas glycine is coreleased only in a subset of presynaptic vesicles. With maturation, the glycinergic fraction increases and glycine becomes the major inhibitory transmitter after hearing onset. In juvenile gerbils, particularly large mIPSC are evoked by glycinergic vesicles containing GABA in addition.

Differential distribution of synaptosomal associated protein 47 kDa isoform (SNAP47) in the mouse and rat hippocampus.

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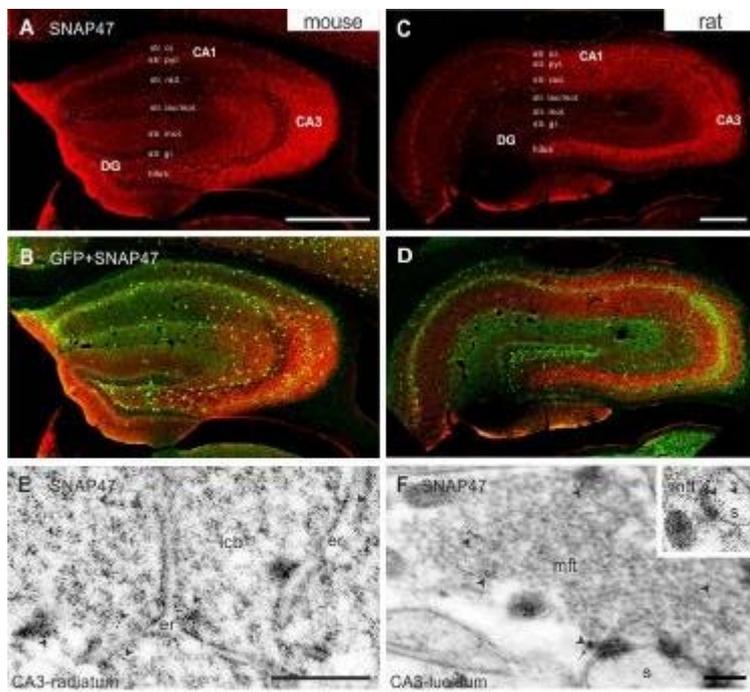
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Synaptosomal-associated protein of 47 kDa (SNAP47) isoform is the most recently identified neuronal SNAP which shows a widespread distribution on intracellular membranes, including synaptic vesicles (SVs) but also other intracellular membrane pools in central neurons (Holt et al., 2006, Takamori et al., 2006). SNAP47 is associated with synaptobrevin-2 (Syb2) and SNAP25, but does not contribute directly to exocytosis and recycling of SVs. While some aspects of the function and expression pattern of this protein in the nervous system are known, its precise cellular and subcellular localization in glutamatergic (excitatory) projections and GABAergic (inhibitory) interneuron (IN) populations remains largely unexplored.

In order to address this question, in this study we applied immunofluorescent and postembedding immunogold labeling combined with confocal- and electron microscopic analysis in mouse and rat hippocampus with a focus on the pre- and postsynaptic localization in the two classes of neurons. For our analysis we used wild-type and VGAT-Venus (YFP) transgenic mice and rats selectively expressing YFP-Venus in INs under the VGAT promoter.

Immunofluorescent labeling revealed a broad distribution of the protein in the hippocampus in both species, however, the pattern of SNAP47 distribution was divergent: in the mouse the immunofluorescence signal was high over the CA3 stratum radiatum, oriens and the cell body layer. In contrast, in the rat the labeling was stronger over the CA1 neuropil and in the CA3 stratum lucidum. In the mouse hippocampus high level of somatic labelling for SNAP47 antibody was observed in VGAT-Venus (YFP)-positive INs. In the rat, while INs were mostly positive, they blended in with the relatively high neuropil labeling. Co-staining for SNAP47 and glutamatergic postsynaptic marker PSD95 or presynaptic vesicular markers ZnT3 and VGLUT1 revealed a strong co-localization postsynaptically in dendritic spines of pyramidal cells (PCs) but also presynaptically, in glutamatergic mossy fiber terminals (MFTs) in CA3 stratum lucidum as well as in asymmetric synapses of local CA3 axon collaterals in CA3 stratum radiatum in the rat hippocampus. Our quantitative immuno-electron microscopy confirmed the preferential localization of SNAP47 postsynaptically in spines and dendritic shafts and at lower level also presynaptically in glutamatergic axon terminals such as MFBs, in the rat hippocampus.

These results demonstrate divergent subcellular localization of SNAP47 protein in mouse and rat hippocampus indicating species- and cell type-specific differences. Nevertheless, the SNAP47 is preferentially localized to postsynaptic compartments in both species. The protein seems to be involved in a fusion machinery distinct from the one used during presynaptic neurotransmitter release. Nonetheless, our data indicate that SNAP47 may impact not only postsynaptic but also presynaptic functions.



A, B: Confocal images of double immunofluorescent labeling for SNAP47 (A, in red) and YFP marker (B, green pseudocolor, channels are merged) in hippocampus of VGAT-Venus (YFP) mouse. Note that the scattered YFP-positive interneurons (B) are positive for SNAP47 (A, B).
C, D: Confocal images of double immunolocalization for SNAP47 and YFP in hippocampus of VGAT-Venus (YFP) rat. Note that the scattered YFP-positive interneurons (D) show no strong labeling for SNAP47 (C, D).
E: Postembedding immunogold labeling for SNAP47 (10 nm gold, arrowheads) associated to the endoplasmic reticulum (er) in the cytoplasm of a putative interneuron cell body (icb) in the CA3 stratum radiatum.
F: Electron micrograph of a mossy fiber terminals (mft) and a postsynaptic complex spine in the CA3 stratum lucidum labeled for SNAP47 (10 nm immunogold particles, arrowheads). The inset illustrates the high postsynaptic localization of SNAP47 in a spine head (s). Abbreviation: m, mitochondrium; thin arrow, dense-core vesicle.

Scale bars represent: A-D, 500 μ m; E-F, 500 nm.

Distinct functions of Piccolo and Bassoon at the calyx of Held

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Bassoon and Piccolo are two large, presynaptic scaffolding proteins located at the active zone of vertebrates. Both proteins share a high degree of sequence homology, although there are important differences; e.g. Piccolo possesses two calcium binding C2 domains and a PDZ domain which are absent in Bassoon. Accordingly, Piccolo and Bassoon share a number of interaction partners, but there are also interactions that are specific for either of the two proteins. Bassoon has been implicated in active zone assembly, localization of voltage-gated calcium channels to release sites, synaptic vesicle priming and the replenishment of synaptic vesicles at release sites during ongoing synaptic activity. The function of Piccolo on the other hand is less well studied, but it has been shown to dynamically regulate the assembly of the actin cytoskeleton. Moreover, both proteins seem to be necessary to maintain the structural integrity of the active zone, as its components become degraded by the proteasome in the absence of both, Bassoon and Piccolo. The function of the two proteins in terms of synaptic transmission at central synapses, is not well established. Bassoon knock-out mice display deficits in synaptic vesicle replenishment that is apparent during high frequency transmission. The function of Piccolo is unknown. Since the two proteins display a considerable amount of homology, it is possible that they functionally compensate for each other. However, a double-knock-out study in cultured hippocampal neurons failed to detect a functional phenotype albeit the ultrastructure of the synapses was drastically altered.

We investigated the function of Piccolo and Bassoon in synaptic transmission simultaneously at the calyx of Held, a giant terminal in the auditory brainstem. Therefore, we generated an *in vivo* double knock-down of both proteins, as well as the corresponding single knock-downs. This was achieved using adeno-associated virus mediated shRNA expression in globular bushy cells of the cochlear nucleus, the projection neurons forming the calyx of Held. The reduction in protein expression was confirmed by 3D Immunohistochemistry in identified single calyces. Calyceal knock-downs did not affect spontaneous synaptic transmission or the properties of single, evoked EPSCs. However, during high frequency stimulation, double knock-down calyces as well as synapses reduced in Bassoon content showed accelerated short-term depression, due to impaired synaptic vesicle replenishment. Short-term depression was largely normal at Piccolo knock-down calyces. However, double knock-down calyces and Piccolo knock-down synapses showed alterations in the recovery from short-term depression. While recovery kinetics of wild type and Bassoon knock-down calyces could be well fitted with a single exponential function, double knock-down and Piccolo knock-down calyces showed a biphasic recovery that was best fitted with a double exponential function. These findings suggest distinct, non-overlapping roles in synaptic transmission for the highly homologous proteins Piccolo and Bassoon at the calyx of Held. While Bassoon seems to play a major role in synaptic vesicle replenishment at the active zone, the data presented here is in line with a scenario in which Piccolo plays a role in defining the fast and slowly releasing subpools of the readily releasable pool, possibly by limiting the spread of the entering calcium.

Distinct roles of auxiliary $\alpha 2\delta$ subunits of voltage-gated calcium channels in excitatory/inhibitory neurotransmission and network activity.

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In the central nervous system Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCC) regulate a large number of neuronal functions including neurotransmitter release. VGCC are membrane multi-complexes consisting of pore-forming $\alpha 1$ subunit and auxiliary $\alpha 2$, $\alpha 3$ and $\alpha \delta$ subunits. $\alpha 2$ subunits play a role in the trafficking of $\alpha 1$ pore-forming subunits to the membrane surface, modulation their properties as well as promote excitatory synapse formation. They are implicated in many neurological disorders and are the target of gabapentinoids - drugs with anti-epileptic and antiallodynic properties. However, it remains still unknown how different $\alpha 2$ isoforms are distributed in neuronal compartments and can affect synaptic transmission or network activity. Here, we addressed these questions using combination of molecular, electrophysiological and imaging techniques. We found that over-expression of $\alpha 2 1$ subunit in hippocampal culture increased surface expression of calcium channels and distributed $\alpha 2 1$ in presynaptic boutons that led to enlarged active zones. An $\alpha 2 3$ subunit, on the other hand, localized in the axonal membrane with smaller accumulation of calcium channels in presynaptic boutons. Accordingly, $\alpha 2 1$ subunit increased the frequency of miniature postsynaptic currents in excitatory synapses (mEPSCs) and this presynaptic release was operated by the large contribution of PQ-type calcium channels. In contrast, $\alpha 2 3$ selectively increased the frequency of miniature postsynaptic currents in inhibitory synapses (mIPSCs) via recruitment of N-type channels with no impact on the amplitudes of mPSCs. Moreover, we found elevated number of glutamatergic synapses in both $\alpha 2 1$ - and $\alpha 2 3$ overexpressing cultures, whereas number of GABA-ergic synapses was increased only upon over-expression of $\alpha 2 3$. The spontaneous network activity investigated on microelectrode arrays demonstrated that upregulation of $\alpha 2 3$ enhances neuronal activity in immature cultures whereas $\alpha 2 1$ improves network activity in mature hippocampal culture. Furthermore, chronic inactivation of $\alpha 2 1$ by gabapentin led to the dramatic enhancement of spontaneous neuronal activity in young $\alpha 2 3$ over-expressing culture.

In sum, our findings demonstrate synapse specific role of $\alpha 2$ subunit on channel trafficking, synapse formation as well as dual function in the network activity in developing culture. We found that $\alpha 2 1$ subunits boost excitatory synapses via involvement PQ-type channels whereas $\alpha 2 3$ has dominant effects in inhibitory synapses with a contribution of N-type channels. Both subunits promote excitatory synapse formation but only $\alpha 2 3$ subunit triggers inhibitory synaptogenesis in hippocampal cultures. $\alpha 2$ subunits have different time-points in the development of synaptic connectivity in neuronal network: $\alpha 2 3$ plays a critical role in early development whereas $\alpha 2 1$ modulates the network activity in later developmental stages. Therefore upregulation of $\alpha 2$ subunits during development can affect the excitation/inhibition balance, alter neuronal network activity and connectivity and result in pathological states.

Foundations of high-fidelity synaptic transmission at intra-cortical synapses between layer 5 pyramidal neurons

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Foundations of high-fidelity synaptic transmission at intra-cortical synapses between layer 5 pyramidal neurons

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Highly reliable synapses between neighboring layer 5 pyramidal neurons (L5PNs) are a major source of neocortical feed-forward excitation^{1,2}. The synaptic foundations of their high reliability remain incompletely understood. We analyzed these synapses in paired recordings from connected L5PNs in the barrel cortex of 3-4 weeks old mice. Consistent with previous work¹, we found that the rate of transmission failures was exceptionally low (< 2%). Using multi-probability fluctuation analysis of EPSC amplitudes, we show that the synapses operate at high vesicular release probability ($pr = 0.63 \pm 0.09$; with $-DGG = 0.60 \pm 0.07$) and ~10 release sites (binomial parameter $N = 10 \pm 9$; with $-DGG = 9 \pm 3$). Cumulative amplitude analysis indicates a large pool of readily releasable vesicles (RRP 10-25), indicating multi-vesicular release. Consistent with high pr , we observed strong depression of the EPSC amplitudes during trains of activation at 100 Hz (see also 1,2). This depression remained pronounced in the presence of the competitive low affinity AMPA receptor antagonist $-DGG$ (2 mM), indicating that its main origin is not postsynaptic. Two-photon Ca^{2+} imaging of individual presynaptic terminals showed no failures in the induction of AP-mediated Ca^{2+} influx with low trial-to-trial signal variability upon repeated activation of the same terminal, indicating a large open probability and/or a large number of voltage-gated Ca^{2+} channels. Ca^{2+} signals summed linearly, indicating that depression of Ca^{2+} influx is not the source for EPSC depression. The majority of the Ca^{2+} influx occurred through ω -agatoxin IVA sensitive Cav2.1 (P/Q-type) Ca^{2+} channels (56%) and a smaller fraction through ω -conotoxin GIVA sensitive Cav2.2 (N-type) channels (15%). The contribution of SNX sensitive Cav2.3 (R-type) channels was negligible. From our data we conclude that the high reliability of synaptic transmission between L5PNs is ensured at all stages translating electrical activity into transmitter release, ranging from the highly reliable AP-mediated Ca^{2+} influx to the high pr of synaptic vesicles contained in a large RRP.

1 Frick, A., Feldmeyer, D., Helmstaedter, M. & Sakmann, B. Monosynaptic connections between pairs of L5A pyramidal neurons in columns of juvenile rat somatosensory cortex. *Cerebral cortex* 18, 397-406, doi:10.1093/cercor/bhm074 (2008).

2 Markram, H., Lubke, J., Frotscher, M., Roth, A. & Sakmann, B. Physiology and anatomy of synaptic connections between thick tufted pyramidal neurones in the developing rat neocortex. *J Physiol* 500, 409-440 (1997).

Functional role of Bassoon at mouse cone photoreceptor ribbon synapses

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The sensory photoreceptor ribbon synapses of the retina are specialized both for fast and sustained neurotransmitter release. A presynaptic organelle, the synaptic ribbon, extends from the active zone into the photoreceptor terminal and tethers hundreds of synaptic vesicles. The presynaptic cytomatrix protein Bassoon anchors the ribbon to the active zone, and lack of functional Bassoon in a Bassoon mutant (mt) mouse leads to impaired neurotransmitter release as seen in electroretinographic recordings¹.

Here, we studied the function of Bassoon in cone photoreceptor ribbon synapses in a Bassoon mt and wild-type (wt) mouse line. Horizontal cell bodies postsynaptic to cone photoreceptors were voltage-clamped, and the effects of a non-functional Bassoon on release properties of the ribbon synapse were investigated by measuring (1) tonic glutamate-induced activity, (2) current responses to changes in light intensity, and (3) electrically evoked postsynaptic responses.

Lack of functional Bassoon decreased the frequency and amplitude of tonic events. Light response amplitudes were also smaller but showed unaltered kinetics. Whole cell calcium currents recorded from Bassoon mt cone photoreceptors showed ~5 fold smaller peak amplitude. Postsynaptic horizontal cell currents evoked by single electrical stimulation displayed smaller peak amplitudes due to a decrease in phasic synaptic vesicle release. Furthermore, trains of electrical stimuli revealed that Bassoon mt synapses cannot follow high frequency stimulation because of reduced synaptic vesicle replenishment rates and smaller readily releasable synaptic vesicle pool sizes. Recovery from short term synaptic depression was also slower in Bassoon mt cone photoreceptor ribbon synapses compared to wt controls.

Our data suggest that in the absence of functional Bassoon, mouse cone photoreceptor ribbon synapses are still functional but they are less efficient triggering both fast and sustained components of neurotransmitter release. Synaptic impairment in Bassoon mt cone photoreceptors is partly a consequence of smaller calcium currents.

Supported by the DFG (BR 1643/6-1 / FE 464/11-1)

¹Dick O et al. (2003) Neuron 37:775-786.

Functional spine analysis with Activity-based Automatic Region of interest Generation (AARG)

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Imaging calcium or voltage transients along CNS neuronal dendrites enables synaptic activity to be linked to specific postsynaptic sites. This is advantageous because postsynaptic sites are highly heterogenous and it is often desirable to study different populations of these sites in isolation.

Dissociated neurons were transfected with GCaMP6, tetrodotoxin was added to the extracellular solution and extracellular magnesium was removed. Under these conditions, spontaneous vesicle fusion events can be detected in the form of transient dendritic calcium elevations. Hundreds of postsynaptic sites can be imaged together, which requires the use of a fast, automated analysis software to establish regions of interest (ROIs). AARG pins ROIs to sites of activity and updates the precise position of each ROI, frame-by-frame. By testing AARG with model data, we have shown the AARG algorithm accurately performs the task of detecting when events occur at established ROIs and when it is appropriate to establish a new ROI. Furthermore, we have measured changes in the frequency of quantal calcium transients and fluorescence intensity changes following application of phorbol esters and recombinant BDNF.

AARG analysis potentially offers a unique insight into the downstream signaling events at individual spines arising from exposure to recombinant proteins or following genetic or pharmacological manipulations. In general, AARG provides a bias-free, non-labour-intensive and fast approach for analysing synaptic activity occurring across hundreds of synaptic sites simultaneously.

Glutamate dynamics in the cleft of Schaffer collateral synapses

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Glutamate release may be indirectly detected by electrophysiological recordings of postsynaptic AMPA receptor mediated currents (EPSCs). The complex nature of postsynaptic responses makes it difficult to distinguish between multisynaptic connections and multivesicular release from a single synaptic site. Here we show that it is possible to monitor neurotransmitter release at individual Schaffer collateral synapses using the genetically encoded glutamate indicator iGluSnFR (Marvin et al., Nat. Meth. 2013) and two-photon microscopy. We expressed the indicator in CA3 pyramidal neurons via single cell electroporation. The method is sensitive enough to detect the release of single transmitter vesicles at individual presynaptic terminals.

To investigate the influence of glutamate diffusion, indicator kinetics and cleft orientation on the optical signals, we set up a Monte Carlo-type simulation of molecular events occurring at an archetypical bouton-spine contact site. The stochastic nature of spatial locations of molecules and binding events reflects some of the variability seen at the experimental level. Combining microscope specific focus size (PSF) and calculated 3D positions of iGluSnFR in both glutamate unbound and bound state can be used to simulate two-photon fluorescence transients which then can be compared to experimental data. The results of the simulation suggest critical parameters for further development of genetically encoded glutamate sensors. Furthermore, we quantified the effect of fusion site variability on AMPA receptor activation.

Hippocampal output to the medial entorhinal cortex: functional monosynaptic projections to layer Va neurons

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The entorhinal cortex (EC) constitutes a major interface between the hippocampus and various regions of the neocortex and plays a key role in spatial representation, navigation and episodic memory. Sensory signals enter the hippocampal formation via neurons located in superficial layers of the EC. In turn, information from the hippocampus reaches the neocortex through deep layers of the EC (mainly layer V). Based on distinct molecular markers, layer V of the medial entorhinal cortex (mEC) can be subdivided into layers Va and Vb (Sürmeli et al. 2015). The differing expression of transcription factors is mirrored by the differential integration of layer V neurons into the hippocampal-entorhinal system. While Ctip2 positive pyramidal-like neurons of layer Vb are the main targets of projections from CA1 and the subiculum, Etv1 positive horizontal neurons of layer Va are the major source of intra-telencephalic projections. This segregation of input and output components suggests active signal computation within deep layers of the mEC. Here, we investigated the functional connectivity between CA1 and mEC layer Va horizontal neurons as well as their integration into the hippocampal-entorhinal network. We performed field potential recordings from the pyramidal layer of CA1 and layer V of the mEC together with sharp microelectrode intracellular recordings from layer Va neurons in acute horizontal mouse brain slices. Location and morphology of recorded neurons were confirmed by biocytin staining. The position of neighboring layer Vb pyramidal neurons was marked by Ctip2 immunolabeling. We found that electrical stimulation of the alveus in CA1 induced robust postsynaptic potentials (PSPs) in identified layer Va horizontal neurons after a delay of 4.4 ± 0.8 ms. The PSP amplitude recorded at a membrane potential of -74.6 ± 1.1 mV was 7.1 ± 1.8 mV and the PSP half-width was 23.7 ± 14.2 ms ($n = 7$). In addition, spontaneous network activity in the hippocampus presented as propagating sharp wave-ripple complexes (SPW-R) was regularly associated with subthreshold postsynaptic potentials in mEC layer Va neurons. To rule out that the detected synaptic potentials are a result of polysynaptic activation (e.g. via subicular or mEC layer Vb neurons), we then applied an elevated divalent cation (EDC) solution (4 mM Ca²⁺ / 6 mM Mg²⁺). Increased divalent cation concentrations restrict transmission to monosynaptic responses. Both PSPs induced by electrical stimulation and PSPs following SPW-R were detected under EDC conditions. At -74.9 ± 0.9 mV PSPs evoked by stimulation had a mean amplitude of 5.8 ± 1.7 mV ($n = 7$, $p > 0.05$ compared to control, paired t-test). These findings suggest direct monosynaptic connections between CA1 and mEC layer Va neurons in horizontal slices as well as direct propagation of SPW-R from CA1 to mEC layer Va. We conclude that, in addition to the previously described projections from CA1 and the subiculum onto mEC layer Vb neurons, layer Va cells receive direct input from hippocampal CA1 neurons. Furthermore our results indicate an input-output integration in layer Va of the mEC. Supported by the DFG (SFB1134, Project A1).

Sürmeli, G., Marcu, D. C., McClure, C., Garden, D. L., Pastoll, H. und Nolan, M. F. Molecularly Defined Circuitry Reveals Input-Output Segregation in Deep Layers of the Medial Entorhinal Cortex. *Neuron* 2015;88:1040-1053.

Imaging glutamate release at individual Schaffer collateral synapses

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Information transmission at chemical synapses is most likely a quantal process based on the release of transmitter-filled vesicles from the presynaptic terminal. This process can be analyzed in detail by comparing the amplitude distribution of stimulation-evoked postsynaptic potentials to spontaneous release events using binomial statistics. This method works well at the neuromuscular synapse, but is of limited use in the central nervous system, where each neuron receives thousands of synapses at different electrotonic distances from the soma. Optical methods based on fluorescent calcium indicators have been used to isolate the response of individual synapses on dendritic spines in intact brain tissue. However, the non-linear relations between glutamate concentration, receptor activation and spine calcium concentration complicate quantal analysis of calcium signals. Here we introduce direct optical measurements of glutamate concentration in the synaptic cleft based on the genetically encoded glutamate sensor iGluSnFR, using a fast scanning two-photon microscope. All Schaffer collateral synapses we sampled showed a large dynamic range and were capable of multivesicular release in high calcium saline. In physiological calcium concentration, however, these synapses had a high failure rate and typically released only one vesicle. The amplitude distributions from individual synapses were well fit by a quantal model if photon shot noise was taken into account. Furthermore, localizing the fusion site on the surface of the presynaptic bouton with an accuracy that exceeds the resolution limit of the two-photon microscope, we show that release was confined to a single active zone which did not expand during multivesicular release.

Impaired sound encoding in PSD-95 knockout mice

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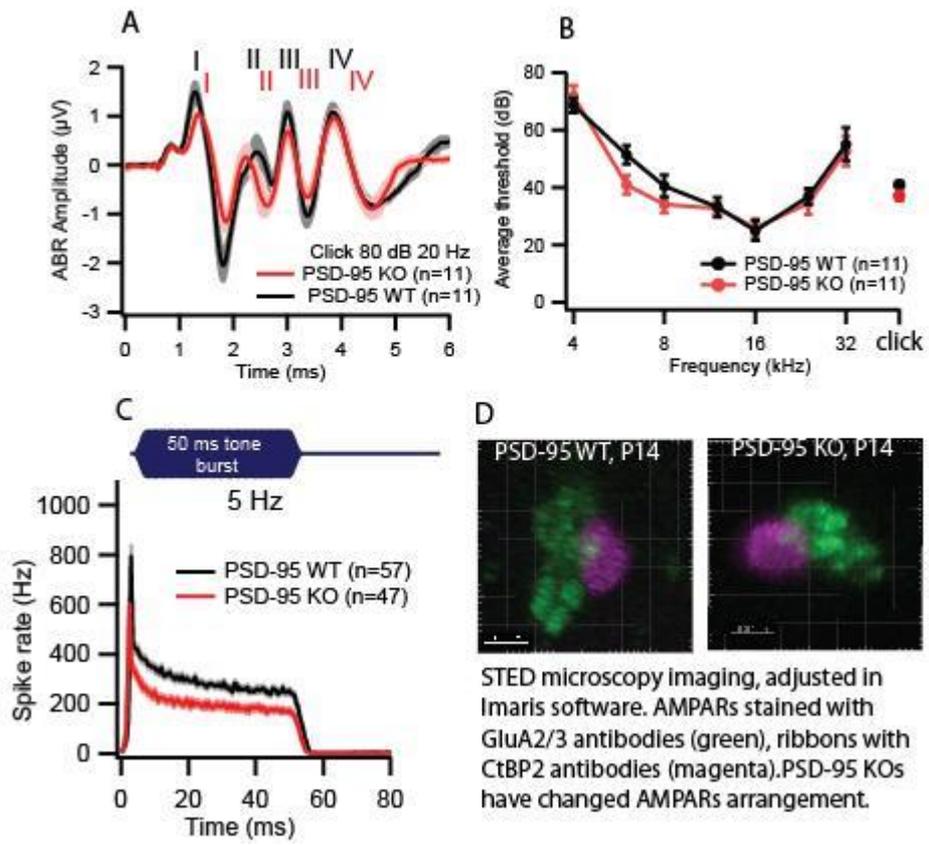
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Introduction: Sound is encoded by inner hair cells, each forming 8-20 ribbon synapses with spiral ganglion neurons (SGNs). The scaffolding protein PSD-95 contributes to clustering of AMPA receptors in the postsynaptic membrane. Here, we used PSD-95 knockout mice (PSD-95 KO) to investigate the role of PSD-95 in AMPA receptor clustering and synaptic sound encoding in the auditory system.

Methods: We performed recordings of auditory brainstem responses (ABRs) and extracellular in vivo single unit recordings from SGN and cochlear nucleus neurons. Furthermore, we imaged inner hair cell ribbon synapses using confocal and STED microscopy.

Results: Adult PSD-95 KO mice had normal auditory brainstem response thresholds (Fig.B), but a reduced amplitude of the wave I (Fig.A), suggesting impaired temporal precision and/or rates of synaptic transmission, while the other ABR waves were normal. Single unit recordings revealed lower spontaneous spike rates in SGNs. Single unit thresholds and frequency tuning were normal. Onset and adapted spike rates in response to suprathreshold tone burst stimulation were reduced (Fig.C) and the time constant of fast adaptation was significantly shorter. The delay and jitter of the first spike in response to stimulus onset was increased. Rate-level functions for 50ms tone bursts showed normal thresholds, but decreased maximal rates. The number of inner hair cell ribbon synapses appeared unchanged. Preliminary STED data indicated alterations in the arrangement of postsynaptic glutamate receptor clusters of PSD-95 KO SGNs (Fig.D).

Conclusion: PSD-95 scaffolding protein is essential for glutamate receptor clustering in SGNs. The absence of this protein results in impaired sound encoding in PSD-95 KO SGNs, presumably due to changes in the number, arrangement or mobility of AMPA receptors.



***In vivo* STED imaging of PSD-95 and Gephyrin in the visual cortex of the living mouse**

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Synapses are the key functional information processing units of neural circuits. The vast majority of excitatory synapses are made on the heads of dendritic spines. Dendritic spines are tiny membranous protrusion on a neuron that receive mainly excitatory synaptic input of another neuron and compartmentalize postsynaptic responses. Spines are known to be highly dynamic, especially during postnatal development, and their morphological changes are associated with long term plasticity [1].

We are using STED (STImulated Emission Depletion) a super-resolution microscopy technique which is capable to resolve tiny structures with a resolution of less than 70 nm in the living mouse brain [2]. With a home-built upright STED-microscope and fluorescently labeled Lifeact (F-actin binding protein) we could nicely trace highly dynamical morphological changes of spine heads, with a resolution down to ~ 50 nm [3]. Going one step further not to see only changes in the morphology of the spine in their entirety, we want to find out proteins which are involved in this morphological changes and are associated with long term plasticity. For this approach we fluorescently labeled the scaffolding proteins postsynaptic density protein 95 (PSD-95) and Gephyrin, by using AAV-transduction into the visual cortex of a living mouse. Due to the fact, that we are interest in morphological changes or trafficking of these proteins we prevent pushing overexpression artefacts by not expressing the fluorescent labeled protein itself, instead we are using transcriptionally regulated intrabodies named PSD95.FingR and GPHN.FingR (FingR = Fibronectin intrabodies generated with mRNA display) [4]. Three weeks after AAV-transduction, an optical window is implemented into the skull of the anaesthetized mouse, directly followed by the *in vivo* STED-measurement within the molecular layer of the visual cortex. Focusing on the fluorescent-labeled PSD95.FingR we could visualize differences in sub-structures of PSD-95 within hours of measurement. So far we are able to visualize changes in spine morphology by labeling F-actin simultaneously with the localization of either PSD-95 or Gephyrin in the living mouse down to a depth of 40 μ m.

[1] Segal M. (2005). Dendritic spines and long-term plasticity. *Nat. Rev. Neurosci.* 6, 277–284.

[2] Berning S, Willig KI, Steffens H, Dibaj P, Hell SW (2012). Nanoscopy in a living mouse brain. *Science* 335, 551-551.

[3] Willig KI, Steffens H, Gregor C, Herholt A, Rossner MJ, Hell SW (2014). Nanoscopy of filamentous actin in cortical dendrites of a living mouse. *Biophys J.* 106, L01-L03.

[4] Gross GG, Junge JA, Mora RJ, Kwon H-B, Olson CA, Takahashi TT, Liman ER, Ellis-Davies GCR, McGee AW, Sabatini BL, Roberts RW, and Arnold DB (2013). Recombinant Probes for Visualizing Endogenous Synaptic Proteins in Living Neurons. *Neuron* 78, 971–985.

***In vivo* time lapse imaging of axonal dense core vesicle trafficking in anaesthetized and awake mice**

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Dense core vesicles (DCV) are large, electron-dense vesicles designed to transport a variety of cargo molecules, including neuropeptides or neurotrophins, from their site of production at the neuronal soma towards their respective release sites in dendrites or axons. Trafficking and movement characteristics of DCVs have been typically investigated in chromaffin cells or primary cell culture models. Yet, DCV trafficking characteristics and their responsiveness to physiological release signals could differ fundamentally in the mammalian brain *in vivo* due to the densely packed tissue neuropil, neuronal connectivity, neuron-glia interactions or different functional brain states. In this study, we used multiphoton-*in vivo*-imaging to visualize DCV trafficking in the central nervous system in anaesthetized or awake mice through a chronically implanted cranial window. Viral co-expression of live fluorescent DCV-markers and axonal markers in thalamic projection neurons allowed us to specifically visualize axonal projections from the thalamus to upper layers of the cortex.

Taking advantage of semiautomated tracking approaches we were able to analyze the movement of hundreds of vesicles in individual axons of different animals. These data for the first time reveal speed, directionality and number of moving DCVs and their movement characteristics at axonal en passant boutons *in vivo* in awake and anaesthetized mice. This approach will enable future studies of the physiological mechanisms triggering DCV cargo-release and studies examining DCV trafficking in pathophysiological situations potentially linked to defects in DCV trafficking, such as neurodegenerative disorders and the aging brain.

Interaction of Piccolino and RIBEYE at the photoreceptor ribbon synapse

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Photoreceptor- and bipolar cells of the retina contain a specialized type of chemical synapse, the so called ribbon synapse. The hallmark of retinal ribbon synapses is the synaptic ribbon, a plate-like structure extending from the release site into the terminals' cytoplasm and tethering hundreds of synaptic vesicles. The synaptic ribbon is mainly built by the ribbon specific protein RIBEYE¹. Another ribbon synapse specific protein is Piccolino, the recently identified C-terminal truncated splice-variant of the multi-domain protein Piccolo².

A knockdown of Piccolino leads to a disruption of the plate-like shape of the synaptic ribbon in photoreceptors³. Most of the ribbon material appears spherical and membrane attached. These findings suggest that Piccolino, respectively an interaction of Piccolino with another synaptic ribbon protein, might play a role in the assembly of the synaptic ribbon.

In this study we show the interaction between both ribbon synapse specific proteins Piccolino and RIBEYE. In GST-pull down assays as well as in co-aggregation experiments in transfected 3T3 cells the C-terminus of Piccolino (AS 2567-2984) is able to interact with RIBEYE. This interaction occurs specifically with the B-domain of RIBEYE, which is identical to the transcriptional co-repressor CtBP2 and highly related to CtBP1¹. Recently it was shown that a PXDLS-like motif in full-length Piccolo is responsible for the interaction with CtBP1⁴. We found several PXDLS-like motifs at the C-terminus of Piccolino. Each of these motifs is able to bind to the B-domain of RIBEYE, which contains a PXDLS binding cleft conserved in CTBPs⁵. The interaction between Piccolino and the B-domain of RIBEYE can be inhibited by mutating the amino acids of the PXDLS-like motifs in the sequence of Piccolino.

Based on the published data and the results of our experiments we propose that the interaction between Piccolino and RIBEYE has a function in the assembly of the photoreceptor synaptic ribbon.

Supported by the DFG (RE 3143/2-1)

¹Schmitz F et al. (2000) *Neuron* 8:857-872.

²Regus-Leidig H et al. (2013) *PLoS ONE* 8:e70373.

³Regus-Leidig H et al. (2014) *Front. Cell. Neurosci.* 8:259.

⁴Ivanova D et al. (2015) *Embo J* 34:1056-1077.

⁵Nardini M et al. (2003) *Embo J* 22:3122–3130.

Intracellular sodium loading under ischemic conditions *in situ* and *in vivo*

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The brain is strictly dependent on a steady supply with oxygen and glucose. Most energy is needed by the Na⁺/K⁺-ATPase (NKA). The NKA sets the steep inwardly directed sodium gradient, emphasizing the direct, imperative link between sodium regulation and metabolism in the brain. In ischemic stroke, the disruption of blood flow leads to a breakdown of energy homeostasis and, as a consequence, a decrease in intracellular ATP and NKA activity. This causes a decrease or even failure in sodium export, mandatory to recover from sodium influx following opening of voltage- and ligand-gated channels and/or activation of secondary active transporters like the sodium-calcium exchanger (NCX). Despite its fundamental role and direct link to cellular energy metabolism, there is a surprising lack of experimental data on the effects of metabolic failure on intracellular sodium of neurons and astrocytes in the intact tissue and, up to date, no *in vivo* measurements exist. Moreover, the consequences of changes in intracellular sodium concentrations on NCX activity and on cellular calcium homeostasis are largely unclear.

In the present study, we analyzed the consequences of energy deprivation on sodium concentrations in astrocytes and neurons of the somatosensory cortex *in vivo* and in acute cortical tissue slices. Moreover, we studied the interplay between sodium and calcium signaling during energy depletion by blocking NCX with KB-R7943. To this end, we performed a permanent middle cerebral artery occlusion (pMCAO) for stroke induction *in vivo* or classical chemical ischemia (induced by sodium azide and 2-deoxyglucose) to mimic stroke-like conditions *in situ*. Intracellular ion changes were monitored using two-photon or wide-field fluorescence microscopy.

Our data reveal that acute pMCAO induces massive transient increases in the intracellular sodium concentration of neurons and astrocytes in the somatosensory cortex that spread to neighboring cells in a wave-like manner, indicating a peri-infarct depolarization wave. Sodium increases reached 20-30 mM in both cell types and lasted over minutes, before slowly recovering to baseline. In acute cortical brain slices, chemical ischemia caused similar wave-like sodium elevations, albeit of smaller amplitudes. In addition, prominent transient elevations in intracellular calcium were observed. These calcium signals were strongly dampened during perfusion with KB-R7943, indicating a significant involvement of NCX in their generation.

Taken together, our study provides the first experimental data on sodium changes *in vivo* in response to pMCAO. In addition, we found evidence for a significant contribution of NCX reversal to calcium signalling during energy deprivation. This indicates that changes in intracellular sodium in neurons and glia are among the very first consequences of ATP shortage following ischemic stroke, triggering secondary calcium influx and cellular damage.

Supported by the DFG [RO 2327/8-1].

Multivariate Analysis of Synaptic Parameters at the *Drosophila* Neuromuscular Junction

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Chemical synaptic transmission is crucial for adaptive animal behavior. Despite growing evidence for heterogeneous functional properties of synapses, heterogeneity in synaptic function has not been systematically studied in a large set of synapses yet. We here systematically analyze inter- and intra-synaptic heterogeneity by correlating synaptic function to synapse morphology, synapse number, and the expression levels of various pre- and postsynaptic markers at the larval *Drosophila* neuromuscular junction (NMJ). Specifically, the functional 'quantal' synaptic parameters N , p , and q of individual NMJs are estimated by various electrophysiology-based approaches (mEPPs, EPSCs, cumulative EPSC analysis, nonstationary variance mean analysis). In a next step, these functional parameters are correlated to a set of 'non-functional' parameters (i.e. synapse number per NMJ, fluorescence intensity of the presynaptic GFP-tagged calcium channel), which are estimated by immunohistochemical analysis and light microscopy approaches. Together, this work is anticipated to reveal new insights into the statistics of neurotransmission considering synapse-to-synapse heterogeneity. This in turn may inform us about the roles of synapse function during neural physiology and pathology.

Nanoscopic Organization of Bassoon and Piccolo at the Active Zone of the Calyx of Held

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Regulated neurotransmission implies a very precise structural organization of synaptic vesicle release sites, called active zones (AZ). Bassoon and Piccolo are large scaffold proteins essential for the assembly of the mammalian AZ. Consistent with their high structural similarity, Piccolo and Bassoon show several overlapping and only few divergent functions. In line with these similarities, the two proteins have been previously shown to colocalize at presynaptic specializations when imaged by conventional microscopy. However, the detailed geometrical arrangement of the AZ remains unresolved due to the diffraction-limited resolution of light microscopy. Using 3D dual-color direct stochastic optical reconstruction microscopy (dSTORM), we determined the precise spatial arrangement of Piccolo and Bassoon at a model synapse of the mammalian central nervous system – the calyx of Held. Intriguingly, we found that Piccolo and Bassoon formed non-overlapping nanoclusters arranged in a chessboard pattern. This newly resolved pattern is very similar to the “sandwich”-like distribution of Piccolo and Bassoon recently reported at the mammalian neuromuscular junction.

Nrg3 is a major Bace1 substrate and controls synaptogenesis.

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Human genetics has correlated the occurrence of neuropsychiatric disease with sequence variants in particular genes. Among the genes implicated in schizophrenia are Neuregulin 3 (NRG3) and ERBB4. Nrg3 encodes a growth factor that contains an EGF-like domain and binds to the tyrosine kinase receptor ErbB4 with low affinity. Nrg3 is exclusively expressed in the nervous system, and produced in many if not all neurons of the cortex and hippocampus. In contrast, the ErbB4 receptor is exclusively expressed by inhibitory interneurons in the cortex, in particular by parvalbumin+ fast-spiking interneurons. Nrg3 is processed and stabilized by Bace1 in-vitro and in-vivo. Nrg3 and ErbB4 accumulate in excitatory synapses on interneurons in-vivo and in-vitro on the pre- and postsynaptic side, respectively. To define the function of Nrg3, we generated Nrg3 mutant mice. Brains of mutant mice display no major morphological abnormalities, but behavior and electrophysiological brain activity are disturbed. Using a reconstituted in vitro model, we demonstrate that Nrg3 promotes synapse formation on ErbB4+ interneurons, causes enrichment of ErbB4 in postsynapses and, vice versa, modulates presynaptic structure and function.

Orientation and organization of Bassoon: from the Golgi to the synapse

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Bassoon is one of the largest scaffolding proteins found in the cytomatrix of the active zone (CAZ) of a neuron's presynaptic terminal. The CAZ is a specialized sub-compartment assembled in close proximity to the neurotransmitter release site, or active zone, and is comprised of interconnected active zone proteins. The CAZ and its proteins have been shown to promote short-term plasticity and long-term plasticity by enabling priming and docking of synaptic vesicles and binding to Ca²⁺ channels. Despite its integral role in presynaptic transmission, the mechanisms of mammalian CAZ formation, synapse assembly, and maturation are still poorly understood.

Bassoon, one of the two large CAZ proteins, binds to other AZ proteins and provides structural stability to the protein complex, and is one of the first proteins to be incorporated into young synapses. It is also the protein with the largest number of well-established tools for studying trafficking of active zone precursor organelles, as well as AZ assembly and maintenance, making it the ideal candidate protein to study CAZ formation.

To visualize the steps involved in the biogenesis of the CAZ, we generated single and double tagged full-length Bassoon constructs optimized for visualizing the orientation and organization of Bassoon molecules with super-resolution imaging techniques. Using specific nanobodies against the fluorescent tags and stimulated emission depletion (STED) nanoscopy, we are able to resolve and characterize the orientation of the N- and C-termini of the protein, and using FLIM imaging the intermolecular organization of neighboring Bassoon molecules in maturing cultured neurons.

This study describes, for the first time, the orientation and organization of Bassoon at different subcellular compartments of neurons. Understanding the changes in conformation of Bassoon, during neuronal development, enhances our knowledge of AZ assembly and synapse maturation, and serves as a reference to compare orientation of other CAZ and synaptic proteins present at mammalian presynaptic sites.

Tight distribution of synaptic vesicle release sites generated by Unc13A limits and synchronizes neurotransmission

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Information processing in our brains depends on the exact timing of synaptic transmission. Physiological analysis indicates that synaptic transmission requires precisely controlled Ca²⁺ activated exocytosis of synaptic vesicles (SVs) from highly specialized release sites within the active zone (AZ). However, molecular principles of site formation and positioning remained elusive. Here, we identified a function of the AZ protein Unc13A in both. By studying the local distribution and stability of the essential release factors Unc18, Syntaxin-1 and Unc13A with super resolution microscopy and intravital imaging at AZs of *Drosophila* neuromuscular junction synapses we found that Unc13A was the only protein positioned with nanometer precision within defined sub-AZ domains where it was stable for hours. The N-terminal portion of Unc13A conferred sub-AZ localization and stabilization, but did not form release sites. In contrast, a C-terminal fragment was sufficient for release site generation, but had promiscuous and instable localization that led to the generation of excessive release sites at positions atypically distant- but surprisingly also too close to Ca²⁺ channels. Unspecific release site positioning resulted in reduced synaptic transmission, excessive facilitation and loss of temporal precision. Scaffold protein-mediated, tight positioning of Unc13A confers formation and stable localization of SV release site, which restricts SV fusion in order to ensure precise timing of synaptic transmission.

Periodic F-actin structures shape the neck of dendritic spines

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Most of the excitatory synapses on principal neurons of the forebrain are located on specialized structures called dendritic spines. Their morphology, comprising a spine head connected to the dendritic branch via a thin neck, provides biochemical and electrical compartmentalization during signal transmission. Spine shape is defined and tightly controlled by the organization of the actin cytoskeleton. Alterations in synaptic strength correlate with changes in the morphological appearance of the spine head and neck. Therefore, it is important to get a better understanding of the nanoscale organization of the actin cytoskeleton in dendritic spines. A periodic organization of the actin/spectrin lattice was recently discovered in axons and a small fraction of dendrites using super-resolution microscopy. Here we use a small probe phalloidin-Atto647N, to label F-actin in mature hippocampal primary neurons and in living hippocampal slices. STED nanoscopy reveals that in contrast to α -II spectrin antibody labelling, phalloidin-Atto647N stains periodic actin structures in all dendrites and the neck of nearly all dendritic spines, including filopodia-like spines. These findings extend the current view on F-actin organization in dendritic spines and may provide new avenues for understanding the structural changes in the spine neck during induction of synaptic plasticity, active organelle transport or tethering.

Persistent sodium current modulates distal axonal excitability in CA1 neurons

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Axonal excitability is an important determinant of neuronal signalling. While most action potentials are generated at the axonal initial segment (AIS), spikes can also arise at more distal locations, largely independent from dendritic excitation. Such ectopic action potentials may support the fast network oscillations and can contribute to pathological activity, e.g. following demyelination. Ectopically generated action potentials appear as antidromic spikes in somatic recordings, lacking the typical pre-depolarization phase. While it is known that persistent sodium current (I_{NaP}) lowers action potential threshold in the distal part of the AIS, its functional role in the periphery of the axon is unclear. Here, we investigated the influence of the I_{NaP} on ectopic axonal excitability in hippocampal CA1 pyramidal neurons. We performed field potential recordings from the pyramidal layer together with conventional intracellular recordings from CA1 neurons in acute horizontal mouse brain slices. In order to probe distal axonal excitability, antidromic spikes were induced by local electrical stimulation of the alveus close to the subiculum (~ 200 μm from recording electrodes) using single short pulses (duration 0.1 ms). The stimulus intensity was determined as 60% of the maximal field response of an individual input-output curve. To prevent spontaneous or recurrent synaptic excitation, all experiments were performed in the presence of CNQX (10 μM), APV (30 μM) and picrotoxin (100 μM). I_{NaP} was blocked with bath-applied riluzole (10 μM) or phenytoin (100 μM). Block of I_{NaP} significantly reduced the antidromic field population responses. In the presence of riluzole, amplitude of antidromic population spikes was reduced to 68.5% of control, while onset and peak latency were not significantly altered. Likewise, perfusion of phenytoin resulted in a reduction of population spike amplitude to 83.7% of control without change of latencies. In order to investigate the mechanisms behind this effect, we performed intracellular recording from CA1 pyramidal neurons ($n = 6$). We found that ectopic action potentials induced by minimal intensity stimulation were lost after adding riluzole in 5 of 6 neurons. However, in 4 of these cells an increase in the stimulus intensity was able to recover spike generation. In the remaining neuron, antidromic spikes could be induced following membrane depolarization. We conclude that I_{NaP} modulates excitability of distal axons of hippocampal CA1 neurons. Furthermore, our results indicate that block of I_{NaP} increases the threshold for ectopic action potential generation. Supported by the DFG (SFB1134, Project A1).

Presynaptic mitochondrial calcium release enhances short-term facilitation during brief high-frequency stimulation

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Mitochondria are known to play important roles in generating ATP and calcium sequestration. However, little is known about the role of mitochondrial calcium dynamics in synaptic transmission induced by a brief high frequency stimulation (HFS). We have previously shown that 2 μM tetraphenylphosphonium (TPP+) specifically blocks mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger (mNCX) without affecting the peaks and fast decaying phase of calcium transients (CaTs) at the calyx of Held. Nevertheless, we found that TPP+ reduces paired pulse facilitation at mature calyx of Held but not at immature one. We further investigated the differential effects of TPP+ using patch-clamp techniques, which allowed us to measure presynaptic calcium currents and postsynaptic excitatory postsynaptic currents (EPSCs) simultaneously during different pulse protocols while Ca^{2+} chelator, EGTA and ATP, are provided through the presynaptic pipette.

When HFS, a train of 30 pulses at 100 Hz, was applied under 0.1 mM EGTA that mimics the physiological calcium buffer concentration of immature calyx of Held, TPP+ had no effect on short-term plasticity of EPSCs. Under the conditions of 0.5 mM EGTA, however, TPP+ reduced short-term facilitation (STF) of EPSCs evoked by the HFS, suggesting that high calcium buffering may be required for mitochondrial Ca^{2+} release via mNCX during HFS to modulate short-term plasticity (STP). Consistent with this hypothesis, TPP+ had no effect on STP when HFS was followed after 10 conditioning pulses (10 Hz, 1 s) even under the 0.5 mM EGTA conditions. To confirm the role of mNCX in STP, the same experiment was repeated using CGP37157, another mNCX blocker. Like TPP+, CGP37157 reduced STF of EPSCs evoked by HFS, and was ineffective when the conditioning pulses were applied before HFS. We studied effects of CGP37157 and TPP+ on mitochondrial ($[\text{Ca}^{2+}]_m$) and cytoplasmic global Ca^{2+} ($[\text{Ca}^{2+}]_i$) during HFS using 10 μM rhodFF-AM and 100 μM Fura-4F, respectively. CGP37157 enhanced the peak $[\text{Ca}^{2+}]_m$ level caused by HFS under 0.5 mM EGTA, but not under 0.1 mM EGTA conditions. In contrast, 2 μM TPP+ had no effect on global $[\text{Ca}^{2+}]_i$, under the 0.5 mM EGTA conditions, indicating that mitochondrial calcium release regulates microdomain calcium during HFS, and thus enhances STF in the presence of high calcium buffers. These results may explain the reason why TPP+ affects STP specifically at mature calyx of Held but not at immature one.

Regulation of activity-dependent compensatory endocytosis at central synapses by N-cadherin

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The synaptic adhesion molecule N-cadherin has been described to play an essential postsynaptic role in long-term potentiation and spine stabilization at mammalian central synapses. In addition, N-cadherin has also been found to influence presynaptic function. However, the specific role of N-cadherin in presynaptic vesicle exo- and endocytosis remains to be elucidated.

To study the influence of N-cadherin on both vesicle exo- and endocytosis in one experiment, we performed synaptophysin-pHluorin (SypHy) fluorescence imaging of individual release sites during extracellular stimulation. Upon overexpression of N-cadherin in cultured mouse cortical neurons, vesicle exocytosis was enhanced similar to overexpression of another synaptic adhesion molecule, Neuroligin1. Most interestingly, overexpression of N-cadherin also strongly accelerated vesicle endocytosis, while overexpression of Neuroligin1 did not. Conditional knockout of N-cadherin in individual cultured neurons by Cre expression only weakly changed vesicle endocytosis induced by low release activity. However, upon strong vesicle release, endocytosis was strongly defective in N-cadherin-deficient neurons.

To begin to investigate the molecular mechanisms underlying the activity dependence of the regulation of endocytosis by N-cadherin, we studied the alterations of the synaptic localisation of N-cadherin upon strong synaptic activation. We performed super-resolution structured illumination microscopy (SIM) imaging of immunocytochemically stained N-cadherin clusters and quantitatively analysed their spatial relation to pre- (vGlut1) and postsynaptic (PSD95) sites. We observed that strong vesicle release led to a recruitment of N-cadherin to the peri-active zone of synapses largely devoid of N-cadherin prior to stimulation.

In summary, we hypothesize that N-cadherin has an essential inductive role in compensatory endocytosis at mammalian central synapses.

Role of Bassoon in the regulation of synaptic vesicle pool size

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The active zone (AZ) is a region of the presynaptic bouton, where neurotransmitter release takes place. It is characterized by the presence of an electron-dense structure called the cytomatrix at the active zone (CAZ). This cytoskeletal matrix is composed of multidomain scaffold proteins (RIM, RIM-BP, Munc-13, CAST/ELKS, Liprins, Piccolo and Bassoon), which act as functional and structural organizers of the release sites and regulators of synaptic vesicle exocytosis. The role of CAZ protein Bassoon (Bsn) in presynaptic function has been investigated in several studies, which showed that this protein does not have an essential role in synapse formation but rather contributes to the plasticity of neurotransmitter release. At conventional and ribbon synapses Bassoon is involved in the reloading of vesicles and the regulation of RRP size, but the molecular mechanisms underlying this process are still not well understood. In order to tackle the molecular mechanism of this Bassoon function, we characterized presynaptic composition and function in primary hippocampal neurons derived from mice lacking Bsn expression. We observed decrease in the synaptic abundance of most CAZ proteins in Bsn lacking synapses and in line with our previous studies defects in synaptic vesicle release. To dissect this phenotype we used the synaptophysin-pHluorin-based reporter technique allowing analysis of synaptic vesicle pools and of release characteristics. We found that ready-releasable pool (RRP) and recycling pool (RP) were reduced and proportion of resting vesicles that do not participate in release was increased in the absence of Bsn. By using pharmacological intervention, we identified the signalling pathway contributing to this process. Along with this, we also demonstrated that neurons lacking Bsn were unable to adjust their presynaptic vesicle recycling in response to the network silencing, showing importance of Bsn for induction of presynaptic homeostatic scaling. Together our study provides new insight into mechanistic understanding of Bassoon function at presynapse.

Role of the TrkB receptor kinase domain in ligand-independent TrkB transactivation

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Neurotrophins are a family of secreted proteins that promote growth, survival, and differentiation of neurons in the central and peripheral nervous system. The neurotrophin BDNF (brain-derived neurotrophic factor) is the high-affinity ligand of the receptor tyrosine kinase TrkB, thereby determining its role in neuronal survival and differentiation as well as in synaptic structure, function, and plasticity. Trk receptor activation is induced by dimerization of receptor monomers on binding of BDNF. The C-terminal intracellular domain of TrkB carries a protein tyrosine kinase core that catalyses the phosphorylation reaction between ATP phosphate and hydroxyl groups of tyrosine. Locally increased kinase activity causes the autophosphorylation of three kinase domain tyrosines enabling opening of the activation loop. Tyrosine residues outside of this domain are phosphorylated and serve as docking sites for target proteins. Although ligand-induced dimerization or oligomerization of receptor tyrosine kinases of the Trk family is a well-established mechanism for growth factor signalling, there is strong evidence that biological responses of TrkB are ligand-independent and can be mediated by two or more receptor systems. Here we show that both BDNF-dependent activation and BDNF-independent activation of TrkB were blocked when either the ATP-binding site or tyrosine 705 in the kinase domain of TrkB were mutated. Immunofluorescence labeling of phosphorylated TrkB mutants shows that the vast majority of phosphorylated TrkB is found at intracellular locations, thus raising the question how cell-surface TrkB, in contrast to intracellular TrkB, becomes active. In TrkB mutants carrying a glutamate or aspartate residue to mimic Try705 phosphorylation, Shc- or PLC adaptor sites remained accessible for phosphorylation. This shows that negative charges in position 705 are required for TrkB activation in absence of BDNF. Notably; this critical residue is not necessarily required for intramolecular phosphate transfer in transactivation. These data raise the question of how the activation loop of the TrkB kinase domain interferes with an external transacting factor to mediate TrkB downstream signalling in the absence of BDNF or whether TrkB itself is responsible for this intracellular effect. Identifying the mechanism underlying Trk activation in absence of the high-affinity ligands is not only important for a better understanding of fundamental processes in development of the nervous system, but also in cancer research, as active Trk being an oncogene can reduce the positive outcome of certain cancer therapies.

Separate pathways for high and low frequency signals in the cerebellar cortex

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Cerebellar granule cells (GCs) are the most numerous neurons in the entire vertebrate brain. According to classical theories about cerebellar computation, each GC detects a specific pattern of active mossy fibers. Yet, mossy fibers convey broad-bandwidth neuronal signals ranging from several hertz up to kilohertz frequencies. How GCs detect this great diversity in the temporal patterns of the mossy fiber inputs remains unclear. Here we show that GCs closer to the white matter are gradually tuned to detect signals with higher frequency, and are less excitable than granule cells close to the Purkinje cell layer. The inner-zone GCs have parallel fibers that are tuned for high speed signal propagation, project preferentially to the base of the Purkinje cell dendritic tree, and elicit faster postsynaptic potentials in the Purkinje cells. Thus, our data suggest the following framework for information processing in the cerebellar cortex: broad-bandwidth mossy fiber signals are separated by GCs with gradually changing biophysical properties. After this frequency dispersion within the GC layer the separated signals were send via specialized downstream pathways to the Purkinje cells. This framework provides exciting implication for theories of cerebellar computation.

Single action potential evoked disynaptic inhibition in vivo

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Cortical excitatory neuronal activity is thought to be control by feedback, disynaptic inhibition from local GABA-ergic interneurons (Galaretta and Hestrin, 1998). In vitro, it has been shown that trains of action potentials can recruit somatostatin (SST) expressing interneurons and lead to disynaptic inhibition of neighbouring pyramidal neurons (Silberberg and Markram, 2007; Kapfer et al., 2007). However, in vivo, layer 2/3 excitatory neurons activity is very sparse firing only single or doublet of action potentials (Barth and Poulet, 2012). To examine which circuit could underlie disynaptic inhibition in layer 2/3 in vivo, we made in vivo multiple two-photon targeted whole cell patch clamp recordings in somatosensory cortex of neighbouring layer 2/3 excitatory neurons and parvalbumin (PV) and SST expressing interneurons in anaesthetized mouse (Jouhanneau et al., 2015). Averaging all paired recording from excitatory neurons shows that the net effect of single excitatory action potential on the excitatory network is a hyperpolarization. Then, we found that a single excitatory action potential fails to recruit SST expressing interneurons but is sufficient to recruit neighbouring PV expressing interneurons that subsequently inhibits the local excitatory network. Thus the net impact of a single excitatory action potential is inhibition through PV expressing interneurons. Our findings provide a rapid inhibitory feedback circuit that may underlie a common organizational principle and might explain the sparse firing of cortical layer 2/3 pyramidal neurons in vivo.

Synaptic performance of lemniscal input fibers onto inferior colliculus neurons

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The mammalian auditory system, especially the periphery and early brainstem stations, is known for fast and precise synaptic transmission. Signals can be transferred with high fidelity and temporal precision even at high frequencies above 200 Hz. The performance of inputs from the cochlear nucleus (CN) and the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO), which are of excitatory and inhibitory nature, respectively, has been intensively studied over the past years. These synapses transmit robustly, implying low failure rates and resistance to synaptic fatigue with half-maximal depression of evoked postsynaptic current (ePSC) amplitudes at 17 Hz (MNTB-LSO) and 11 Hz (CN-LSO). In addition, transmission is fast and precise with peak latencies of ~2 ms and temporal jitter below 100 μ s. Finally, ePSCs are short, lasting 4.8 ms (MNTB-LSO) and 4.0 ms (CN-LSO). Are these features of medullary neurons preserved at higher stations of the auditory brainstem, where temporal precision may be less important? To address this question, the performance of both excitatory and inhibitory synaptic inputs to the inferior colliculus (IC) was analyzed. Whole-cell patch-clamp recordings of IC neurons were performed in acute coronal slices of mice with an age of P11 \pm 1. Synaptic inputs were electrically stimulated via a theta electrode placed within the lateral lemniscal tract. Based on their firing pattern, IC neurons were categorized as onset, sustained, and adapting. Furthermore, ePSC kinetics as well as the performance of the inputs were analyzed upon prolonged high-frequency stimulation (60 s; up to 200 Hz). eEPSCs recorded from onset neurons were shorter than eEPSCs from sustained and adapting neurons (6.1 ms, 8.0 ms, 8.4 ms). Peak latencies amounted to 2.8, 2.9, and 3.4 ms with temporal jitters of 130, 300, and 290 μ s. Regarding the performance of synaptic inputs upon prolonged high-frequency stimulation, similar half-maximal depression values were obtained for the three types (3.8 Hz, 4.6 Hz, 4.8 Hz). Taken together, our results show that onset neurons appear to be superior compared to their counterparts in the IC in most respects. Nevertheless, they do not display the features towards fast and precise synaptic transmission seen at medullary neurons, namely synapses in the LSO. Therefore, we conclude that precise and robust synaptic transmission is not as important as at early stations of the auditory pathway.

Supported by grant Fr 1784/18-1 within the DFG Priority Program 1608.

Synaptic translation of neuroligin 1, 2 and 3

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Neuroligins (NLGNs) are postsynaptic cell-adhesion molecules that bind to presynaptic neuroligins and connect pre- and postsynaptic neurons at synapses. Thus, NLGNs are crucial for the formation, maturation and maintenance of synapses. Here, we aimed at determining whether local translation of NLGNs at the synapse is controlled by Fragile X mental retardation protein (FMRP). As a model we used synaptoneuroosomes isolated from wild type and Fmr1 knock-out mice. Using immunoprecipitation with anti-FMRP antibody, we observed the association of mRNA for all three studied NLGNs with FMRP in synaptoneuroosomes. We analyzed the profiles of polyribosomes isolated from synaptoneuroosomes and assessed polyribosome association of Nlgn1, Nlgn2 and Nlgn3 mRNAs. We observed elevated translation of all three NLGNs at the basal state in Fmr1 KO mice and the lack of response to stimulation, that was observed in WT synaptoneuroosomes. However, the level of total synaptic Nlgn1, Nlgn2 and Nlgn3 mRNAs was unchanged. Moreover, our results indicate that excessive local synthesis of neuroligins in Fmr1 KO mice leads to their elevated levels in synaptoneuroosomes. We have also detected the nascent protein synthesis of NLGNs in synaptoneuroosomes, by incorporation of OP-puromycin followed by click chemistry. Altogether, our results indicate, that neuroligins are locally translated at the synapse and this process is controlled by FMRP.

Systematic investigation of the roles of proteins with calcium-binding domains in synaptic transmission and presynaptic calcium buffering

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Changes in intracellular concentration of free calcium are the most diverse way of regulating intra- and intercellular signaling. Calcium ions entering a nerve terminal upon AP-stimulation are rapidly bound by proteins with calcium-binding domains (CaBPs). Maladaptive calcium-homeostasis and several CaBPs have been linked to neurological disorders, making it important to understand how CaBPs regulate synaptic transmission. We used an electrophysiology-based RNAi screen at the *Drosophila* neuromuscular junction to unravel the roles of presynaptic CaBPs in neurotransmitter release. Specifically, we quantified synaptic transmission after presynaptic overexpression of RNAis targeting 213 CaBP-genes, most of which encode EF-hand and/or C2-calcium-binding domains. This screen identified several candidate mutants with increased or decreased synaptic transmission without apparent changes in synapse morphology. We are in the process of analyzing candidates in terms of genetics, presynaptic calcium dynamics, synaptic physiology and morphology. This approach is expected to reveal new insights into the basic mechanisms of presynaptic calcium-signaling and its role in neural physiology and pathology.

The discrepancy between the presynaptic vesicle fusion rate and the postsynaptic spike rate at the first auditory synapse

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The auditory inner hair cell releases synaptic vesicles at its 10 to 20 presynaptic active zones in response to sound stimuli. The mechanical stimulus causes a graded membrane potential, which leads to the opening of voltage gated Ca²⁺ channels triggering neurotransmitter release. According to Fuchs et al. (2003) only few vesicles are released extrasynaptically. Excitatory postsynaptic potentials (EPSPs) are large and vary in size (Glowatzki and Fuchs 2002). Yet, the transmitter content of one synaptic vesicle is enough to trigger a postsynaptic action potential with a very low failure rate (Rutherford et al., 2012).

In this study, we performed presynaptic recordings of exocytosis at near physiological temperature, and compared this to spike rates in auditory nerve fibers evoked by sound stimuli 30dB above threshold.

Our data indicate that one presynaptic active zone is capable of releasing up to 2300 (normal sized) synaptic vesicles per second during sustained stimulation. The sustained spike rate in the postsynaptic neuron is about 250 Hz and thus one order of magnitude lower.

Where does this discrepancy come from? First, for presynaptic recordings of exocytosis, the cell is depolarized to -14 mV, while sound stimulation depolarizes the cell presumably only to -25 mV (Russell and Sellick, 1983). Still, the difference in Ca²⁺ influx between in vivo and in vitro recordings is presumably less than two-fold.

Is the discrepancy a proof for multivesicular release? The synchronous fusion of two or more vesicles has been shown for central synapses, for which the release is triggered by action potentials. However, at the hair cell synapse, a graded membrane potential and not an action potential elicits vesicle fusion. Modelling studies revealed that this graded change in potential is very unlikely to trigger the simultaneous fusion of two or more vesicles (Chapochnikov et al., 2014). As an alternative, homotypic fusion of synaptic vesicles to form a large vesicle which then fuses to the plasma membrane could provide an explanation. However, large vesicles have only rarely been observed near the active zone membrane at the inner hair cell synapse, and might as well originate from improper vesicle reformation (Strenzke et al., 2016). Modelling predictions revealed that the release probability of a vesicle increases with increasing size (Chapochnikov et al, 2014), which might be an explanation why large vesicles have not been observed. Thus, further studies need to clarify why the postsynaptic spike rate is at least five-fold lower than the number of released synaptic vesicles.

The impact of NKCC1-mediated GABAergic depolarization on the development of hippocampal network activity in mice

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It is widely accepted that GABA undergoes a developmental shift from depolarizing to hyperpolarizing responses in various brain regions. This reflects a change in the intracellular chloride concentration in postsynaptic neurons due to a differential expression of the chloride importer NKCC1 and the chloride exporter KCC2. Although the fundamental mechanisms for the developmental shift of GABAergic responses seem to be well understood, the impact on the maturation of a functional network is still under debate. By knocking out NKCC1 exclusively in cortical glutamatergic neurons early in development, we analyzed the function of depolarizing GABA at different stages of development. Gramicidin-perforated patch recordings from CA3 pyramidal neurons showed that the conditional deletion of NKCC1 resulted in a decreased GABAergic depolarization and accordingly in a reduced probability of GABA-mediated action potential firing. Functionally, we observed a substantial difference at the level of spontaneous network activity at postnatal days 3-4. Confocal Ca²⁺ imaging and simultaneous field potential recordings revealed a complete blockade of synchronized network activity (GDPs) in hippocampal slices of conditional knock out mice similarly to the acute blockade with bumetanide. We conclude that a reduced GABAergic depolarization in excitatory neurons alone suffices to profoundly attenuate spontaneous hippocampal network activity.

The instantaneous time constant as a measure of conductance changes of neurons during excitatory synaptic inputs

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The hippocampus plays an essential role in many cognitive processes, such as spatial navigation or memory formation. In this brain region, there are different network oscillations associated with different behavioral states. These oscillations rely on very synchronous synaptic inputs.

AMPA synapses are the main excitatory inputs in the central nervous system. Their effect on post synaptic cells has been thoroughly studied in patch clamp studies. On the one hand, voltage clamp (VC) configuration can record post synaptic currents, which are an indirect measure of conductance changes due to synaptic activation. As VC sets the neuron to a fixed holding potential, no information about this membrane potential is available. Conversely, current clamp (CC) configuration allows to record membrane potential evolution as a reaction to synaptic input, but gives no information about conductance changes.

We propose a method to estimate the conductance changes due to excitatory synaptic input, while still having information about the membrane potential course. The method relies on the calculation of the instantaneous time constant, τ . τ reflects the value of the overall conductance of the neuron as a function of time and thus capturing the variation of conductance. The method is based in recording an excitatory post synaptic potential (EPSP) preceding along an sinusoidal current injected through the patch pipette. With this procedure, we obtain a set of (I, V, dV/dt) points for each time along the time course of the synaptic event. These points are fitted into an equation of the membrane to calculate τ . Furthermore, averaging the events removes the sinusoidal oscillation and results in the waveform of the EPSP.

We applied this procedure in vitro to CA1 pyramidal cells. Our results show that τ presents faster kinetics than EPSCs, suggesting the effect of space clamp is less pronounced for τ obtained in CC as it is for excitatory post synaptic currents (EPSCs) recorded in VC.

Computational simulation further shows that both τ and EPSCs are affected by space clamp. This distortion is greater for increasing electrotonic distance between synaptic activation and the recording site, although it happens to a lesser extent in the case of τ .

In conclusion, τ is a measure of excitatory conductance due to synaptic input. It is less affected by space clamp than EPSCs and it does not require to have the membrane potential fixed, it may be used to analyze the effect of conductance changes, i.e., passive properties, in synaptic integration.

The presynaptic scaffold-Bassoon-acts as a master regulator of the ubiquitin-proteasome system

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Ubiquitin proteasome system (UPS) is a cellular pathway that modulates protein abundance, activity, and localization with high substrate specificity and in a strictly controlled temporal and spatial manner. Emerging evidence indicates a fundamental role of UPS in the regulation of synaptic function. It has been recently shown that UPS processivity and substrate-specificity are differentially controlled by neuronal activity in the pre- and postsynaptic compartments (Lazarevic et al, 2011). While most of the research is focused on the postsynapse, our goal is to elucidate the role of the UPS at the presynapse.

Bassoon, a presynaptic scaffolding protein involved in the regulation of neurotransmission, is a target of proteasome degradation and can, in turn, control the activity of the UPS in the presynaptic compartment. UPS-mediated degradation of bassoon is a fine-tuned process dependent on the level of the ongoing synaptic activity (Lazarevic et al., 2011). Furthermore, it has been demonstrated that bassoon promotes synaptic stability by directly binding the E3 ligase SIAH, which prompts sequestration and functional inhibition of SIAH (Waites et al., 2013). Our efforts are directed to investigate molecular mechanisms, by which bassoon regulates UPS and how this regulation contributes to the presynaptic function and plasticity. Using imaging and biochemical approaches we are characterizing alternations in the UPS activity as well as proteasome assembly and their reliance on bassoon. Additionally, we study a possible effect of bassoon-proteasome interaction on the synaptic vesicle cycling. Our data reveal changes in the UPS activity as well as synaptic vesicle pool sizes in the bassoon knockout mice. Together, our experimental data substantiate the prime role of the UPS in the fine-tuning of presynaptic function with bassoon as a principal player.

Lazarevic V, Schone C, Heine M, Gundelfinger ED, Fejtova A (2011) Extensive remodeling of the presynaptic cytomatrix upon homeostatic adaptation to network activity silencing. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31:10189-10200.

Waites CL, Leal-Ortiz SA, Okerlund N, Dalke H, Fejtova A, Altmann WD, Gundelfinger ED, Garner CC (2013) Bassoon and Piccolo maintain synapse integrity by regulating protein ubiquitination and degradation. *The EMBO journal* 32:954-969.

The role of the Alanine-Serine-Cysteine-1 transporter in glycinergic transmission

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Glycine is a major inhibitory neurotransmitter of the spinal cord and the brainstem involved in vital functions such as motor control and breathing. Mutations of genes related with glycine receptors or glycine transporters cause hyperekplexia. It has been shown that the deletion of the Alanine-Serine-Cysteine-1 transporter (Asc-1) gene in mice induce a phenotype similar to hyperekplexia mouse models, such as the Glycine Transporter 2 (GlyT2) knock-out mice. Asc-1 Knocked-out mice also displayed low levels of glycine in the brain and decreased glycine transmission. The phenotype was rescued by Glycine or L-serine intraperitoneal injection, indicating that Asc-1 plays a major role in hyperekplexia phenotype involving glycinergic transmission. The aim of this study is to investigate the role of Asc-1 transporter in glycinergic transmission in the brainstem respiratory network. Using whole-cell patch-clamp experiments on neurons of the Pre-Bötzinger complex area in acutely isolated brainstem slices of Mice (postnatal day (P) 1-10, we tested the effects of inhibitors of Asc-1 transporter on miniature inhibitory synaptic currents (mIPSC). CNQX 20 μ M, AP5 100 μ M and Bicuculin 20 μ M were used to isolate mIPSCs. Application of D-Isoleucine (D-ILE), a competitive inhibitor of Asc-1, reduced both the amplitude and frequency of mIPSC. The mIPSC frequency were reduced from 1.16 ± 0.65 Hz before D-ILE to 0.12 ± 0.05 Hz with D-ILE, and mIPSC amplitude was reduced from 28.58 ± 5.9 pA to 16.77 ± 4.2 pA ($p=0.0137$ and $p=0.0195$, respectively). This data confirms an important role of Asc-1 in inhibitory transmission in the respiratory network. The reduction of mIPSC amplitude points towards a role Asc-1 in presynaptic glycine levels.

The SNAP-25 linker is an integral regulator of SNARE-mediated exocytosis.

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The fast and reliable release of neurotransmitters from presynaptic terminals is essential for synaptic transmission. On the molecular level, the formation of ternary SNARE complexes between vesicle and plasma membrane constitutes the central mechanistic step inducing membrane fusion. While the transmembrane domain-containing SNARE proteins Syntaxin-1A and Synaptobrevin-2 each insert a single SNARE domain into the SNARE complex, SNAP-25 is membrane-attached via palmitoylation and contributes two SNARE domains (Q_b and Q_c) to the assembly. As the functional implications of the specialized structural features of SNAP-25 are poorly understood, we have investigated whether the integrity and length of the linker between Q_b and Q_c as well as the positioning of the palmitoylation site are functionally important. Using adrenal chromaffin cells as a model system to study the mechanisms of transmitter release, we co-expressed separated SNARE motifs with adjoining sequences in SNAP-25^{-/-} cells and tested for the restoration of transmitter release using membrane capacitance measurements and amperometry. Intriguingly, we found that separated Q_b and Q_c reestablish transmitter release only at a very low level, even though the employed SNAP-25 fragments readily formed stable SNARE complexes in biochemical experiments. Also highlighting the importance of an unperturbed linker, expression of a mutant SNAP-25 variant, in which the whole linker was substituted by a flexible G/S-containing peptide of the same length, completely failed to rescue secretion. Biochemical assays demonstrated that this mutant variant is less efficient in forming t-SNARE dimers with syntaxin-1A and also delays the assembly of ternary SNARE complexes in comparison to the wildtype protein, which suggests that not only the physical linkage of Q_b and Q_c but also sequence-specific properties of the linker are required for normal SNARE-mediated secretion. A more detailed structural analysis pointed out that N- and C-terminal regions of the linker contain motifs that support exocytosis, in particular by stabilizing primed vesicle pools and triggering fusion. While increasing total linker length did not affect release, shifting the position of the palmitoylation site by insertion of flexible G/S-peptides directly after Q_b selectively slowed down triggering and altered fusion pore behavior. Since flexible insertions at this site also additively decelerated release-rate in a palmitoylation-deficient mutant, the SNAP-25 lipid-anchor is obviously not used for force-transfer from SNARE motif to the plasma membrane. Rather, the palmitoylated N-terminal region of the linker seems to mediate a stabilizing effect on C-terminal SNARE complex assembly. In summary, we show here that the linked arrangement of SNARE motifs in SNAP-25 is essential for fast secretion and that the linker itself acts as a positive regulator of SNARE assembly and membrane fusion.

Ultrastructural analysis of rod photoreceptor ribbon synapses in a Piccolino KO rat

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At conventional chemical synapses, the presynaptic active zone (AZ) is the hotspot for neurotransmitter release from synaptic vesicles (SV). Before SVs are considered fusion competent, they are docked close to calcium channels and primed for exocytosis. This coupling step is mediated via the interaction of a tripartite complex composed of the proteins Rab3A, RIM, and Munc13. In contrast to direct binding of SVs to the AZ at conventional chemical synapses, photoreceptors in the retina possess a presynaptic organelle, the synaptic ribbon (SR), which tethers 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, and whether the proteins Rab3A, RIM, and Munc13 play a role in this process. Recent results from a study in our lab speak against an involvement of RIM.

A candidate protein involved in SV tethering at photoreceptor ribbon synapses is Piccolino, the C-terminally truncated splice variant of the AZ protein Piccolo, sharing multiple homologous domains with RIM^{1,2}. In the current study, we performed an ultrastructural analysis of rod photoreceptor ribbon synapses in a Piccolino knock-out (KO) rat. First, we analyzed synaptic ribbons from Piccolino KO and wild-type (WT) rats in 3D reconstructions from serial sections. In Piccolino KO rats, ribbons exhibited a marked morphological phenotype with small and amorphous synaptic ribbons compared to the large, plate-like ribbons in WT. These findings are in line with a preceding study examining the Piccolino knock-down phenotype in mouse photoreceptors³. Next, we examined whether the loss of Piccolino has an effect on the density of ribbon-associated SVs. SVs were counted at the ribbon site and, as a control, some distance away in the photoreceptor terminal. We found that the density of ribbon-associated SVs in WT rod photoreceptors was about 40% higher than in the control region. In contrast, there was no significant difference in SV densities between ribbon site and control region in the Piccolino KO rod photoreceptors. Our data suggest that Piccolino may play a role in accumulating SVs at the synaptic ribbon.

Currently, electrophysiological and biochemical experiments are performed in order to generate a mechanistic model of Piccolino function at photoreceptor ribbon synapses.

Supported by the DFG (RE 3143/2-1)

¹Wang X et al. (2001) J Biol Chem 276:32480-488.

²Regus-Leidig H et al. (2013) PLoS ONE 8:e70373.

³Regus-Leidig H et al. (2014) Front Cell Neurosci 8:259.

VISUAL PROCESSING AND NETWORK REMODELLING WITHIN AN EPILEPTIC FOCUS IN MOUSE VISUAL CORTEX

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Epilepsy is a group of neurological disorder, affecting over 0.5% of the world population, with a high socio-economic impact. A percentage of patients (30%) remain resistant to drug treatments, in particular patients with focal epilepsy. Electrophysiological studies of refractory epilepsy are currently in progress to get insights into the mechanisms of circuit remodelling, thus paving the way for alternative therapeutic options. However, how plastic rearrangements within the epileptic focus trigger cortical dysfunction and hyperexcitability is still incompletely understood.

Recently, we used the mouse visual cortex to assess plasticity in pathological conditions. In particular, we focus on plastic rearrangements induced by epileptic seizures. We employ a model of neocortical, non-lesional epilepsy based on local delivery of the clostridial enzyme tetanus neurotoxin (TeNT) in mouse visual cortex. TeNT is a metalloprotease that enters synaptic terminals and cleaves the synaptic vesicle protein VAMP/synaptobrevin, resulting in preferential blockade of inhibitory neurotransmission. Delivery of TeNT to the adult cortex results in refractory epilepsy with electrographic seizures persisting for several months, even after the toxin has been cleared from the system. Delivery of the toxin into the visual cortex allowed us to produce a model of focal neocortical epilepsy, that resembles occipital epilepsy in humans.

We used anesthetized TeNT mice to investigate how epileptic rearrangements impact on sensory processing. We recorded local field potentials and spiking activity both during baseline conditions and after presentation of visual stimuli, in control and TeNT-injected mice, at the completion of TeNT effects (i.e 45 days following injection). In TeNT-treated cortices we found that spontaneous neuronal discharge was increased and visual responses were less reliable, with a higher proportion of failure trials, associated to higher spiking activity before stimulus presentation. These findings are accompanied by an increased expression of GABAergic markers. Remarkably, visual acuity was lower than normal, both at electrophysiological and behavioral level. We also investigated how contrast-driven modulations in spiking activity and local field potential spectra were affected by TeNT injection.

To further understand layer-specific rearrangements within the epileptic focus, we recorded control and TeNT mice using multichannel linear probes (16 channels), spanning the whole cortical thickness, allowing a layer-specific analysis of firing rate. Interestingly, we found a clear modulation of gamma-band activity. We also found a significant remodeling of the dendritic arbors of pyramidal neurons, with increased dendritic length and branching, and overall reduction in spine density but significant preservation of mushroom, mature spines. We are also analyzing interneurons population in the different cortical layers, in order to individuate possible effectors of circuit remodeling.

These data demonstrate robust, long-term alteration of visual cortical circuitry associated with specific disturbances of network function in focal neocortical epilepsy, and will be useful to understand how to restore normal circuit activity.

What guides Bruchpilot and RIM-binding protein into the active zone? A domain analysis of two presynaptic core components.

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The presynaptic site of synaptic vesicle fusion, the active zone (AZ) is decorated with membrane close scaffold proteins which aid exo- and endocytosis of synaptic vesicles. A set of conserved core components of the active zone matrix is described, including RIM-binding protein, ELKS/CAST family proteins, SYD-1 and SYD-2/Liprin-alpha. To date it remains largely unknown how these proteins localize and anchor into the AZ. Here, we focus on the two major scaffold proteins RIM-binding protein (RBP) and the *Drosophila* ELKS family homologue Bruchpilot (BRP). First, we precisely investigated BRP localization and orientation at the AZ of *Drosophila* neuromuscular synapses with six different BRP antibodies spread over the entire protein length using super-resolution microscopy (STED). Secondly, through expression analysis of truncated BRP constructs, we identified a minimal localisation domain sufficient for Bruchpilot integration into the active zone and an auto-interaction domain, required for BRP-BRP interaction. Thirdly, deletion analysis of RBP, revealed distinct functions in protein localization and trafficking for specific RBP domains, including the SH3-, FN3- domains and the N-terminus. Finally, we investigated BRP and RBP interaction by biochemical means. In summary we provide novel and detailed insights in the architecture of the active zone scaffold.

μ -opioid receptor-mediated attenuation of midline thalamic inputs to the amygdala

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The dorsal midline thalamus (dMT) is implicated in the retrieval of fear memories. The region is innervated by a vast array of neuropeptidergic fibers, containing e.g. enkephalin, and dMT neurons highly express μ -opioid receptors (MORs). The dMT is connected to a variety of subcortical structures that regulate emotional behavior, among them the basal (BA) and centrolateral amygdala (CeL). Within the amygdala, BA projects excitatory to neurons of the centromedial amygdala (CeM), which mediates conditioned fear behavior via projections to brain stem and hypothalamic nuclei. CeL, on the contrary, exerts inhibitory control over CeM.

We performed patch clamp recordings in combination with retrograde tracing or optogenetics to electrophysiologically characterize dMT-BA and dMT-CeL synaptic connections and investigate how they are modulated by MORs. We found that MORs mediate the hyperpolarization of both BA-projecting and CeL-projecting dMT neurons. Further, we showed that both BA and CeL neurons receive excitatory input from dMT neurons and generate fast postsynaptic responses (eEPSCs). Activation of MORs attenuated transmission at both dMT-BA and dMT-CeL synapses. Interestingly, dMT and CeL exhibited lower apparent synaptic connectivity as compared to dMT and BA. Moreover, dMT inputs evoked substantially smaller eEPSCs in CeL neurons than in BA neurons and the MOR system exerted significantly weaker modulatory influence on dMT inputs to CeL as compared to BA. We finally provided evidence that feed-forward excitation of CeM neurons by dMT afferents is reduced by the MOR system.

Together, these results suggest that MORs are important negative modulators of synaptic transmission between dMT and amygdala, a circuit that is critically involved in the expression of emotional behaviors such as fear.

Poster Topic

T8: Synaptic Plasticity, LTP, LTD

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- [T8-7A](#) Contribution of single and multiple postsynaptic action potentials to dopamine signaling
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- [T8-8A](#) D1-like dopamine receptor activation affects the perisynaptic extracellular matrix in a protein kinase A - dependent manner
Jessica Mitlöhner, Alexander Dityatev, Constanze Seidenbecher, Renato Frischknecht
- [T8-1B](#) Enhanced neuronal excitability and increased number of glutamatergic synapses promote network oscillations in a human stem cell-derived model of autism
Katharina Behr, Philippe Valmaggia, Ravi Jagasia, Josef Bischofberger
- [T8-2B](#) Fast dynamics of endoplasmic reticulum in relation to spine plasticity
Alberto Perez Alvarez, Shuting Yin, Christian Schulze, John A Hammer, Wolfgang Wagner, Thomas G. Oertner
- [T8-3B](#) Ghrelin Stimulates Fyn-mediated Phosphorylation of GluN2B Subunit at Tyr-1336 through the activation of GHSR1a in the Rat Hippocampus
Masako Isokawa

- [T8-4B](#) Hippocampal mossy fiber synapses represent individual computational units
Alexander Drakew, Urban Maier, Michael Frotscher
- [T8-5B](#) Homeostatic plasticity in the brain is facilitated by proteolysis of the extracellular matrix
Armand Blondiaux, Alessandra Pellerito, Eckart D. Gundelfinger, Constanze Seidenbecher, Renato Frischknecht
- [T8-6B](#) Investigating Interactions of MicroRNAs and their Targets in Learning and Memory in the Honeybee (*Apis mellifera*)
Susanne Kraft, Julia Michely, Fabian Kobel, Uli Müller
- [T8-7B](#) Local translation of actin-binding proteins in the central nervous system
Jonas Feuge, Martin Korte, Kristin Michaelsen-Preusse
- [T8-1C](#) Long-term depression (LTD) at hippocampal mossy fiber-CA3 synapses in rodents is independent of BDNF signaling unlike Schaffer collateral-CA1 synapses
Machindra Chandrakant Garad, Elke Edelmann, Volkmar Lessmann
- [T8-2C](#) Madm controls synapse development and maintenance
Kumar Aavula, Ingrid Kieweg, Victoria Bulat, Jan Pielage
- [T8-3C](#) Modulation of dendritic GABA_A receptors rescues impaired NMDA receptor activation in a mouse model of Down Syndrome
Jan Michael Schulz, Frederic Knoflach, Maria-Clemencia Hernandez, Josef Bischofberger
- [T8-4C](#) Patterned stimulation of the piriform cortex induces hippocampal synaptic plasticity in vivo
Denise Manahan-Vaughan, Christina Strauch
- [T8-5C](#) Mover: A novel vertebrate-specific modulator of transmission at specialized synapses
Julio Viotti, Hermes Pofantis, Thomas Dresbach
- [T8-6C](#) Novel mechanism for studying LTM formation: Behavioral Tagging
Shruti Vishnoi, Suhel Parvez
- [T8-7C](#) Optogenetic manipulation of cyclic nucleotides and hippocampal synaptic plasticity
Oana Constantin, Ulrike Scheib, Daniel Udvari, Peter Hegemann, Thomas G. Oertner, Christine E. Gee
- [T8-8C](#) Palmitoylation of Cdc42 maintains its neuronal functions
Alexander Wirth, Norihiko Yokoi, Masaki Fukata, Evgeni Ponimaskin
- [T8-1D](#) Tuning Synaptic Plasticity via Neurogranin-dependent Regulation of Neuronal Phosphoproteome and PP2B Activity
Weifeng Xu, Hongik Hwang, Mathew J. Szucs, Lei J. Ding, Fan Gao, Steven A. Carr, Rushdy Amhad
- [T8-2D](#) Potentiation of input-output relationships during mGluR-dependent LTD at Schaffer collateral-CA1 synapses is mediated by endocannabinoid-dependent LTD of inhibitory synapses

Hye-Hyun Kim, Suk-Ho Lee, Won-Kyung Ho

[T8-3D](#) Priming of hippocampal synapses by dopamine
Annika Briese, Volkmar Leßmann, Elke Edelmann

[T8-4D](#) Repetitive magnetic stimulation modulates inhibitory neurotransmission
Maximilian Lenz, Christos Galanis, Florian Müller-Dahlhaus, Alexander Opitz, Corette J. Wierenga, Gábor Szabó, Ulf Ziemann, Thomas Deller, Klaus Funke, Andreas Vlachos

[T8-5D](#) Ultrastructural reorganization of recycling vesicle pools mediated by long-term plasticity in hippocampus
Stephanie Rey, Catherine Smith, Kevin Staras

[T8-6D](#) Unaltered hippocampal synaptic transmission and plasticity in mice deficient in the actin-binding protein Drebrin
Claudia Gisela Willmes, Till G. A. Mack, Julia Ledderose, Dietmar Schmitz, Christian Wozny, Britta J. Eickholt

[T8-7D](#) Within-gap recovery and rebound effects of LSO inputs
Elisa Krächan, Tatjana Schmitt, Martin Fuhr, Alexander Mehring, Isabelle Römer, Eckhard Friauf

Activity-dependent spatially-localized miRNA maturation in neuronal dendrites

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miRNAs play a key role in the regulation of gene expression in diverse systems. They bind to the 3' untranslated region of target mRNAs and prevent their translation or promote mRNA degradation. A mature miRNA is usually generated by the sequential processing of 2 different precursors: the primary miRNA (pri-miRNA) and the precursor miRNA (pre-miRNA). The pri-miRNA is processed in the nucleus by Drosha resulting in a ~70 nucleotide pre-miRNA. The pre-miRNA, with its characteristic hairpin structure, is then exported to the cytoplasm where it is processed by Dicer to the mature ~22 nucleotide miRNA. In general, the number of potential miRNA targets in a cell is orders of magnitude greater than the miRNA copy number, suggesting that mechanisms exist to increase the likelihood of miRNA-target interactions. To explore regulation of miRNA maturation by external stimuli in space and time, we developed an inducible fluorescent probe to report dicer-mediated cleavage of pre-miRNAs. In neurons, a pre-miRNA-181a probe delivered intracellularly during whole-cell recordings exhibited an activity-dependent increase in fluorescence, indicating the stimulation of miRNA maturation by external stimuli. Single-synapse stimulation using a caged neurotransmitter resulted in a local maturation of miRNA-181a that required the activity of NMDA receptors. The local maturation of miRNA-181a was associated with a spatially restricted and dramatic reduction in the protein synthesis of a target mRNA, CamKII. These data suggest that the spatially and temporally regulated maturation of pre-miRNAs can be used to increase the precision and robustness of miRNA-mediated translational repression.

Age-Dependent Effect of TH-9 on Synaptic Plasticity in the Rat Hippocampus *in vitro*

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Dementia is a general term referring to cognitive deficit, including memory impairment. Alzheimer's disease (AD), the most common form of dementia is one of the most disabling and burdensome health conditions worldwide. Memory loss, the main and initial complaint in AD, is associated with defects in synaptic transmission and plasticity in the hippocampus and other brain areas. Since AD is largely an age-dependent disease and its prevalence continues to rise due to increasing human life expectancy, there is an urgent need for novel drugs that can cure AD. This study investigates the effect of TH-9 on synaptic transmission, long-term potentiation (LTP) and long-term depression in young and old rats.

350- μ m coronal hippocampal slices were generated from brains of male Sprague-Dawley rats aged 1 and 20 months. Evoked, field excitatory postsynaptic potentials (fEPSPs) were recorded from the dendritic layer of area CA1 of the hippocampus by stimulating appropriate afferents. LTP was induced using high-frequency stimulation (HFS; 100 Hz for 1 second) while LTD was elicited using low-frequency stimulation (LFS; 1 Hz for 5 minutes).

TH-9 (10 μ M) increased the slopes of fEPSPs by $34.9 \pm 7.3\%$ ($p < 0.05$) and $38.9 \pm 18.7\%$ ($p < 0.05$) in young and old rats respectively. LTP induction resulted in an increase of $59.9 \pm 11.0\%$ ($p < 0.05$) and $47.4 \pm 16.5\%$ ($p < 0.05$) in fEPSP slopes in slices from young and old rats, respectively. Induction of LTP in the presence of TH-9 resulted in a greater total increase in fEPSP slopes in old rats compared to young rats ($58.3 \pm 10.1\%$ and $89.1 \pm 27.8\%$, respectively). LFS depressed fEPSP slopes by $24.7 \pm 3.4\%$ ($p < 0.05$) and $26.7 \pm 3.9\%$ ($p < 0.05$) in young and old rats respectively. However, pre-treatment with TH-9 abolished LTD responses in old but not young rats. This effect was blocked by rolipram but not H-89

TH-9 enhances LTP in hippocampal slices of both young and old rats while preventing LTD maintenance only in older rats. This action of TH-9 is consistent with a potential to be used for dementia.

Array tomography – high-resolution localization of synaptic proteins in the honeybee brain

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Colonies of the honeybee, *Apis mellifera*, consist of thousands of individuals and have often been described as “superorganisms”. The workers, as the main individual ‘unit’ for the functioning of the superorganism, show a highly adaptive behavioral repertoire enabling the colony to express emergent responses to varying environmental conditions. Variations in behavior as well as learning and memory processes on an individual level have also been shown to be associated with plastic neuronal changes in the brain. Such changes are not only triggered by sensory stimuli but are also linked to age-related maturation processes. Therefore, a detailed understanding of the underlying neuronal architecture and localization of key proteins playing a role in neuronal plasticity and memory formation appear as a fundamental prerequisite to gain insights in the neuronal and molecular processes mediating behavioral plasticity.

Thus far, high-resolution information on neuronal structures exhibiting synaptic plasticity such as the prominent microglomerulus complex synapse in the mushroom body calyx (a higher sensory integration as well as learning and memory center) mainly derives from electron microscopy (EM) and confocal laserscanning microscopy. Both methods, however, have certain disadvantages: while EM in combination with immunogold labeling allows for precise protein detection in specific cell compartments, it clearly lacks possibilities for advanced colocalization analyzes. In contrast conventional confocal fluorescence microscopy offers the tools for multi-protein labeling, but lacks the high resolution of EM for subcellular detection levels. In the present study we applied and adapted, for the first time, array tomography (Micheva and Smith, Neuron, 2007) for use in the brain of the honeybee. This technique combines the advantages of two worlds by correlation of images taken from the same ultra-thin resin sections with high-resolution EM and super-resolution fluorescence microscopy (structured illumination microscopy – SIM). We established a protocol using high-pressure freezing and LR-White embedding that preserves the tissue in a close to in-vivo status allowing for ultrastructural analyzes while leaving antigens intact. The obtained overlay of EM and fluorescent images of ultra-thin serial sectioned tissues provided high-resolution 3D morphological models of synapses with the additional super-resolution 3D information of synaptic protein localization. This approach allowed us to push the limits of the so far achieved resolution in all three dimensions, the analysis of pre- and postsynaptic structures and distribution of synaptic proteins (e.g. synapsin, pCaMKII) in the mushroom body neuropil. Thus, array tomography offers the chance to create reference models for the 3D localization of a bibliography of synaptic proteins.

We believe that array tomography can easily be adapted for the characterization of other neuropils and for other insect species. It seems likely that this combination of ultrastructural synapse morphology in combination with high-resolution protein localization will take neuroanatomical analyzes to new levels.

Supported by DFG SFB 1047 to WR.

BDNF-dependent regulation of hippocampal neuron architecture, activity and plasticity upon Fingolimod treatment

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Brain-derived neurotrophin factor (BDNF) has been shown to promote neuronal survival and differentiation during the development of the central nervous system (CNS). In the adult CNS BDNF signaling, via its receptor TrkB regulates neuronal architecture, adult neurogenesis, synaptic transmission as well as activity-dependent structural and functional plasticity. Moreover, reduced BDNF levels typically correlate with impaired neuronal function as well as deficits in cognitive processes such as episodic memory. Thus, the possibility of increasing BDNF levels with well-tolerated drugs diffusing into the CNS is of extreme interest. In this context, Fingolimod is the first approved oral drug for the treatment of Multiple sclerosis, it has been shown to cross the blood-brain barrier and to increase BDNF levels in neuronal cultures in an activity- and MAPK-dependent way. Moreover, Fingolimod application counteracts NMDA-induced neuronal death in a BDNF-dependent manner indicating its function as “neuroprotectant”.

Whether Fingolimod application influences neuronal morphology, synaptic transmission as well as plasticity in the hippocampus is still unknown. To address this questions, here we applied Fingolimod for 24 hours to hippocampal neuronal cultures. We found that compared to controls, Fingolimod treated cultures showed higher number of cFos positive neurons indicating an increased neuronal activation. This conclusion was confirmed via calcium imaging showing an increase both in the amplitude and in the frequency of calcium transients at spines upon a 24 hours Fingolimod application. Finally, we observed that Fingolimod treatment results in an increase in dendritic complexity, spine density and number of active synapses at hippocampal pyramidal neurons. Importantly, all these effect of a Fingolimod treatment could be prevented by co-application of TrkB receptor bodies to scavenge BDNF. This indicates that the observed effects are indeed depend on BDNF release. Next we will assess the molecular mechanisms mediating the activity of Fingolimod in regulating BDNF levels. Moreover, we are currently testing whether Fingolimod might act in a biphasic way by acutely (within minutes of application) inducing the release of BDNF and chronically (after 24 hours) by increasing its new synthesis.

Our results, indicate that in the intact CNS Fingolimod modulates several aspects of neuronal architecture and function in a BDNF-dependent manner. This is extremely interesting first from the therapeutic point of view to improve CNS function in conditions that have been linked with a decrease of BDNF levels. Moreover, Fingolimod application can be used as a gain-of-function approach to further analyze BDNF functions in the intact CNS.

Blockade of brain angiotensin II AT₁ receptors abolishes capsaicin-mediated deficits in synaptic plasticity in mouse lateral amygdala

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The brain renin-angiotensin system (RAS) has an important neuromodulatory function and its dysfunction seems to play a role in several brain disorders including major depression and bipolar disorders. Indeed, the physiological function of RAS triggered signal transduction pathway and how it is implicated in brain disorders is poorly understood. The RAS generates a family of bioactive angiotensin peptides including angiotensin II which acts through two different high specific receptors, AT₁ and AT₂ expressed in several brain structures including the lateral amygdala. Recently a co-localization and functional interaction between AT₂ receptors and TRPV1 receptors had been described in DRG neurons. Additionally, activation of TRPV1 receptors by capsaicin have been shown to inhibit angiotensin II induced vasoconstriction in mice. These findings raised the question whether AT₁/AT₂ receptors and TRPV1 receptors might interact in neurons of the lateral amygdala, where both receptors are expressed. We have previously found that activation of AT₁ receptors as well as activation of TRPV1 receptors suppress long term potentiation (LTP) induced by high frequency stimulation (HFS) in the lateral amygdala in horizontal mouse brain slices. Using in vitro field potential recordings, we now show that blockade of AT₁ receptors by losartan (100 nm) abolished capsaicin (1 μM) mediated suppression of HFS-induced LTP. In the presence of the L-type calcium channel blocker nifedipine (10 μM) this modulatory effect of losartan was diminished. Application of losartan in the absence of capsaicin had no effect on HFS-induced LTP. Patch-clamp recordings of spontaneous IPSCs indicated that co-application of losartan and capsaicin had an effect neither on GABAergic inhibition nor on membrane potential. Although further investigations are necessary to analyze the underlying mechanisms, our data indicate that a specific blockade of AT₁ receptors might be a useful approach to recover synaptic plasticity and support the hypothesis of losartan acting as a cognitive enhancer.

Chronic manipulation of activity at identified synapses

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Neuroplasticity refers to the brain's ability to reorganize itself by forming new neural connections throughout life. In particular, long term plasticity of synaptic connections between neurons is generally believed to be a key mechanism for learning and memory. What is unknown, however, is whether information storage in the brain relies on the analog strength of synapses or whether changes in neuronal connectivity underlie a more stable, binary form of memory. Altered neuronal activity is known to affect the turnover of dendritic spines, but little is known about the specificity and molecular mechanisms of this process. Previous studies in hippocampal slice cultures have shown that long term depression (LTD), induced by stimulating Channelrhodopsin2-expressing CA3 neurons at 1 Hz, leads to increased elimination of Schaffer collateral synapses during the following days, whereas activation at higher frequencies prevented removal. In addition, it was found that initial low release probability is associated with higher elimination probability after LTD suggesting that the rate of ongoing synaptic transmission determines whether a depressed synapse is retained in the circuit, or not.

Based on this assumption, by chronically changing activity levels at identified synapses during the days following plasticity induction, we can directly assess whether this is the case. Here, we combine optogenetic stimulation of identified Schaffer collateral synapses and two-photon imaging of postsynaptic calcium signals with DREADD technology to chronically dampen synaptic transmission selectively in the pathway under scrutiny. After a first electrophysiological characterization of the hM4D-DREADD silencing tool, we tested the effect of such manipulations on synapse survival by following the fate of individual synapses for 7 days.

Contribution of single and multiple postsynaptic action potentials to dopamine signaling

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Increasing evidence suggests that dopamine has an important neuromodulatory role in spike timing-dependent synaptic modifications in the hippocampus. However, the synaptic mechanisms that govern such effects are not fully understood.

Experimentally, long-term synaptic modifications can be achieved by repetitive (classically 60-100 times) synchronous pre- and postsynaptic activation (i.e., spike pairings) within millisecond time intervals at low frequency, which is known as spike timing-dependent plasticity (STDP). Here we examined the actions of endogenous dopamine on timing long-term potentiation (t-LTP) at Schaffer collateral (Sc)-CA1 synapses in the hippocampus induced with very few repetitions (6 times) of spike pairings in which one excitatory postsynaptic potential (EPSP) was paired with a single or short barrages of 4 postsynaptic action potentials (1EPSP/1AP and 1EPSP/4AP, respectively).

We found that both STDP paradigms induced a robust t-LTP (30-60min after induction), which, however, rely on different dopamine signaling mechanisms. The t-LTP induced with 1EPSP/1AP was NMDA and L-type Ca⁺² channel dependent and required both, D1-like and D2-like dopamine receptor activation. In contrast, the t-LTP induced with 1EPSP/4AP was independent of these Ca⁺² sources and only D2-like receptor activity was required. Furthermore, synaptic plasticity induced with 1EPSP/4AP led to a significant increase of AMPA receptor mediated currents compared to unpaired controls, whereas the 1EPSP/1AP protocol did not induce any changes in AMPA receptor conductance. These results suggest that enhancement of GluA1 subunit trafficking is required selectively for synaptic plasticity induced with the 1EPSP/4AP paradigm.

All together these data indicate, that the specific pattern of pre- and postsynaptic spikes used for STDP induction decides which signaling events (i.e. source for Ca⁺² elevation, dopamine receptor subtype activation) are recruited to establish t-LTP (see more at *symposium 25*). Thus, STDP represents a powerful tool to determine the impact of variations in the strength, frequency or number of spike pairings on molecular signaling that underlies long-term synaptic modifications. This approach could give us a new insight into the computational properties and information storage at the single cell level.

The work was funded by the DFG (SFB779, B06).

D1-like dopamine receptor activation affects the perisynaptic extracellular matrix in a protein kinase A - dependent manner

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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. A specialized form of the brain's ECM has been shown to surround pre- as well as postsynapses and to be formed and remodeled in an activity-dependent manner, thus being important for synaptic plasticity, learning, and memory formation.

Dopamine, a crucial neuromodulator for motivated learning, acts through either D1- or D2-like dopamine receptors in the brain. Activation of protein kinase A (PKA) via stimulated D1/D5 dopamine receptors was shown to lead to enhanced extracellular tissue-type plasminogen activator (tPA) activity probably associated with an increased release of this protease. We hypothesized that a similar mechanism may underlie ECM remodeling by specific extracellular proteases, such as ADAMTS 4/5.

Here, we studied the impact of dopamine in ECM modification by proteolytic cleavage of brevican, a major chondroitin sulfate proteoglycan component of mature brain ECM. Therefore, in primary neuronal cultures D1-like or D2-like receptors were activated by dopamine receptor agonists SKF81297 and Quinpirole, respectively. Immunocytochemical analysis with a cleavage-indicating neo-epitope-specific antibody revealed that perisynaptic brevican cleavage is increased only after D1-like receptor activation. We could block this effect by the D1-like dopamine receptor antagonist SCH23390 as well as by using a PKA inhibitor (cAMPS-Rp) or an inhibitor of ADAMTS 4 (TIMP-3). Taken together, these findings strengthen our hypothesis of an interplay between the dopaminergic system and the integrity of the perisynaptic ECM, though the exact molecular and cellular mechanisms remain to be clarified.

Enhanced neuronal excitability and increased number of glutamatergic synapses promote network oscillations in a human stem cell-derived model of autism

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Valproic acid (VPA) is an anticonvulsant drug with histone deacetylase (HDAC) inhibition activity which is frequently used to treat epilepsy and bipolar disorders. VPA intake during pregnancy is associated with an increased risk of the unborn child to develop autism spectrum disorder (ASD). Using rodent models it could be shown that in utero VPA exposure results in an ASD-related behavioral phenotype in the offspring. On the neuronal level, alterations in both excitatory and inhibitory synaptic transmission as well as enhanced NMDA-mediated long-term potentiation have been proposed to contribute to these ASD phenotypes.

To investigate the effect of VPA on developing human neurons, we used hESC-derived human neural stem cells that can be differentiated into synaptically connected neuronal networks within 6 to 10 weeks. Neuronal cultures are treated with low concentrations of VPA (0.6 to 1 mM) throughout the entire differentiation process. We used whole-cell current-clamp and voltage-clamp recordings to study the maturation of passive and active membrane properties, miniature postsynaptic currents as well as spontaneous network activity, which revealed several important functional differences.

First, passive membrane properties were similar during early differentiation (2 weeks) but developed significantly slower with time in VPA treated neurons. At about 10 weeks, the input resistance was higher ($1.44 \pm 0.62 \text{ G } \Omega$; versus $0.54 \pm 0.3 \text{ G } \Omega$; , $P < 0.001$, $n = 9$, and 7) and the membrane capacitance was smaller than in control condition ($50.1 \pm 20.7 \text{ pF}$ versus $102.3 \pm 40 \text{ pF}$, $P < 0.004$, $n = 8$, and 9). In accordance with the increased input resistance, VPA treated neurons showed enhanced electrical excitability. Action potentials could be elicited by significantly smaller current pulses (10 ms duration) in VPA than in control neurons ($163.3 \pm 66.2 \text{ pA}$ versus $442.9 \pm 278.2 \text{ pA}$, $P < 0.02$, $n = 6$, and 7). Second, miniature excitatory postsynaptic currents (mEPSCs) in the presence of TTX and gabazine showed significantly higher frequency ($1.46 \pm 0.73 \text{ Hz}$ vs. $0.52 \pm 0.43 \text{ Hz}$, $P < 0.02$, $n = 9$, and 6) and amplitude ($25.3 \pm 3.8 \text{ pA}$ vs. $17.4 \pm 6.2 \text{ pA}$, $P < 0.03$, $n = 9$, and 6) in VPA treated neurons compared to control neurons indicating enhanced formation of glutamatergic synapses upon VPA treatment. By contrast, frequency and amplitude of miniature GABAergic currents (mGPSCs), measured in TTX, CNQX and AP5, were unchanged. Finally, we observed a strong increase in spontaneous synaptic activity in VPA treated networks. At about 6 to 7 weeks of differentiation ~83% of the VPA treated cultures (1 mM) showed rhythmic burst activity which could not be detected in control neurons at this developmental stage.

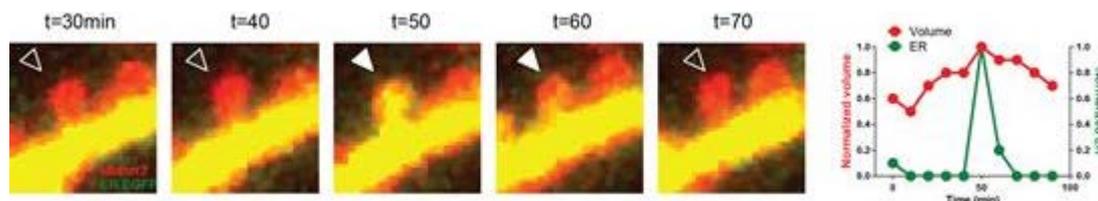
Taken together, our results indicate that early VPA exposure of human neurons during embryonic development leads to network hyperactivity, which is most likely generated by enhanced electrical excitability combined with a shift in synaptic excitation-inhibition balance. These mechanisms could substantially contribute to the increased ASD susceptibility upon in utero VPA exposure.

Fast dynamics of endoplasmic reticulum in relation to spine plasticity

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The precise role of intracellular organelles in spines is poorly understood, but there is evidence for effects on synaptic plasticity. The presence of endoplasmic reticulum (ER) allows mGluR-dependent depression and local calcium signalling in voluminous dendritic spines which bear strong synapses (Holbro et al., 2009 PNAS). Knocking out synaptopodin, a protein that is essential for ER organization into a 'spine apparatus', has modest effects on synaptic plasticity (Deller et al., 2003 PNAS; Vlachos et al., 2008 Hippocampus). However, what determines acquisition or loss of the endoplasmic reticulum by dendritic spines and the impact on synaptic properties remains unanswered. Therefore, we set out to monitor the temporal dynamics of ER and synaptic structure. Using multiphoton microscopy, we followed GFP-labelled ER and the volume of dendritic spines over time in organotypic hippocampal slices at physiological temperature. ER movements in and out of spines were much more dynamic than previously thought, occurring on a time scale of minutes rather than days (Toresson and Grant, 2005 EJM). We could distinguish 2 classes of ER dynamics: In some spines (~10%), the ER remained present for hours. The majority of ER intrusions, however, were short-lasting (< 20 min). About 20% of CA1 hippocampal spines possess ER at any given time point but more than 50% were visited within 2 hours, and progressively more in longer time periods. Interestingly, the volume of dendritic spines was at its maximum at the time of ER insertion, pointing to a tight correlation between ER and structural plasticity (see example shown below). A spine apparatus, revealed by the simultaneous presence of ER and synaptopodin, was mostly found in spines that contained stable ER. Inducing spine structural plasticity (sLTP) by repetitive two photon glutamate uncaging, we observed that spines were typically invaded by ER immediately after sLTP induction. Spines that contained stable ER before sLTP induction did not grow further. We found that fast ER dynamics depended on myosin Va activity and were positively modulated by glutamate receptors. Expressing in single CA1 neurons a myosin Va (MyoVa) dominant negative (DN) pointed to a role of this molecular motor in spine ER insertion. MyoVa DN abolished the aforementioned fast ER dynamics, reduced the fraction of the stable ER-positive spines and blocked LTP induction as revealed by whole cell patch clamp. Our time-lapse analysis agrees with the concept that spine ER acts as a 'brake' on spine growth and synaptic potentiation (Holbro et al., 2009 PNAS).



Ghrelin Stimulates Fyn-mediated Phosphorylation of GluN2B Subunit at Tyr-1336 through the activation of GHSR1a in the Rat Hippocampus

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Increasing evidence suggests ghrelin and its cognate receptor the ghrelin receptor (GHSR1a) have numerous physiological functions in the brain besides their orexigenic role in the hypothalamus. For example ghrelin is reported to stimulate the brain's reward center as well as to improve memory retention, suggesting the potential importance of ghrelin as a key molecule for reward-related learning. Although GHSR1a is highly localized in the hippocampus, a molecular mechanism for ghrelin-mediated hippocampal function is poorly understood. The NMDA receptor (NMDAR) is a tetrameric assembly of GluN1 and GluN2 subunits and essential for hippocampal plasticity and learning. We previously reported that ghrelin stimulated phosphorylation of GluN1 and enhanced NMDAR-mediated synaptic currents. In the present study, the effect of ghrelin on GluN2B is studied as GluN2B is a primary GluN2 subunit and participates in plasticity-associated synaptic changes. Exogenous application of ghrelin activated GHSR1a and increased phosphorylation of GluN2B (pGluN2B) at Tyr1336 in the cultured rat hippocampal slices. This increase was independent of the NMDAR activity because it was insensitive to ifenprodil and MK801. However, it depended on Fyn activity as it was blocked by PP2 (the inhibitor of Fyn). Forskolin, Rp-cAMP, TBB, ryanodine, and thapsigargin amplified pGluN2B up to three-folds when they were applied together with ghrelin, suggesting a negative involvement of cytosolic calcium, cAMP, PKA, and CKII signaling pathways. In contrast, KN-93 did not affect ghrelin-induced pGluN2B indicating CaMKII was not involved. In conclusion, GluN2B is likely a molecular target of ghrelin and GHSR1a-mediated phosphorylation of NMDAR. It has been suggested that GluN2B phosphorylation at Tyr-1336 contributes to the mobility and surface expression of NMDAR. Thus, ghrelin-induced phosphorylation of GluN2B may play a critical role in the successful acquisition of metabolic demand-based synaptic plasticity and adaptive appetitive behavior. Supported by NIH grant R15DA021683.

Hippocampal mossy fiber synapses represent individual computational units

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The dentate gyrus relays information flow from the entorhinal cortex to the hippocampus proper. Individual dentate granule cells (GC) projecting via the mossy fibers (MF) to hippocampal CA3 and to mossy cells (MC) receive differential input from entorhinal axons. MCs in turn innervate GCs thereby forming a feed-back loop that potentially enables GCs to modulate AP firing of other GCs. If indeed GCs contribute individually to microcircuits, one would expect that individual MF synapses are encountered in individual states of synaptic transmission at a given time point. However, this is difficult to assess experimentally. In order to analyze the structure and function of single MF synapses, we combined single-bouton stimulation and two-photon imaging of spines postsynaptic to the stimulated mossy fiber bouton. We labeled MCs in organotypic entorhino-hippocampal slice cultures in the whole-cell patch clamp configuration using dye-filled pipettes. Alexa 594 was used to visualize the morphology of spines, whereas Fluo-4ff served to report calcium transients in single spines. Alexa 488 released from a second pipette transiently stained the extracellular space and allowed for targeted cell-attached patching of unlabeled boutons presynaptic to labeled identified spines. Stimulation of the bouton evoked very heterogeneous excitatory synaptic responses pointing to a varying contribution of different glutamate receptors of MF synapses even on the same MC. Some of the excitatory postsynaptic potentials were suprathreshold resulting in AP firing of the MC. We identified three synaptic states defined as all subthreshold, all suprathreshold, and mixed sub- and suprathreshold responses. Stimulation of individual MF synapses with a defined activity pattern modified these synaptic states depending on the initially encountered state. These results suggest that each mossy fiber synapse contributes individually to computation in the hippocampus.

Supported by the Deutsche Forschungsgemeinschaft (FOR 2419: FR 620/14-1) and Landesforschungsförderung Hamburg (LFF-FV27b) to M.F.; M.F. is Research Professor for Neuroscience of the Hertie Foundation.

Homeostatic plasticity in the brain is facilitated by proteolysis of the extracellular matrix

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Homeostatic mechanisms of neurons and synapses are important for maintenance of neuronal networks' baseline function and stability despite of multiple plastic challenges. Synaptic homeostasis and synaptic scaling involve structural reorganization processes in synaptic contacts.

Pre- and postsynaptic elements are surrounded by a brain-specific form of extracellular matrix (ECM). Brevican is one of its main components and we have found that its cleavage is increased around synapses after network silencing. Here, we hypothesize that the perisynaptic extracellular matrix contributes to homeostatic changes of synapses. As experimental model to induce homeostatic plasticity we used long-term treatment of primary neuronal cultures with the sodium channel blocker tetrodotoxin (TTX). It has been shown that this treatment affects total and synaptic expression levels of both pre- and postsynaptic proteins upon network silencing. We wondered whether proteolytic processing of ECM components is necessary for the regulation of synaptic proteins and homeostatic plasticity in the CNS.

To test this, we used 21 days in vitro (DIV) dissociated cortical cultures from rats and treated them with TTX for 48 hours to silence neuronal networks. We then measured the abundance of synaptic proteins by immunocytochemistry in the presence or absence of matrix metalloprotease inhibitors or before and after ECM dissociation by hyaluronidase treatment.

Expression levels of synaptic markers such as Homer, GABAR 2, Synapsin and vGlut were incremented after metalloprotease inhibition during network silencing, but not upon ECM degradation. Simultaneous treatment with TTX and hyaluronidase seems to have a cooperative effect.

Our results indicate that proteolytic processing of the ECM via matrix metalloproteases is necessary for the regulation of many synaptic markers during homeostatic plasticity upon network silencing. Now we want to clarify if the extracellular matrix proteolysis is implicated in disturbed homeostasis in disorders like epilepsy.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 642881

Investigating Interactions of MicroRNAs and their Targets in Learning and Memory in the Honeybee (*Apis mellifera*)

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MicroRNAs are small regulatory non-coding RNAs mediating degradation of transcripts or post-transcriptional silencing by antisense interaction with a target mRNA. They are key players in neuronal circuits like maturation, connectivity and plasticity of neurons. Only little is known about their modes of action in learning. We therefore took up investigating microRNA-target interactions that might be involved in memory formation and synaptic plasticity in the honeybee brain. The study focuses on the well-conserved neuron-specific miR-124 and its previously identified target the AMPA-type glutamate receptor (GluA2). The obvious approach was to look at changes in associative learning behaviour and changes in miR-124 and GluA2 levels after functional inhibition of miR-124. Moreover, we investigated associative learning behavior after induction of Glutamate receptor agonists. For further understanding of underlying mechanisms, we established an in situ hybridization to localize the miR-124 and its target GluA2 in order to find possible interaction sites. Additionally we wanted to investigate the amounts of GluA2 mRNA in three age-groups of honeybees since it was already proven that there are age-dependent changes of the miR-124, which might thus be a result of microRNA mediated regulations. By our approach investigating the miR-124 and its target with different experimental setups, we will gain a better understanding of miRNA-target interactions and their implications in memory formation.

Local translation of actin-binding proteins in the central nervous system

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Memory storage is widely believed to involve long-lasting modifications of synaptic efficacy and is associated with either strengthening (long-term potentiation (LTP)) or weakening (long-term depression (LTD)) of single synapses. The mechanisms involved have been shown to be dependent on de novo gene transcription but also translation of pre-existing mRNAs. Interestingly, several thousands of mRNAs as well as the whole protein synthesis machinery were found in dendrites and it could be shown that localized stimuli can induce rapid local mRNA translation in dendrites. These local translation events are tightly regulated in space and time and allow synapses to autonomously change their structural and functional properties in response to incoming action potentials on a short timescale. Morphological changes are based on modifications of the actin cytoskeleton as actin is the most prominent cytoskeletal protein in the pre- and postsynaptic compartment. During different forms of synaptic plasticity, a fast remodeling of the actin cytoskeleton can be observed which is mediated by actin-binding proteins (ABPs). As a large number of ABPs is involved, the question arises how these processes are regulated and if ABPs are locally translated at dendritic spines upon need and if mRNA localization of ABPs is influenced by neuronal activity.

This study aims to analyze the mRNA localization of the three ABPs profilin 1 (PFN1), profilin 2a (PFN2a) and cofilin (CFL) using fluorescence in situ hybridization (FISH). For this purpose, mRNA localization was analyzed in vivo using hippocampal slices as well as in vitro using 21d old primary embryonic hippocampal cultures gained from C57 BL6 GFP mice. To investigate if the mRNA localization of neuronal profilins or cofilin is influenced by neuronal activity, mRNA localization was additionally analyzed in primary embryonic hippocampal cultures 20, 40 and 60 min after chemical induction of NMDA receptor dependent LTP using a glycine/strychnine protocol. Finally, this work aims to prove that all three ABPs analyzed here are locally translated. Therefore, 21d old primary embryonic hippocampal cultures will be transfected with a vector expressing membrane-bound GFP fused to either one or both of the untranslated regions of the respective ABP and fluorescence recovery after photobleaching will be analyzed in dendrites with or without the presence of the translation inhibitor anisomycin.

Our results show that the mRNA of PFN1, PFN2a as well as CFL is present in dendrites and moreover directly at dendritic spines in vitro and in vivo suggesting that all three actin-binding proteins are locally translated. Additionally, we could show that the amount of PFN2a mRNA present in the cell significantly increases overall after induction of cLTP accompanied by an increase in the amount of mRNA located in dendrites. In contrast to this, the mRNA localization of PFN1 and CFL remains unaltered.

In summary, this work provides evidence for the dendritic localization of ABP mRNAs and their regulation by neuronal plasticity.

Long-term depression (LTD) at hippocampal mossy fiber-CA3 synapses in rodents is independent of BDNF signaling unlike Schaffer collateral-CA1 synapses

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Long-term depression (LTD), underlying learning and memory, is an activity-dependent reduction in synaptic efficacy that lasts for hours or longer. The hippocampal synapses reveal LTD and the hippocampus itself is thereby a crucial structure in information processing. The neurotrophin brain-derived neurotrophic factor (BDNF) is most likely the key instructor for plasticity-related processes underlying long-term memory; diverse studies indicate a critical role of BDNF particularly in long-term potentiation (LTP). Mature (m) BDNF and its precursor, proBDNF preferentially activate tropomyosin related kinase-B (TrkB) and pan neurotrophic receptors (p75NTR), respectively. Although hippocampal mossy fiber (MF) synapses are rich in both pro- and mBDNF, their role in MF synaptic plasticity, mainly LTD, remains elusive.

Using extracellular field potential recording in the Cornu Ammonis (CA)3 region of acute hippocampal slices, we show that acute and chronic interference in p75NTR signaling does not influence LTD induced with standard low frequency stimulation (LFS; 900 pulses @ 1 Hz). Similarly, acute inhibition of TrkB signaling does not affect LTD. Thus, evidence from the present study specifies standard LFS induced LTD to occur independent of p75NTR and TrkB signaling. In future, we will apply either p75NTR specific REX antibodies or the BDNF scavenger TrkB-Fc during LTD recordings to test our preliminary data interpretation. In addition, we perceived impairment in LTD induced with strong LFS (2 X 900 pulses @ 1 Hz; 5 min interval) in the presence of LM11A31 (p75NTR ligand, 100 nM) and K252a (tyrosine kinase inhibitor, 200 nM) in comparison to respective vehicles. K252a was administered at a concentration that is believed to be selective for Trk tyrosine kinase inhibition.

Several previous studies indicate the crucial role of BDNF-TrkB and proBDNF-p75NTR signaling in LTP and LTD, respectively, at Schaffer collateral (SC)-CA1 synapses. Earlier, we showed that acute and chronic interference in BDNF-TrkB signaling impairs LTP at MF synapses (Schildt et al., *Neuropharmacology* 71: 247-54, 2013). The results from current study indicate that LTD at MF synapses is different compared to SC synapses and might involve expression mechanisms independent of pro- and mature BDNF signaling.

Madm controls synapse development and maintenance

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The precise regulation of synaptic growth and maintenance is essential for the development and functionality of neural circuits. Here, we aim to identify the molecular mechanisms controlling synapse formation and stability *in vivo*.

In a large-scale RNAi-based genetic screen using the *Drosophila* neuromuscular junction (NMJ) as a model system, we identified the Mlf1 adapter molecule (Madm) as a novel regulatory factor coordinating synaptic growth and stability. Loss of Madm results in severe impairments of NMJ growth and morphology and resulted in inappropriate synaptic retractions. Using tissue-specific rescue assays we demonstrate that Madm is required both pre and post synaptically to regulate synaptic growth and development. Using genetic interaction studies in combination with high resolution imaging assays, we then demonstrate that Madm functions through the mTOR pathway to regulate synaptic growth. Together, our study identifies Madm as an important novel regulator of synaptic development and maintenance.

Modulation of dendritic GABA_A receptors rescues impaired NMDA receptor activation in a mouse model of Down Syndrome

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Increased inhibition is believed to cause cognitive deficits in Down syndrome. Selective modulation of δ -subunit containing GABA_A receptors by negative allosteric modulators (5-NAM) has been shown to improve hippocampus-dependent learning. In the current study, we investigated the underlying cellular mechanisms using whole-cell patch-clamp recordings in the CA1 region of hippocampal brain slices. Application of the δ -5-NAM RO4938581 reduced slow dendritic inhibitory postsynaptic current (IPSC) amplitude to $64.4 \pm 14.4\%$ of the control, whereas somatic IPSCs remained unaffected. The dendritic nature of the δ -5-NAM-sensitive inputs was confirmed in mice expressing channelrhodopsin (ChR2) in dendrite-targeting interneurons as well as by spatially localized GABA release in the presence of tetrodotoxin from interneurons expressing ChR2 under the control of the vesicular GABA transporter promoter. Remarkably, application of the δ -5-NAM increased burst EPSPs up to $137.0 \pm 15.8\%$ and rectified deficient NMDA receptor activation in Ts65Dn mice. Furthermore, impaired theta-burst stimulation-induced LTP in Ts65Dn mice ($110.1 \pm 3.1\%$; wt: $127.1 \pm 7.2\%$) was rescued by acute application of the δ -5-NAM ($122.9 \pm 6.2\%$). These results demonstrate that δ -5-NAM counteracts the inhibition-to-excitation imbalance in Down Syndrome to allow for normal dendritic integration and plasticity essential for learning and memory.

The work was supported by the Roche RPF-program and the Swiss National Science foundation (SNF, 31003A-153276)

Patterned stimulation of the piriform cortex induces hippocampal synaptic plasticity in vivo

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Synaptic plasticity is an underlying mechanism of learning and memory. Olfactory stimuli comprise potent cues for memory retrieval and are readily integrated into new associative memory engrams, whereby associative memory is encoded by the hippocampus. How exactly the olfactory system influences hippocampal information storage is not known, however. But learning about olfactory spatial cues promotes hippocampal long-term depression (André and Manahan-Vaughan, 2013, *Hippocampus* 23:963) and hippocampal place fields anchor to spatial localized olfactory stimuli (Zhang and Manahan-Vaughan, 2015, *Cereb Cortex* 25:423). Furthermore, olfactory discrimination learning alters hippocampal neuronal excitability (Chaillan et al., 1996, *J Physiol Paris* 90:343; Truchet et al., 2002, *Hippocampus* 12:600). The question therefore arises as to whether the primary olfactory (piriform) cortex relays information to the hippocampus that directly supports synaptic information storage.

We therefore investigated the influence of patterned stimulation of the piriform cortex on evoked field responses in the hippocampus of freely behaving adult rats. High-frequency stimulation of the piriform cortex resulted in hippocampal long-term potentiation, whereas low-frequency stimulation induced short-term depression. These results indicate that olfactory information derived from the piriform cortex, potently influences synaptic information storage in the form of hippocampal synaptic plasticity. This may comprise a mechanism whereby olfactory information is integrated into associative memories.

This work was supported by a German Research Foundation (DFG) grant to DM-V (SFB 874, B1).

Mover: A novel vertebrate-specific modulator of transmission at specialized synapses

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The release of neurotransmitters is a highly complex sequence of events taking place in the presynaptic terminals, resulting in one or more synaptic vesicles fusing with the presynaptic membrane. The probability that such a release takes place is governed by a plethora of molecules, and it greatly varies among subsets of synapses.

Mover is a vertebrate-specific synaptic vesicle protein with an unusually heterogeneous distribution among synapses. For example Mover is present at the glutamatergic synapses of the area CA3 of the hippocampus but not at the GABAergic synapses of that region. In addition, we detected Mover in subsets of excitatory and inhibitory synapses in the cerebellar cortex and the auditory brainstem. At the Calyx of Held, knockdown of Mover increases release probability and short-term depression, suggesting that Mover has a modulatory role at subsets of synapses.

Here we show that Mover is heterogeneously expressed at subsets of excitatory terminals in the auditory brainstem and that its expression is regulated by neuronal activity. To determine the role of Mover at specialized synapses, such as the Calyx of Held and the hippocampal mossy fiber terminals, we generated a knockout mouse strain. These knockout mice have enhanced frequency facilitation, increased paired-pulse ratio and high-frequency facilitation in the Hippocampal Mossy Fiber - to CA3 synapse, but not in the Schaffer Collateral - to CA1 synapse. These data suggest that Mover boosts release probability and dampens frequency facilitation in hippocampal Mossy Fibre terminals.

These discoveries, together with the changes in the Calyx of Held, suggest that Mover is an activity-dependent, synapse-specific regulator of presynaptic plasticity. Moreover, they suggest that a) Mover has distinct roles at different synapses; b) generally acts to dampen the extent of presynaptic events; c) acts as a brake that can be released during low activity. At the hippocampal Mossy Fibre terminal, activity-dependent regulation of Mover could increase the dynamic range for the induction of frequency facilitation and working memory.

Novel mechanism for studying LTM formation: Behavioral Tagging

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Novel mechanism for studying LTM formation: Behavioral Tagging

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Abstract

Learning can change an animal's behavior and an animal can store that information acquired through learning for much longer time in their memory. New information that is acquired is stored in the form of two temporally and mechanistically different memory types: a short term memory (STM) and a long term memory (LTM). Memories are intertwined in nature but science has limited knowledge about the mechanisms behind memory formation. Here, we tried to understand this, using two behavioral learning tasks viz. Novel Object Recognition (NOR) and Conditioned Taste Aversion (CTA). Here we have shown that in animals subjected to weak training that is capable of producing only STM, LTM can also be promoted and produced in contingency with a novel, not familiar event within a critical time window. Novelty promotes synthesis of Plasticity Related Proteins (PRP's). These PRPs when captured at tagged sites allow memory consolidation. Weak training generally sets a "learning tag" which is used by PRP's to form LTM. A learning process leading to LTM formation initiates both setting of a tag and synthesis of PRP's side by side. The mechanism revolving around has been termed as "Behavioral Tagging" which is an analogue of synaptic tagging and capture process.

Keywords: NOR, CTA, PRP, STM, LTM

Optogenetic manipulation of cyclic nucleotides and hippocampal synaptic plasticity

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The cyclic nucleotides, cAMP and cGMP are ubiquitously expressed in eukaryotic cells. In neurons, cAMP in particular has been implicated as a key second messenger mediating activity-dependent synaptic plasticity. Various pre- and post-synaptic pathways have been invoked depending on the synapse being studied. Most studies have used forskolin stimulation of endogenous adenylyl cyclases combined with inhibition of phosphodiesterases to induce synaptic potentiation. While effective at inducing synaptic potentiation, such tools affect all cells in the preparation. We have taken advantage of recent developments in optogenetic tools to study the effects of selectively raising the cyclic nucleotides cAMP and cGMP in only the presynaptic or postsynaptic compartments of hippocampal Schaffer collateral synapses. Surprisingly, stimulation of bPAC (*Beggiatoa* photoactivated adenylyl cyclase) with blue/UV light in the postsynaptic CA1 neurons did not alter excitatory postsynaptic potentials (EPSCs) or alter the threshold for synaptic plasticity. Likewise, EPSCs recorded in CA1 neurons in response to action potentials evoked in presynaptic CA3 neurons co-expressing bPAC and ChrimsonR were not affected by raising cAMP with blue/UV light. The endogenous adenylyl cyclases that are thought to be responsible for the synaptic plasticity-inducing rises in neuronal cAMP are membrane associated and it has been postulated that the cAMP is largely confined to and mediates its actions within a microdomain adjacent to the plasma membrane. As bPAC is a soluble adenylyl cyclase, activating it will increase cAMP throughout the cytoplasm, possibly accounting for the lack of effect on synaptic transmission when compared with activating endogenous adenylyl cyclases. Rhodopsin guanylyl cyclase (RhGC) from *Blastocladia emersonii* (beRhGC) has recently been described (Avelar et al., 2014, Curr. Biol.; Scheib et al., 2015, Sci. Signalling; Gao et al., 2015, Nat. Commun.). As there are also suggestions that cGMP may be important for setting synaptic strength, we have expressed RhGC in CA1 neurons to see if raising cGMP close to the plasma membrane would affect EPSCs. Green light applied to CA1 neurons expressing beRhGC with and without concomitant block of phosphodiesterases also had no effect on EPSCs, suggesting that increasing postsynaptic cGMP is also insufficient to increase synaptic transmission. In conclusion, increases in only presynaptic or only postsynaptic cAMP appear to be insufficient for increasing the strength of synaptic transmission and additional mechanisms must be involved.

Palmitoylation of Cdc42 maintains its neuronal functions

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Nearly the half of synaptic proteins can undergo post-translational S-palmitoylation, which has emerged as an important regulator of signalling-protein functions involved in synaptic plasticity. Acting as dynamic hydrophobicity switch, palmitoylation can influence membrane insertion and sub-compartmentalisation of modified proteins. It plays a crucial role in the regulation of multiple processes in the central nervous system, including modulation of neuronal morphology and synaptogenesis. Here we investigated molecular mechanisms of the brain-specific small GTPase Cdc42-palm, which undergoes palmitoylation. As a key modulator of cellular morphology, Cdc42 plays an important role in regulating dendrite and spine structural plasticity. We addressed functional consequences of palmitoylation in respect to regulation of the neuronal signalling pathways. Our investigations revealed that a small fraction of Cdc42 is mono-palmitoylated in the hippocampus. We further identified palmitoyltransferase DHHC5 as an enzyme responsible for Cdc42 palmitoylation that favours cysteine residue 188 of Cdc42 as a site of palmitoylation. Functionally, DHHC5-mediated mono-palmitoylation of the Cdc42 isoform is responsible for the ability of Cdc42 to modulate dendritic morphology and gene activation.

Tuning Synaptic Plasticity via Neurogranin-dependent Regulation of Neuronal Phosphoproteome and PP2B Activity

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Long-term plasticity of excitatory synaptic transmission is an important cellular mechanism underlying learning and memory. It is known that coincident of pre- and post-synaptic neuron firing induces calcium (Ca) influx through glutamatergic NMDA receptors (NMDARs). This Ca influx is transduced via Ca-binding protein, calmodulin (CaM) to engage downstream Ca/CaM-dependent signaling events that eventually lead to either long-term potentiation (LTP) or long-term depression (LTD). However, it is still not known how regulation of Ca/CaM dynamics is achieved to control the directionality of synaptic plasticity. Additionally, we know little about the molecular substrates that are critical for regulating synaptic plasticity. Using a combination of molecular approaches, electrophysiology and mass spectrometry-based quantitative phosphoproteomics, we find that neurogranin (Ng), a neuron-specific CaM binding protein, controls the threshold of spike-timing-dependent long-term potentiation (STDP-LTP) by regulating Ca/CaM-dependent protein phosphatase 2B (PP2B; Calcineurin) activity. Decreasing Ng levels blocks STDP-LTP by enhancing synaptic PP2B activity, which dephosphorylates the Grin2A subunit of NMDARs and accelerates the decay of NMDAR-mediated synaptic currents. Conversely, increasing Ng levels prolongs the STDP-LTP timing window at Schaffer Collateral-CA1 synapses by suppressing PP2B activity. Taken together, our results suggest that the dynamics of Ng levels at different behavioral and/or pathological states influence the basal phosphorylation state of neurons by tuning PP2B activity, and therefore, influence the expression of synaptic plasticity.

Potential of input-output relationships during mGluR-dependent LTD at Schaffer collateral-CA1 synapses is mediated by endocannabinoid-dependent LTD of inhibitory synapses

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Input-output relationships in neural networks are determined not only by synaptic efficacy but also by neuronal excitability. In spite that alterations of synaptic efficacy by various forms of synaptic stimulations have been extensively investigated, it is relatively less investigated how signaling mechanisms that induce synaptic plasticity affect neuronal excitability and changes input-output relationships. Here we induced metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) of Schaffer collateral (SC)-CA1 synapses by using low-frequency stimulus (paired stimuli with 50 ms inter-pulse interval at 1 Hz for 15 min; PP-LFS), and investigated intrinsic excitability changes and action potential outputs during LTD. We measured intrinsic excitability parameters before and 30 min after induction of LTD, and found that input resistance was increased and action potential threshold (V_{th}) was hyperpolarized during LTD without significant changes in resting membrane potentials or sagging. Interestingly, in spite of the decrease in the amplitude of excitatory post-synaptic potentials (EPSPs) during LTD, the EPSP-to-spike (E-S) coupling was significantly potentiated during LTD when APs were evoked by a train of 5 pulses at 50 Hz. These results suggested that the positive effect of hyperpolarization of V_{th} exceeds the negative effects of decreased EPSPs on input-output relationship. Potentiation of E-S coupling and increases in intrinsic excitability during LTD were abolished by GABA receptors blockade, suggesting that disinhibition of GABAergic pathways is involved. Indeed, we confirmed that LTD of inhibitory synapses (i-LTD) is induced by PP-LFS. Finally, we found that PP-LFS dependent E-S coupling and intrinsic excitability changes are abolished by blocking endocannabinoid signaling, especially CB1Rs. Together, we propose that mGluR activation during PP-LFS induces LTD of inhibitory synapses via retrograde endocannabinoid signaling as well as LTD of glutamatergic synapses and that final outcome of mGluR activation is the increase in input-output relationship of CA1 pyramidal neurons.

Priming of hippocampal synapses by dopamine

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Long-term potentiation (LTP) is an important form of synaptic plasticity and a cellular correlate for learning and memory processes. Depolarization of the postsynaptic membrane is induced via excitatory postsynaptic potentials (EPSPs). Summated EPSPs give rise to a local field-EPSP (fEPSP) that can be measured with extracellular electrodes. Using high-frequency or theta-burst-stimulation, a stable early-LTP (e-LTP) is induced and can be measured as increased slope of the fEPSP. As previously shown, the likelihood to induce and the magnitude of LTP are affected by neurotransmitters such as dopamine. Neuromodulators can either directly modulate or can “prime” synapses to undergo a certain type of long-lasting change in synaptic efficacy. This process is called metaplasticity. However, the underlying mechanisms are not fully understood.

To investigate signaling mechanisms underlying metaplasticity on a cellular level we chose field potential recordings in acute hippocampal slices of 8-12 weeks old, male C57BL6/J mice. Recordings of fEPSPs were performed in the CA1 area in response to presynaptic stimulation of Schaffer collateral axons. We first established an appropriate protocol to induce stable and robust e-LTP by using a 3x 100 Hz high-frequency-stimulation (HFS, interval: 30 s between tetani). Since our aim is to better understand the importance of neurotransmitters such as Dopamine and their role in priming or metaplasticity processes, we additionally established a protocol that was subthreshold for LTP induction but which could be restored to full strength by bath application of dopamine during LTP recording. After testing the potency of dopamine to restore or facilitate LTP under subthreshold conditions, we next focused on the time course and duration of dopamine application to prime synapses for successful LTP under subthreshold induction conditions. In these experiments we asked whether 10 min preincubation with dopamine can restore subsequent and/or delayed LTP induced with subthreshold stimulations.

Our experiments will contribute to the understanding of neuromodulation of synaptic plasticity and determine the fate of synapses to undergo a certain change. That knowledge will be helpful to understand concepts such as behavioral tagging by shedding light on the underlying synaptic mechanisms.

This work is supported by the DFG (SFB779/B06).

Repetitive magnetic stimulation modulates inhibitory neurotransmission

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Non-invasive brain stimulation techniques are widely used in neurology and psychiatry for diagnostic and therapeutic purposes. Among them, repetitive transcranial magnetic stimulation (rTMS) is employed as a modulator of cortical excitability. However, the cellular and molecular mechanisms of rTMS-induced neural plasticity remain not well understood. Here, we tested the hypothesis that repetitive magnetic stimulation (rMS) could act on neural networks by dampening inhibitory neurotransmission, i.e., through disinhibition. Using immunohistochemistry, fluorescence recovery after photobleaching, GABA-uncaging experiments and paired whole-cell patch clamp recordings of entorhino-hippocampal slice cultures, we show that 10 Hz rMS induces a calcium-dependent reduction in inhibitory neurotransmission on CA1 pyramidal neurons. Furthermore, our results disclose that rMS acts differentially on dendritic and somatic inhibition. These findings provide an attractive explanation how a seemingly unspecific, i.e., exogenous electromagnetic stimulation could prime the ability of neurons to express endogenous, i.e., task- and input-specific excitatory synaptic plasticity. Accordingly, we propose that rTMS may assert its positive effects, at least in part, through disinhibition of cortical neurons. [supported by Federal Ministry of Education and Research, Germany; GCBS-WP1: 01EE1403B]

Ultrastructural reorganization of recycling vesicle pools mediated by long-term plasticity in hippocampus

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At small central synapses, presynaptic vesicle pools are important possible substrates for contributing to changes in synaptic efficacy associated with forms of plasticity such as long-term potentiation (LTP) or long-term depression (LTD). At CA3-CA1 excitatory synapses in the hippocampus, the expression of LTP is thought to involve changes in presynaptic sites but the specific impact on functional vesicle pools remains unclear. Here, we used FM-dye labelling methods, diaminobenzidine photoconversion and serial electron microscopy in acute hippocampal slices to explore ultrastructural and functional vesicle pool properties after the induction of LTP. We demonstrated an increase in the functional pool size and a change in the docked vesicle pool organization. We also found that the activation of cAMP/PKA signalling pathways is involved in the reorganization of the docked vesicle pool, offering an insight into underlying molecular targets. Our experiments reveal the intrinsic relationship between presynaptic nanoscale reorganisation of vesicle pools and the persistent change in synaptic strength induced by LTP.

Unaltered hippocampal synaptic transmission and plasticity in mice deficient in the actin-binding protein Drebrin

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The dynamic regulation of the actin cytoskeleton plays a key role in controlling the structural integrity and function of synapses. It is vital for activity-dependent modulation of synaptic transmission and long-term changes in synaptic morphology associated with memory consolidation. Several regulators of actin dynamics at the synapse have been identified, of which a salient one is the postsynaptic actin stabilising protein Drebrin (DBN). It has been suggested that DBN modulates synaptic transmission and changes in dendritic spine morphology associated with synaptic plasticity. In light of the fact that a decrease in DBN levels is correlated with cognitive deficits associated with ageing and dementia, it was hypothesised that DBN protein abundance instructs the integrity and function of the synapse.

We created a novel mutant mouse line deficient for DBN. Analysis of gross brain and neuronal morphology revealed no phenotype in the absence of DBN. Electrophysiological recordings in acute hippocampal slices and primary hippocampal neuronal cultures showed that basal synaptic transmission, and both long-term and homeostatic synaptic plasticity were unchanged, suggesting that loss of DBN is not sufficient in inducing synapse dysfunction.

We propose that the overall lack of changes in synaptic function and plasticity in DBN deficient mice may indicate robust compensatory mechanisms that safeguard cytoskeleton dynamics at the synapse.

Within-gap recovery and rebound effects of LSO inputs

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Sound source localization in mammals is achieved by processing interaural time and level differences (ILD) in the auditory brainstem. Synapses involved in these tasks are capable of transmitting signals in a precise and reliable manner, even at periods of sustained high-frequency activity. To do so, they are equipped with several morphological and molecular features, e.g. ribbon synapses in the inner hair cells and giant, calyceal presynaptic terminals (endbulbs and calyces of Held). The lateral superior olive (LSO) is the center of the mammalian ILD pathway and integrates excitatory signals from the ipsilateral path and inhibitory signals from the contralateral path. Although LSO inputs lack any morphological specifications, they have to process binaural signals in the millisecond range over sustained periods of time. In this study, we assessed the synaptic response characteristics of both inhibitory and excitatory LSO inputs to continuous (60 s) high-frequency activity opposed to a more physiological burst-gap activity pattern (insertion of gaps of silence). To do so, we performed whole-cell voltage clamp recordings at LSO principal neurons of juvenile mice (P11) while electrically stimulating excitatory glutamatergic inputs from the cochlear nucleus (CN-LSO) or inhibitory inputs from the medial nucleus of the trapezoid body (MNTB-LSO). During sustained activity at moderate levels (50 Hz), postsynaptic currents (ePSCs) of both LSO inputs depressed to similar extent with steady state levels of ~20% of the baseline. High activity rates of 200 Hz led to stronger short-term depression (STD) with steady state levels below 10%. Insertion of 200 ms gaps at moderate activity rates led to 2-fold higher steady state levels compared to continuous stimulation. The effect was gone at high activity rates where continuous and burst-gap stimulation led to similar steady state levels. Inserting gaps resulted in a higher fidelity and superior temporal precision as evidenced by a lower jitter in ePSC latency. To further elucidate the effect of burst-gap patterns, we analyzed the first ePSC of each burst (ePSC1) and found an unexpected increase of amplitudes after initial STD, which we called rebound effect. The rebound effect was present only at the MNTB-LSO synapses, i.e. it was absent at CN-LSO synapses and only at 200 Hz. ePSC1 amplitudes showed a gradual increase with maximal values of ~15% compared to maximal depression levels reached earlier in the stimulation train. The rebound effect was also prominent at P20 and appears to be preserved into young adulthood (P35). It is probably of presynaptic origin because we observed a constant quantal size, which excludes postsynaptic receptor desensitization and saturation. Although the rebound effect appears to be unaffected by changes in the external Ca^{2+} concentration, we found evidence for a dependency on intracellular Ca^{2+} levels as it shows a strong temporal correlation with the asynchronous release rate. Taken together, we found a rebound effect as a specific functional feature of inhibitory MNTB-LSO synapses. It is probably an adaptation that enables the shift to onset responses under sustained high activity and thereby efficient coding of burst patterns.

Supported by grants Fr 1784/17-1 and /18-1 within the DFG Priority Program 1608.

Poster Topic

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- [T9-5D](#) The impact of astrocytes morphology on their Ca²⁺ characteristics

Andre Zeug, Franziska E. Müller, Volodymyr Cherkas, Evgeni Ponimaskin

[T9-6D](#) The role of PMP22 in CMT disease type 1A
Friederike Art, Robert Fledrich, Ruth M. Stassart, Olaf Jahn, Klaus Armin Nave, Michael W. Sereda

Absence of the astrocytic AP-2 disrupts intracellular calcium and sodium homeostasis and dysregulates glutamate uptake and lysosomal function.

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Astrocytes are the most numerous cells in the mammalian central nervous system where they fulfill diverse functions such as neurotransmitter uptake, control of the extracellular ion balance and pH, supply of nutrients to the nerve tissue, support of the cells that comprise the blood-brain barrier and aid in post-traumatic repair and scarring processes among others. To accomplish these functions, astrocytes express specific ion channels, transporters and receptors whose surface expression needs to be dynamically regulated. However, despite the wealth of information about the membrane proteins involved in metabolic functions in astrocytes, the relationship between these processes and endocytic pathways in glial cells under normal and pathological conditions remains unclear. Upon perturbing endocytosis by means of knocking out AP-2, which is a key protein to coordinate cargo selection into clathrin coated pits, we found that loss of astrocytic AP-2 affects the intracellular trafficking of the endosomal Na⁺/H⁺ Exchangers (eNHEs) which are a subset of transporters linked to a variety of neurological disorders such as autism, attention deficit hyperactivity disorder, intellectual disability and epilepsy. These transporters determine luminal pH and regulate cation content in endosomal compartments. Cell surface biotinylation experiments revealed that eNHEs were strongly mislocalized to the plasma membrane in AP-2-depleted astrocytes, leading to changes in intracellular sodium concentration and compromising the glutamate uptake, one of the most important astrocyte functions. In addition, AP-2 KO astrocytes also undergo changes in intracellular Ca²⁺ resulting in an altered autophagic function and energy metabolism. Moreover we found an abnormal accumulation of late endosomal – lysosomal structures as well as a higher number of autophagosomes, obtaining a phenotype resembling to what is seen in lysosomal storage disorders (LSDs). These results bring out the role of AP-2 in regulating the trafficking and activity of eNHEs and how this is crucial for maintaining a proper ionic homeostasis, very important for the activity of glutamate transporters and lysosomal functionality. This provides new clues for understanding NHE-linked neurological disorders and LSDs.

Analysis of microglial synaptic surveying territory in the stratum radiatum of CA1 of sedentary and exercised Wistar rats.

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There have been significant efforts to investigate the contribution of microglia in surveying their local environment in the brain parenchyma, and recently the focus has been on their role in surveying and monitoring synaptic health. Physical exercise has been shown to enhance neuronal and synaptic plasticity, and a common mechanism underlying the effects of exercise over brain function might be related to inflammation. There are evidences that physical exercise induces the expression of a proneurogenic microglial phenotype in the hippocampus (Kohman et al, 2012). The aim of the present work was to analyze the effects of physical exercise on the synaptic territory surveyed by microglial cells in hippocampal formation. Eight adult Wistar rats (4 months old) were submitted to either 5 weeks of forced treadmill exercise or left sedentary. The brains were removed and processed for selective microglia/macrophage immunolabeling with anti-IBA-1 antibodies. Microglial cells on the stratum radiatum of CA1 were randomly/systematically chosen and three-dimensionally reconstructed using NeuroLucida software. To estimate how many synapses fall within the territory of a single microglia cell we assumed 449 synapses in a block of tissue of 6x6x5 μm^3 of the stratum radiatum neuropil based on previous findings (Bartol et al., 2015). We also assumed a spherical domain for each microglial cell (Jinno et al, 2007). Considering the proposed spatial arrangement of theoretical territory surveyed by a single microglial cell, we used Sholl analysis to estimate the critical radius of the microglia and calculated the average microglial volumes of $310.18 \times 10^{-6} \text{ mm}^3$ and $292.22 \times 10^{-6} \text{ mm}^3$ (radius of 42,1 and 41,8 μm) for sedentary and exercised groups, respectively. Correcting the volume density for shrinkage of 25% we estimated a total between 331,597.00 and 728,926.55 synapses inside a single microglial spherical territory for exercised and sedentary groups, respectively. Our findings suggest that the microglial morphology, and consequently the number of synapses inside a microglial territory is influenced by environmental changes. However, the frequency of microglial contact with synapses, the number of synapses surveyed by a single process, the contact time per synapse as well as the mechanisms and physiological implications underlying these differences are still poorly understood.

Ca²⁺-permeable AMPA receptors in periglomerular astrocytes of the mouse olfactory bulb

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Astrocytes are involved in a plethora of functions in the nervous system. As part of the blood-brain barrier, they provide nutrients to neurons and generally modulate synaptic plasticity and transmission of neurons.

In this study, we focused on periglomerular astrocytes located in the glomerular layer of the olfactory bulb. This astrocyte population intermingles with juxtglomerular interneurons and directly influences membrane properties of mitral cells, the principle neurons of the olfactory bulb, as well as interneurons such as granule cells. Olfactory bulb astrocytes communicate with surrounding neurons via metabotropic neurotransmitter receptors linked to internal Ca²⁺ release, however, whether Ca²⁺ signalling in olfactory bulb astrocytes is also mediated by Ca²⁺-permeable AMPA receptors is not known so far.

We used Ca²⁺ imaging, patch-clamp and immunohistology to investigate the presence of Ca²⁺-permeable AMPA receptors in periglomerular astrocytes. Ca²⁺ imaging was performed on acute slices of the murine olfactory bulb. Periglomerular astrocytes were identified by their responsiveness to ADP (Doengi et al., FASEB J 2008). The cells were stimulated with AMPA to induce Ca²⁺ responses. We observed that Ca²⁺ signals in about 40% of periglomerular astrocytes were persistent after slices were preincubated with bafilomycin to inhibit neuronal transmitter secretion and tetrodotoxin was added to the bath solution to block neuronal activity. These Ca²⁺ signals decreased in a Ca²⁺-free environment and increased after extracellular Ca²⁺ was re-added. Furthermore, the evoked Ca²⁺ signals could be reversibly blocked by 1-naphthyl acetyl spermine (nasp), a selective blocker for the Ca²⁺ permeable AMPA receptor, indicating the presence of Ca²⁺-permeable AMPA receptors in periglomerular astrocytes. Additionally, we used GLAST-CreETR2xGCaMP6sfl/fl mice to measure Ca²⁺ signals in periglomerular astrocytes generated by electrical stimulation. Ca²⁺ signals from electrical stimulation could also be reversibly blocked by nasp. Acutely dissociated periglomerular astrocytes from hGFAP-eGFP mice were used for patch-clamp experiments. Currents induced in periglomerular astrocytes by kainate administration could be partially blocked by nasp. Immunostaining showed an equal distribution of the AMPA receptor subunits GluR1, GluR2 and GluR4 in astrocytes of the glomerular layer. Since it has been shown that AMPA receptors including the GluR2 subunit are not Ca²⁺-permeable (Burnashev et al., Science 1992), the histological data suggest that AMPA receptors in periglomerular astrocytes are at least partly Ca²⁺-impermeable. From our physiological and histological results we conclude that periglomerular astrocytes express several subtypes of AMPA receptors including some with and some without the GluR2 subunit, the latter being Ca²⁺-permeable. Supported by the DFG (LO779/10).

Carbachol-evoked astrocytic calcium signals in hippocampal slices

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Background:

A role of the cholinergic system in patients Alzheimer's disease (AD) was already indicated in the late 1970s by a number of studies. The correlation between reduced cholinergic markers including ChAT, mAChR and nAChR and clinical ratings of dementia led to the formulation of the cholinergic hypothesis of memory dysfunction in AD (Schliebs & Arendt, 2011). Glia influence synaptic transmission at the hippocampal CA3-CA1 synapse. Synaptically released glutamate from the Schaffer collaterals and acetylcholine from the Alveus evoke a calcium elevation in astrocytes in hippocampal slices (Araque *et al.*, 2002). Properties of synaptically evoked astrocyte calcium signals reveal synaptic information processing by astrocytes (Perea & Araque, 2005). It is unclear whether synaptically evoked astrocytic calcium signals modulate synaptic information transfer and whether this process is changed in APP/PS1 (Alzheimer) mice. Here, we first examine the effect of carbachol on calcium signals in astrocytes.

Methods:

Acute hippocampal brain slices (300µm) were prepared from 28-42 days old C57Bl6 mice. Astrocytes were visualized live by incubation with Sulforhodamine 101 (1-2µM). For calcium imaging, astrocytes were bath loaded with Fluo4-AM (2µM) and Pluronic F-127 (0,002%) for 15 min at 32°C. Whole-cell voltage-clamp recordings were obtained from pyramidal neurons. Spontaneous EPSCs were recorded in voltage-clamp mode at -65mV for 3 minutes to obtain a baseline level of activity. During the same period spontaneous astrocytic calcium signals were recorded from 5-9 astrocytes from the same slice. Carbachol (100µM) was bath applied for 5 minutes, where after a second recording period of 3 minutes followed. To exclude a major effect of neuronal firing, the experiment was repeated in the presence of TTX (1µM) which blocks all neuronal spiking activity.

Results:

Under baseline conditions we observed spontaneous unsynchronized calcium activity in astrocytes. Carbachol seems to modulate this activity. The level of synchrony between the astrocytes and the spatial organization of the calcium events are to be determined. Using patch clamp recordings from neurons in the same slice, we are currently investigating whether the astrocytic calcium signals affect neuronal activity.

Conclusions:

- The overlap between Fluo-4 signals and the specific astrocyte marker SR101 indicates that Fluo-4AM can at least qualitatively be used for calcium imaging in astrocytes in vitro
- Bath applied carbachol, which activates acetylcholine receptors, seems to affect the spontaneous calcium signals in the astrocytes. This effect appears to be independent of neuronal activity

References:

Araque, A., Martin, E. D., Perea, G., Arellano, J. I., & Buno, W. (2002). Synaptically Released
2+

Acetylcholine Evokes Ca²⁺ Elevations in Astrocytes in Hippocampal Slices. *J. Neurosci.*, 22(7), 2443–2450. doi:20026212

Perea, G., & Araque, A. (2005). Properties of Synaptically Evoked Astrocyte Calcium Signal Reveal Synaptic Information Processing by Astrocytes. *Journal of Neuroscience*, 25(9), 2192–2203. doi:10.1523/JNEUROSCI.3965-04.2005

Schliebs, R., & Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. *Behavioural Brain Research*, 221(2), 555–563. doi:10.1016/j.bbr.2010.11.058

Characterization of ion channels in astrocytes proliferating in response to acute brain injury

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Glial cells are the most abundant cells in the brain and astrocytes represent one important subclass. They play a major role in brain injury and several neurological diseases by becoming reactive under these conditions. Their reactions show a great heterogeneity ranging from retention of morphology to polarization of processes, hypertrophy and even division. Juxtavascular astrocytes, a distinct subgroup with their somata directly adjacent to blood vessels, are prone to selectively proliferate after traumatic brain injury (TBI) in the somatosensory cortex of mice (Bardehle et al., 2013). We want to characterize the electrophysiological properties and the different types of ion channels of juxtavascular astrocytes and compare them with those of non-juxtavascular ones, because it is known that ion channels play an important role in cell-cycle progression and therefore cell proliferation (Urrego et al., 2014). We use the BAC Aldh1l1 eGFP transgenic mouse strain which expresses eGFP in astrocytes. By means of whole-cell patch-clamp recordings as well as immunohistochemical stainings we identified different ion channel subtypes. We have focused on inwardly rectifying K⁺ (K_{ir}) channels, due to their contribution in maintaining the resting membrane potential and the redistribution of potassium across astrocytic membranes. By means of immunohistochemistry we identified the K_{ir}4.1 and K_{ir}6.2 channel subunits; as well as the K_v4.3 channel giving rise to A-type currents. We confirmed these findings by pharmacologically blocking the channels in patch-clamp experiments. Our astrocytes possess K⁺ currents sensitive to Ba²⁺, an effective blocker of K_{ir}4.1 channels. Furthermore we were able to identify the T-type low voltage activated Ca²⁺ channels Ca_v3.1 and Ca_v3.2 in immunohistochemical stainings. These channels are important for rapid influx of calcium into cells, which contributes to cellular signalling including induction of gene expression. It has also been shown that some T-type Ca²⁺ channels are overexpressed in highly proliferative cancer cells. We found no obvious difference in ion channel expression of juxtavascular and non-juxtavascular astrocytes in the healthy brain. Therefore, by means of electrophysiology and immunohistochemistry we examined whether a stab wound lesion in the somatosensory cortex of the same mouse line induces a difference or an upregulation of ion channel expression after astrocytes become reactive in response to the brain lesion.

Differences in the molecular structure of the blood-brain barrier in the cerebral cortex and white matter

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The blood-brain barrier (BBB) is the main interface controlling molecular and cellular traffic between the central nervous system (CNS) and the periphery. It consists of cerebral endothelial cells (CECs) interconnected by continuous tight junctions, and closely associated pericytes and astrocytes. Different parts of the CNS have diverse functions and structure and may be subjects of different pathologies, in which the BBB is actively involved. It is largely unknown however, what are the cellular and molecular differences of the BBB in different regions of the brain. Using *in silico*, *in vitro* and *ex vivo* techniques we compared the expression of BBB-associated genes and proteins (i.e. markers of CECs, brain pericytes and astrocytes) in the cortical grey matter and white matter. *In silico* human database analysis (obtained from recalculated data of the Allen Brain Atlas), qPCR, western-blot and immunofluorescence studies on porcine and mouse brain tissue indicated an increased expression of GFAP in astrocytes in the white matter in comparison to the grey matter. We have also found increased expression of genes of the junctional complex of CECs (occludin, claudin-5, alpha-catenin) in the white matter in comparison to the cerebral cortex. Accordingly, occludin, claudin-5 and alpha-catenin proteins showed increased expression in CECs of the white matter in comparison to endothelial cells of the cortical grey matter. In parallel, barrier properties of white matter CECs were superior as well. These differences might be important in the pathogenesis of diseases differently affecting distinct regions of the brain.

Visualization of tetrapartite synapse: Towards understanding the logic of structural synaptic plasticity

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Synapses are no longer considered as bipartite communication sites between pre- and postsynaptic compartments. Over the last two decades, many studies have implicated an important role of astroglial processes and extracellular matrix (ECM) in the mechanisms of synaptic plasticity. Both astrocytic processes and ECM seem to be closely associated with synaptic compartments and appear to be dynamic partners that are modulated by neuronal activity and can feedback and regulate various signaling processes pre- and postsynaptically. Neuronal activity can modulate ECM either by the secretion and incorporation of ECM protein into the matrix and/or by digestion of certain ECM protein by extracellular release of matrix degrading proteases such as matrix metalloproteases (MMP's) and a disintegrin and metalloproteinase with a thrombospondin type 1 motif (ADAMTS). Similarly, glial processes are highly dynamic and are centrally involved into synaptic transmission by regulating the mechanisms like neurotransmitter uptake. Considering various evidences, it can be conceived that a functional synapse would have well-organized four components ("Synaptic Quadriga" or "Tetrapartite Synapse") in order to have proper functioning (Dityatev et al., *Results Probl Cell Differ.* 2006; *Trends Neurosci.* 2010; Dityatev and Rusakov, *Curr Opin Neurobiol*, 2011). Various synaptopathies arises due to dysregulation in one or more components of a healthy synapse. So, it is highly desirable and of high relevance to study the sequence and logic of changes in these four components during structural synaptic plasticity in vitro and in vivo. To achieve this, we have developed four AAV based viral vectors that can label all four components of both excitatory and inhibitory synapses and can be used to study dynamic morphological changes under various stimulation conditions using high resolution time-lapse imaging. Simultaneous imaging of all compartments during various stages of development of hippocampal cultures, synaptogenesis, stabilization of synapses and activity dependent changes in mature synapses might give very useful insight about such processes.

Expression of functional inhibitory neurotransmitter transporters and receptors in astrocytes of the inferior colliculus and the hippocampus

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Neuronal inhibition is mediated by glycine and/or GABA. Inferior colliculus (IC) neurons receive mixed glycinergic and GABAergic inputs, whereas inhibition in hippocampus predominantly relies on GABA. Astrocytes heterogeneously express neurotransmitter transporters and are expected to adapt to the local requirements regarding neurotransmitter homeostasis. Here we analyzed the expression of inhibitory neurotransmitter transporters and receptors in IC and hippocampal astrocytes using whole-cell patch-clamp recordings. Acute 270 μm -thick brainstem and hippocampal slices were prepared from C57Bl6 mice at postnatal days 10 to 12. Astrocytes were labeled with the red fluorescent dye sulforhodamine 101 (Kafitz et al., 2008; Stephan and Friauf, 2014). In our study we could show that astrocytes of both regions expressed functional glycine transporters (GlyTs). Upon activation these transporters mediated a profound inward current (I_{Gly}) that was sensitive to the competitive GlyT1 agonist sarcosine. Glycine did not alter the membrane resistance (R_M) arguing for the absence of functional glycine receptors (GlyRs). Thus, I_{Gly} was not compromised by GlyR-mediated currents. Similarly, we found expression of functional GABA transporters (GATs) in IC and hippocampal astrocytes. Upon activation these transporters mediated an eminent inward current (I_{GABA}) that was sensitive to the competitive GAT-1 and GAT-3 antagonists NO711 and SNAP5114, respectively. GABA transiently reduced R_M only in hippocampal astrocytes demonstrating the presence of GABA_A receptors (GABA_ARs). However, I_{GABA} was mainly not contaminated by GABA_AR-mediated currents as R_M changes vanished shortly after GABA application. Interestingly, in both regions I_{GABA} was stronger than I_{Gly} . Furthermore, the hippocampal $I_{\text{GABA}}/I_{\text{Gly}}$ ratio was markedly elevated. Taken together, our results demonstrate that IC and hippocampal astrocytes in part exhibit common features, namely the expression of functional GlyT1, GAT-1, and GAT-3. Moreover, our data indicate a higher adaptation of hippocampal astrocytes to GABA homeostasis than IC astrocytes.

Functional anisotropic panglial networks in the lateral superior olive

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Astrocytes form large gap junctional networks that contribute to ion and neurotransmitter homeostasis. Astrocytes concentrate in the lateral superior olive (LSO), a prominent auditory brainstem center. Compared to the LSO, astrocyte density is lower in the region dorsal to the LSO (dLSO) and in the internuclear space between the LSO and the superior paraolivary nucleus (SPN). We questioned whether astrocyte networks exhibit certain properties that reflect the precise neuronal arrangement. Employing whole-cell patch-clamp and concomitant injection of a gap junction-permeable tracer, we analyzed size and orientation of astrocyte networks in LSO, dLSO, and SPN-LSO in acute brainstem slices of mice at postnatal days 10-20. The majority of LSO networks exhibited an oval topography oriented orthogonally to the tonotopic axis, whereas dLSO networks showed no preferred orientation. This correlated with the overall astrocyte morphology in both regions, i.e. LSO astrocyte processes were oriented mainly orthogonally to the tonotopic axis. To assess the spread of small ions within LSO networks, we analyzed the diffusion of Na⁺ signals between cells using Na⁺ imaging. We found that Na⁺ not only diffused between SR101⁺ astrocytes, but also from astrocytes into SR101⁻ cells. Using PLP-GFP mice for tracing, we could show that LSO networks contained astrocytes and oligodendrocytes. Together, our results demonstrate that LSO astrocytes and LSO oligodendrocytes form functional anisotropic panglial networks that are oriented predominantly orthogonally to the tonotopic axis. Thus, our results point toward an anisotropic ion and metabolite diffusion and a limited glial crosstalk between neighboring isofrequency bands in the LSO.

Glial activity patterns in memory-related networks of mice

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Recent findings using Ca^{2+} activity indicators provide first ideas how and why neuronal activity triggers specific activity patterns in astroglial networks in the hippocampus. However, it is still unsolved whether the controversially discussed neurotransmitter activation of astroglia is involved in learning and memory. Our aim is to elucidate whether specific astroglia activity patterns are correlated with neuronal network activity underlying learning and memory. To unravel putative correlations between specific patterns of neuronal and astroglial activity, we will analyze $[\text{Ca}^{2+}]_i$ events in hippocampal astroglia populations during spontaneous and induced neuronal network activity in mice. We will use multicellular neuronal activation paradigms to describe astroglial $[\text{Ca}^{2+}]_i$ response patterns in acute hippocampal slices of adult and aged mice as well as genetically modified mouse lines. We will also record astroglia activity in vivo in the olfactory bulb, somatosensory cortex and hippocampus.

Intracellular ion signaling influences myelin basic protein synthesis in oligodendrocyte precursor cells

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Myelination in the central nervous system depends on axon-oligodendrocyte precursor cell (OPC) interaction. We suggest that myelin synthesis may be influenced by $[Na^+]_i$ and $[Ca^{2+}]_i$ signaling in OPCs. Experiments were performed in mouse cultured OPCs at day in vitro (DIV) 2–6 or acute slices of the corpus callosum at postnatal days (P) 10–30. Synthesis of Myelin Basic Protein (MBP), an “executive molecule of myelin”, was used as readout of myelination. Immunohistological data revealed that MBP synthesis in cultured OPCs starts around DIV4. Transient elevations of resting $[Ca^{2+}]_i$ and $[Na^+]_i$ levels were observed in the same temporal window (DIV4-5). At DIV4, but not at DIV2, both extracellular $[K^+]_e$ elevation (+5 mM) and partial Na^+ , K^+ -ATPase (NKA) inhibition elicited $[Na^+]_i$ and $[Ca^{2+}]_i$ transients. These responses were blocked with KB-R7943 (1 μ M), a blocker of Na^+ - Ca^{2+} -exchanger (NCX), indicating an involvement of NCX which operates in reverse mode. Treatment of OPCs with culture medium containing elevated $[K^+]_e$ (+5 mM, 24 h) or ouabain (500 nM, 24 h) increased resting $[Ca^{2+}]_i$ and facilitated MBP synthesis. Blockade of NCX with KB-R7943 (1 μ M, 12 h) reduced resting $[Ca^{2+}]_i$ and decreased MBP synthesis. Similar to the results obtained in OPC cultures, OPCs in acute callosal slices demonstrated an increase in resting $[Ca^{2+}]_i$ and $[Na^+]_i$ levels during development. NCX blockade induced $[Ca^{2+}]_i$ and $[Na^+]_i$ responses in OPCs at P20-30 but not at P10. We conclude that local $[Na^+]_i$ and/or membrane potential changes can modulate Ca^{2+} influx through NCX and in turn MBP synthesis. Thus neuronal activity-induced changes in $[K^+]_e$ may via NCX and NKA modulate myelination.

Investigating the phenotype of microglia in an animal model of Autism Spectrum Disorder using Neuroligin-4 Knockout mice

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Microglia as the cells of the brain's immune system are recently appreciated as novel therapeutic targets in mental disorders. There is accumulating evidence that in Autism Spectrum Disorders (ASD) the innate immune system and mainly the microglial compartment are dysregulated. However, a complete molecular and cellular characterization of microglia in this particular model is lacking.

We used the Neuroligin4 (NL4) knock-out mouse as a model to study ASD since NL4 is the most common mutation amongst all genetic modifications in ASD patients. We analyzed the microglia cell density using Iba1 as a marker and activation status using FACS analysis of freshly isolated microglia derived from different subcortical structures: Amygdala, hippocampus, and medial prefrontal cortex (mPFC). We compared these microglial features in the brains of both genders at postnatal day (P) 20 and P90 derived from NL4 knock-out and wild type mice. In the adult stage (P90), we found that microglia density significantly decreased in the hippocampus and mPFC, whereas it increased in the amygdala in both genders, which is in line with recent human post mortem studies. At the P20 developmental stage we observed similar changes in the hippocampus and amygdala, whereas no change was observed in mPFC. Moreover, we found decreased expression of MHCII, CD54, SiglecH in NL4^{-/-} male mice, while NL4^{-/-} female mice displayed increased expression of MHCII, CD54 and CD62L in hippocampus and mPFC at the adult stage compared to the respective wild type controls. On the contrary at the P20 developmental stage, we observed significantly higher expression of MHCI, CD86, SiglecH and SiglecF in NL4^{-/-} male and lower MHCII and SiglecF expression in NL4^{-/-} female compared to their respective wild type controls.

Taken together our data suggest a heterogeneous microglia density and activation profile in the NL4^{-/-} monogenic mouse model of ASD which is specific for each region, gender and developmental stage. This supports the idea that microglia contribute to disease development and/or progression.

Noradrenaline suppresses a chloride current as well as phagocytosis in murine microglia

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Innate as well as adaptive immune cells express adrenergic receptors (AR). Microglial cells, resident macrophage-like cells in the nervous system, express AR and may play a central role in neurodegenerative diseases. As a decrease in noradrenaline (NA) in the central nervous system has been associated with neurodegenerative diseases, responsiveness of microglial cells to catecholamines are of interest. In microglial cells, a Cl⁻ conductance is considered to be involved in cell volume regulation as well as in volume-regulated cellular responses, like migration and phagocytosis. Blockade of Cl⁻ channels suppressed migration and phagocytosis (Zierler et al. 2008, *Cell Physiol Biochem.* 21, 55-62; Harl et al., 2013, *Cell Physiol Biochem.* 31:319-37). Interestingly, NA has been shown to suppress phagocytosis in microglial cells (Steininger, 2011, *Brain Res.* 1407, 1-12) and N-formyl-Met-Leu-Phe (fMLP)-induced migration in neutrophils (Scanzano et al., 2015, *Inflamm Res.* 64(2):127-35). In the present study, we assessed whether NA suppresses phagocytosis via inhibition of a Cl⁻ conductance in microglial cells.

Membrane currents were recorded using the perforated patch clamp technique to leave the cytosolic milieu intact. Exposure of microglial cells to a hypotonic solution caused cell swelling and, accordingly, generated an outwardly rectifying current carried by Cl⁻ (ICl,swell). Addition of NA (1 nM or 1 μM) after induction of ICl,swell reduced the current. Similarly, the beta-adrenergic agonist, isoproterenol, suppressed the ICl,swell. Phagocytosis was quantified by exposure of IgG-coated microspheres to BV-2 cells or to primary murine microglia for 15 minutes and by counting the number of cells containing at least one microsphere using scanning electron microscopy. NA in the pM and nM range suppressed uptake of microspheres by microglial cells. Blockade of the alpha2 AR with yohimbine enhanced the suppressing effect of noradrenaline on microsphere uptake. These findings indicate that at least beta-adrenergic stimulation suppresses phagocytosis in microglia.

We suggest that formation of the engulfment pseudopodia, which is formed after the interaction between a microglial cell and a particle, is due to a locally restricted swelling. Similar to global cell-volume regulation, local volume changes depend on Cl⁻ conductance. If Cl⁻ currents are suppressed, all cell volume-related processes, like formation of lamellipodia and migration or formation of engulfment pseudopodia, are suppressed. Accordingly, reduction of ICl,swell by noradrenaline might cause a decline in phagocytosis.

Oligodendrocyte-Specific Deletion of HIF1 α Leads to Dysfunctional Axonal Mitochondria

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Oligodendrocytes are best known for making myelin, which enables rapid axonal impulse propagation. Recently, we have shown that glycolytic oligodendrocytes support axonal function by providing energy-rich metabolites (pyruvate/lactate) to the axonal compartment. What mechanisms regulate this trophic support function? Hypoxia inhibitory factor 1 (HIF1) is a transcription factor that promotes the expression of all but two glycolytic enzymes, as well as the cellular import of glucose. HIF1 targets also inhibit pyruvate entry into mitochondria thereby promoting pyruvate/lactate export. Through its many transcriptional targets HIF1 is a plausible candidate to drive oligodendroglial glycolysis for trophic support of axons. To test this hypothesis, we selectively and inducibly deleted HIF1 from oligodendrocytes in adult Plp-creERT2*HIF1^{flox/flox} mice. This caused decreased expression of glycolytic enzymes in the corpus callosum, suggesting that HIF1 is necessary to maintain normal levels of glycolysis. By electron microscopy, Plp-creERT2*HIF1^{flox/flox} mice exhibit decreased myelin content and enlarged inner tongues. Interestingly, deletion of oligodendroglial HIF1 led to disturbed ultrastructure and severe enlargement of axonal mitochondria as early as 10 days post tamoxifen administration. These abnormalities increased with time, and eventually led to axonal swellings and neurodegeneration. Axonal pathology preceded neuroinflammation as microgliosis and lymphocyte infiltration were observed only much later. We suggest that HIF1 is necessary to maintain oligodendroglial glycolysis at the rate required to fully support axonal energy metabolism.

Panglial gap-junctional coupling mediates calcium signaling between olfactory bulb glial cells

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Recently, there is upcoming evidence that not only neurons are organized in highly specialized networks, but also different types of glial cells form complex interconnected cell associations. Several histological studies demonstrate gap-junctional coupling among astrocytes and oligodendrocytes, establishing so-called panglial syncytia. However, it has not been shown so far how communication actually occurs between those glial cells. In the olfactory bulb, two spatially separated populations of glial cells exist, namely astrocytes in the glomerular layer and olfactory ensheathing cells (OECs) in the olfactory nerve layer. Neurotransmitter release by olfactory sensory axons lead to calcium transients in both glial cell populations directly. However, whether calcium signaling in one glial cell type can be transmitted to the other glial cell type has not been shown before.

Here we show that the application of NMDA initially induces calcium responses in juxtglomerular cells and with a delay, in OECs. To investigate this communication pathway, we performed confocal calcium imaging using in-toto preparations of mouse olfactory bulbs. Our results indicate that NMDA-induced calcium responses in OECs were inhibited by preincubation with bafilomycin A1 and TTX, suggesting neuronal origin of OEC calcium transients. However, GABAergic and dopaminergic neurotransmission appear not to drive OEC calcium transients. Inhibiting AMPA receptors instantly suppressed NMDA-induced calcium signaling in OECs, suggesting the involvement of glutamatergic neurons. We have shown the expression of AMPA receptors on astrocytes in the olfactory bulb, raising a possible role for astrocytes in this calcium signaling pathway. Furthermore, anti-GFAP stainings showed that astrocytic processes are located within the glomerular layer as well as in the olfactory nerve layer, indicating an interaction of astrocytic processes and OECs. Local activation of astrocytic mGluR5 via photolysis of caged-ACPD in one glomerulus and simultaneous inhibition of neuronal impact resulted in delayed calcium responses in OECs as well. Additional inhibition of gap junctions suppressed cACPD-triggered calcium signaling in OECs, whereas directly evoked calcium signals in astrocytes remained unaffected. Moreover the observed communication pathway also occurred vice versa. Local puff application of DHPG stimulated OECs directly, whereas astrocytic calcium signals occurred delayed. Furthermore immunohistochemical stainings provided evidence that both types of glial cells express connexin 43, necessary for gap junction coupling.

We conclude that the two different populations of glial cells in the olfactory bulb, OECs and astrocytes form panglial networks for functional transmission of calcium signaling.

Supported by the DFG (LO779/10)

Phosphorylation of Focal Adhesion Kinase at Y925: Role in Radial Neuronal Migration

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The adult mammalian cerebral cortex consists of six layers. During development, cortical neurons originating from the ventricular zone migrate radially towards the marginal zone. Cajal-Retzius cells in the marginal zone express Reelin, an extracellular matrix protein. Reelin has been shown to play an important role in the control of neuronal migration. It has been suggested that neuronal migration requires proper successive attachment and detachment of migrating neurons from radial glial cells, which is largely dependent on adhesion proteins. Focal Adhesion Kinase (FAK), one of the tyrosine kinases localized to focal adhesions, has been shown to be activated by Src at tyrosine residue 925. Src is an important downstream molecule of Reelin signaling. Up to date, the precise molecular function of FAK and its phosphorylation at Y925 during development has remained unclear. Using in utero electroporation and live imaging, we have demonstrated that overexpression of FAK and a point mutation at Y925A in late-born neurons disrupted radial neuronal migration. Compared to control neurons, overexpression of FAK and FAK Y925A induced migration defects of late born neurons. Time-lapse imaging demonstrated that these neurons showed abnormal migratory behaviors. Taken together, our findings indicate that FAK and phosphorylation of FAK at Y925 is required for radial neuronal migration and might be regulated by Reelin signaling.

(Supported by DFG: FR 620/12-2 and FR 620/13-1 and CSC(China Scholarship Council))

Reelin from interneurons influences glial cell morphology and adult neurogenesis in the dentate gyrus

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The large extracellular matrix protein Reelin is well known for its important role in the development of layered brain structures. In reeler mice deficient in Reelin the migration of cortical and hippocampal neurons is severely altered, leading to the malformation of cell layers in these regions. Interestingly, the expression pattern of Reelin changes shortly after birth. During development, Reelin is secreted by Cajal-Retzius cells in the marginal zones of cortex and hippocampus. However, Reelin expression by these neurons decreases dramatically after birth, while GABAergic interneurons in the cortex and hippocampus start to express Reelin. Due to the severe developmental defects in classical reeler mice, the function of this late Reelin expression by interneurons is poorly understood.

In the present study we took advantage of a conditional mouse mutant in which the postnatal Reelin expression by interneurons was selectively ablated, while Reelin secretion by Cajal-Retzius cells remained normal. As expected, the overall anatomical organization of neocortex and hippocampus is normal in these mutants. However, we found that the complexity of glial cells and the number of newly generated doublecortin-positive granule cells in the adult dentate gyrus was significantly decreased when compared to controls. The results indicate that postnatal Reelin expression by interneurons is crucial for persisting neurogenesis in the dentate gyrus.

(Supported by DFG: FR 620/13-1; BR 4888/2-1)

Regional heterogeneity in astrocyte sodium signaling

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Glutamate is the main excitatory neurotransmitter in the brain. Its effects are mediated by binding to ionotropic and metabotropic glutamate receptors present on postsynaptic membranes. In addition to neurons, astrocytes have been reported to express ionotropic glutamate receptors. Expression of functional AMPA- and NMDA-receptors on astrocytes, however, differs between brain regions. While hippocampal astrocytes largely lack ionotropic glutamate receptors (Matthias et al., J Neurosci 2003), astrocytes in the cortex show prominent inward currents in response to receptor agonists. Interestingly, NMDA-mediated currents, which are especially prominent in cortical astrocytes, are linearly dependent on the membrane potential and do not exhibit the voltage-dependent block by magnesium ions which is typical for neuronal NMDA receptors (Lalo et al., J. Neurosci 2006). NMDA receptors are main pathways for activity-induced sodium signalling in neurons, and, therefore, might also contribute to sodium signalling in cortical, but not hippocampal astrocytes.

To test this hypothesis, we analyzed glutamate-induced sodium signalling in cortical and hippocampal astrocytes using wide-field imaging with the sodium indicator dye SBFI and whole-cell patch-clamp in acute tissue slices of the juvenile mouse brain. Agonists were applied by pressure application; in addition, synaptic stimulation was performed by electrical stimulation of Schaffer Collaterals or afferent fibers. To study pathways for sodium influx, specific antagonists for NMDA-R, AMPA-R or glutamate transporters were used. Astrocytes in layer 2/3 of the visual cortex exhibited sodium increases in response to glutamate application which were about twice as large as those in astrocytes in the CA1 region of the hippocampus. Cortical sodium signals were blocked by about 50% in the presence of AP5, which blocks NMDA-R. In hippocampal astrocytes, in contrast, AP5 did not alter glutamate-induced sodium signals. Additional inhibition of AMPA-R by application of NBQX cause a slight reduction of sodium signals in both brain regions, while blocking glutamate uptake by TFB-TBOA diminished them largely. Similar results were obtained with synaptic stimulation.

Taken together our data demonstrate a substantial heterogeneity in astrocyte sodium signalling between hippocampus and cortex. Sodium influx through NMDA receptors significantly contributes to strong intracellular sodium signalling in cortical astrocytes, whereas the predominant sodium influx in hippocampal astrocytes is a result of glutamate transporter activity.

Supported by the DFG (SPP 1757 Glial Heterogeneity, Ro 2327/8-1).

Septin filaments scaffold CNS myelin to accelerate nerve conduction

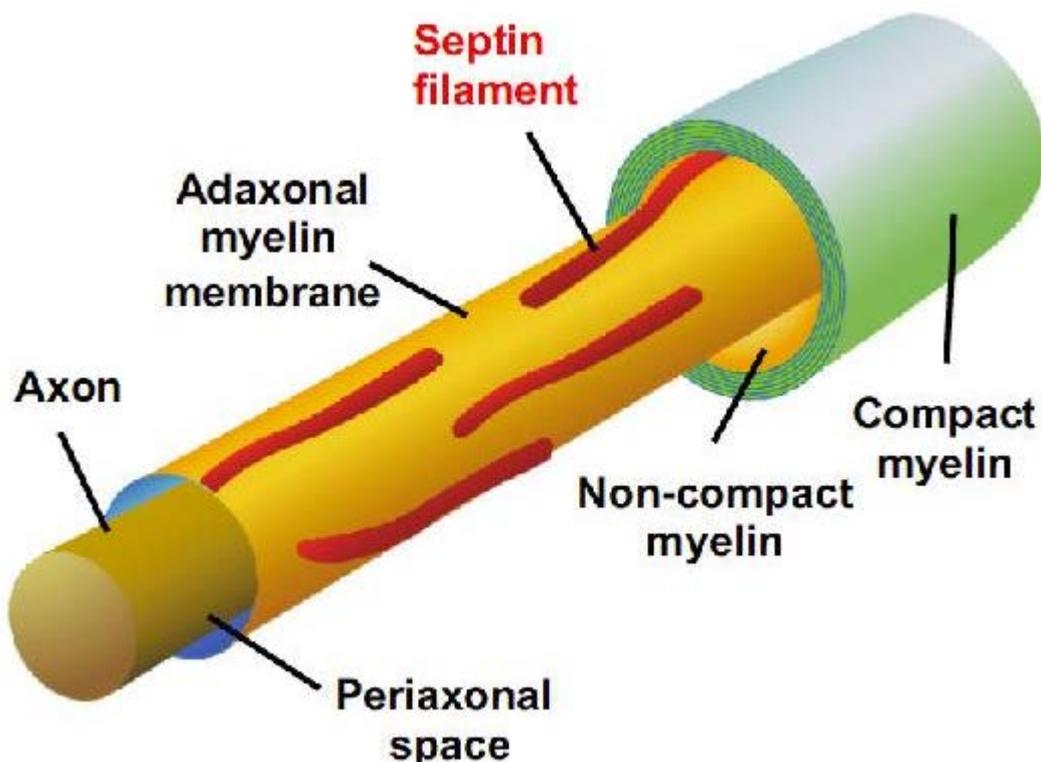
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Myelination of axons facilitates rapid impulse propagation in the nervous system. The axon/myelin-unit becomes impaired in myelin-related disorders and upon normal aging. However, the molecular cause of many pathological features, including the frequently observed myelin outfoldings, remained unknown. Using label-free quantitative proteomics, we find that the presence of myelin outfoldings correlates with a loss of cytoskeletal septins in myelin. Regulated by phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P₂)-levels, myelin septins (SEPT2/SEPT4/SEPT7/SEPT8) and the PI(4,5)P₂-adaptor anillin form previously unrecognized filaments that extend longitudinally along myelinated axons. By confocal microscopy and immunogold-electron microscopy, these filaments are localized to the non-compacted adaxonal myelin compartment. Genetic disruption of these filaments in Sept8-mutant mice causes myelin outfoldings as a very specific neuropathology. Septin filaments thus serve an important function in scaffolding the axon/myelin-unit, evidently a late stage of myelin maturation. We propose that pathological or aging-associated diminishment of the septin/anillin-scaffold causes myelin outfoldings that impair the normal nerve conduction velocity.



Simultaneous activation of interferon-gamma and Toll-like receptors severely impairs neuronal network activity

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Toll-like receptors (TLRs) play a key role as pattern recognition receptors in the innate immune system, which - in the brain - mainly consists of microglia. Activation of TLRs triggers proinflammatory responses such as the production of NO, TNF- α and interleukins. It has been shown that activation of TLR4 leads to severe neurotoxicity but only in the presence of leukocyte cytokine, interferon- γ (IFN- γ). We investigate the properties of TLR2 (activated by bacterial and fungal components) and TLR3 (viral components) regarding their neurotoxic effects by monitoring neuronal network activity, i.e. γ -oscillations (30-100 Hz) that are known to be highly vulnerable to changes in functional interactions of excitatory and inhibitory neurons.

For this purpose, organotypic hippocampal slice cultures were incubated with Poly(I:C) (TLR3 agonist) and Peptidoglycan (TLR2 agonist), with and without IFN- γ for 48h-72h. In succession, local field potential (LFP) recordings of muscarinic agonist-induced γ -oscillations were performed.

We found that single activation of TLR2 or TLR3 only moderately affects neuronal network activity. The combined activation of TLR2 and TLR3 leads to varying functional alterations, ranging from persisting γ -oscillations or δ -oscillations (12.5-30 Hz) to bursting activity with loss of network activity in a small fraction of slice cultures. In combination with IFN- γ , however, the power of γ -oscillations decreases up to complete loss of oscillations in a concentration dependent manner. Slice cultures that were pretreated with clodronate to deplete the microglial population showed none of the functional alterations to the stimuli applied. We conclude that microglia exhibit strong neurotoxic effects when activated through double stimuli, i.e. IFN- γ with TLR2 or TLR3.

Small molecule mediated differentiation of hiPSCs derived NSCs towards astrocytes

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The use of small molecules has been very promising in the field of human induced pluripotent stem cells (hiPSCs) for reprogramming, neural induction as well as differentiation towards specific subtypes of neurons (Zhu et al., Cell Stem Cell, 2010; Chambers et al., Nat Biotechnol, 2009; Han et al., Neuron, 2011). This could be attributed to their ease of use, cost effectiveness and more importantly providing highly defined and reproducible procedures for the desired application. However, till date, no such approach has been shown for the differentiation of hiPSCs towards astrocytes. The previously published growth factor based approach for astrocyte differentiation from hNSCs is very time consuming (Krencik et al., Nat Biotechnol, 2011) which is a major limitation for disease modelling. Furthermore, most of the studies on astrocytes differentiation focus on generating high GFAP expressing astrocytes, excluding protoplasmic subtype and leaving the question of astrocyte heterogeneity unaddressed. Additionally, recently discovered differences between the gene expression profile of rodent and human astrocytes emphasize the importance of studying human astrocytes for disease modeling (Zhang et al., Neuron, 2016).

In this study, we have established an efficient, small molecule based approach for differentiating hiPSCs derived NSCs towards astrocytes. This approach can be used to differentiate astrocytes expressing either low or high levels of GFAP depending on the precursors employed. We further characterized properties of the remarkably homogenous population of low GFAP astrocytes, where these cells were found to express other markers of astrocyte lineage (e.g. ALDH1L1, Cx43, EAAT2, Acsbg1, ApoE) and exhibit cortical identity. Additionally, they display functional properties typical of astrocytes, such as elevating calcium transients upon glutamate stimulation and turning reactive when exposed to inflammatory molecules like TNF- and LPS. Importantly, the low GFAP astrocytes clear glutamate from the extracellular solution in a sodium dependent way. When co-cultured with hiPSCs derived neurons (Nieweg et al., Cell Death Dis, 2015), the presence of low GFAP astrocytes was found to support neuronal survival in minimal media and to predominantly promote formation of GABAergic synapses. Interestingly, the small molecule mediated derivation of astrocytes was found to follow the canonical STAT1 and STAT3 pathway of astrocyte differentiation in a delayed manner. Altogether, we established the first small molecule based approach for astrocyte differentiation from human iPSCs and elucidated the underlying mechanism and signalling cascade. This is the first study demonstrating the generation of an astrocyte subtype, expressing low levels of GFAP, which exhibits a variety of functional astrocytic properties, including support of neuronal survival and maturation in human cocultures. This approach offers a great potential to obtain chemically defined, reproducible human astrocyte cultures which could be largely used for disease modelling.

Sodium signaling in white matter glial cells

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Astrocytes fulfil a wide range of functions in the brain. While much is known about protoplasmic astrocytes and their role in the grey matter, the functions of fibrous astrocytes are not as clear. In white matter tracts, astrocytes interact closely with axons at nodes of Ranvier and react to glutamate released at these sites. Furthermore, there is evidence for physiological interactions with other resident macroglial cells, namely oligodendrocytes and NG2 cells. To gain insight into astrocyte function in white matter, the corpus callosum, a fiber tract connecting the two cerebral hemispheres, was studied via dynamic sodium imaging. The analysis of transient changes in sodium concentrations has been proven to be a useful approach to monitor astrocyte function in brain tissue, as astrocytic sodium gradients play a vital role, e. g. by providing the driving force for the uptake of glutamate released by neurons and thus preventing excitotoxicity.

To study glial sodium signalling in the corpus callosum, acute tissue slices were prepared and bolus-loaded with the sodium sensitive dye SBFI-AM. In addition, slices were stained with sulforhodamine 101 (SR101) to identify astrocytes. Oligodendrocytes and NG2 cells were identified by the use of transgenic reporter mice (PLP-GFP and NG2-EYFP, respectively). Local pressure-application of glutamate (1 mM, 250 ms) reliably induced sodium signals in SR101-positive astrocytes that amounted to up to 10 mM. While glutamate receptor blockers NBQX and AP5 had almost no effect on glutamate-induced astrocyte sodium signals, blocking glutamate transporters by TFB-TBOA reduced their amplitude by about 80%. In contrast to astrocytes, SR101-negative cells displayed either small sodium signals (peak amplitudes ~3 mM) or did not show a visible response (~33% of SR101-negative cells studied). Electrical stimulation of axons (25 pulses/50 Hz) caused sodium transients in astrocytes amounting to ~1 mM. These were completely blocked after application of TTX, showing their dependence on action potential generation. Again, signals were only slightly dampened upon application of glutamate receptor blockers, but strongly reduced by TFB-TBOA. To study the intercellular spread of sodium between glial cells, single SR101-positive astrocytes were electrically stimulated by delivering a square pulse (1 ms/40 V) through a pipette positioned on their soma. Direct electrical stimulation induced an immediate rise of the sodium concentration in the stimulated cell. In addition, we observed sodium transients in neighboring cells. Both amplitudes and slopes of sodium transients monotonically decayed with increasing distance from the stimulated cell. Sodium signalling included SR101-positive cells as well as NG2-cells and oligodendrocytes, albeit with reduced amplitudes in the latter two cell types.

Taken together our data demonstrate that astrocyte sodium signalling is not restricted to grey matter and to perisynaptic astrocyte processes at glutamatergic synapses. Astrocyte sodium signals are also evoked by axonal action potential firing in the corpus callosum upon activation of sodium-dependent glutamate uptake. Moreover, intercellular sodium signalling domains not only encompass gap-junction coupled astrocytes, but also include other macro-glial cells, most likely mediated by panglial gap junctional coupling.

The growth/differentiation factor GDF15 signals the sprouting of new astrocyte processes upon fluoxetine treatment

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Altered cell morphology is a hallmark of several brain pathologies. Astrocyte processes, which impact synaptic and vascular functions, are disrupted in major depressive disorder (MDD) and in an animal model of MDD, the HAB rat. Fluoxetine (FLX) restores the morphological complexity of HAB cortical astrocytes, but molecular mediators of this effect remain unexplored. We identified the releasable growth/differentiation factor 15 (GDF15) as an upregulated target of FLX in primary astrocytes and in their processes around blood vessels of the adult prefrontal cortex (PFC) of HAB rats. Exposure of cortical astrocytes to exogenous GDF15 increased the abundance of short-sized cell processes, suggesting its prevalent sprouting effect on new processes. These results were confirmed by the pharmacogenetic and immunological inhibition of GDF15, which hampered the morphological changes, suggesting its essential role for an astrocytic remodelling. Our results indicate that GDF15 might be explored as a treatment option for brain disorders with a glia cell pathology.

The impact of astrocytes morphology on their Ca²⁺ characteristics

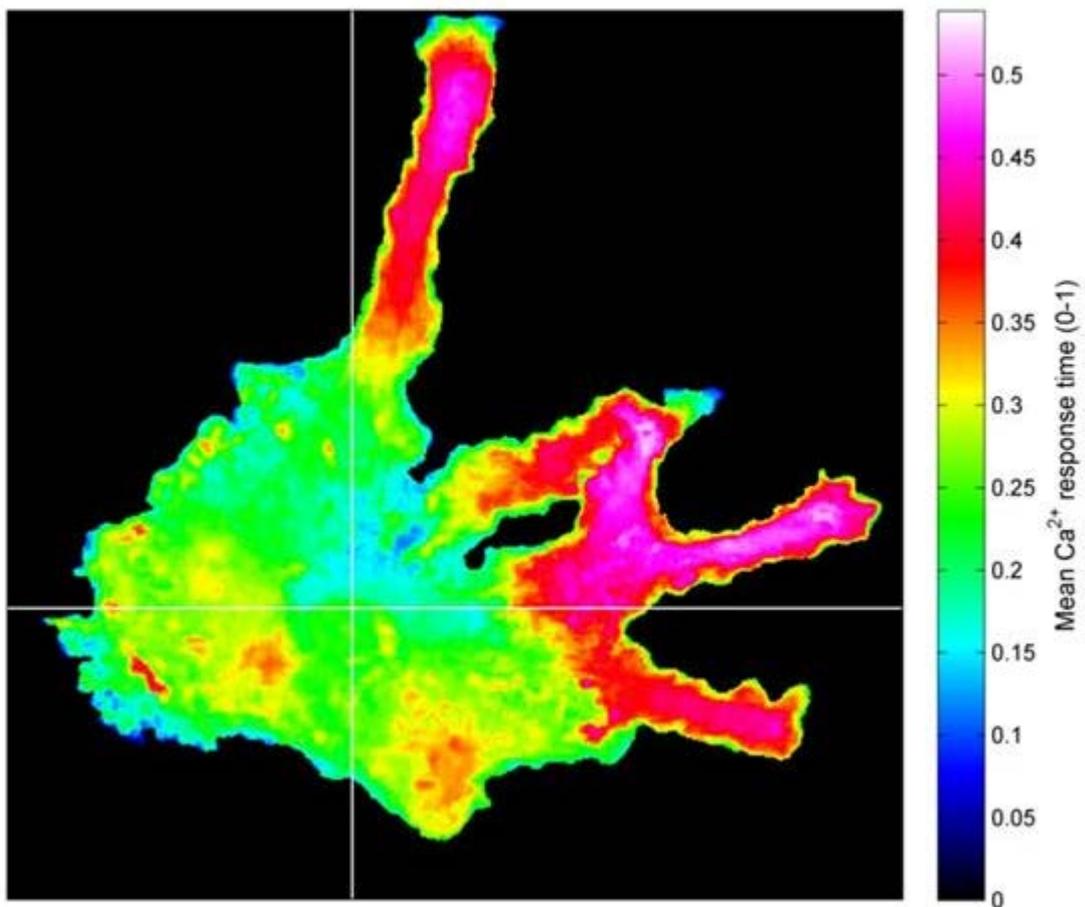
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Astrocytes, an abundant type of glial cells in mammalian brain and spinal cord, play an important role in regulation of neuronal network functions. During the last decade it has become evident that astrocytes can also be directly involved in modulation of synaptic signalling and synaptic plasticity, and that these astrocyte functions are related to the intracellular Ca²⁺ dynamics. Ca²⁺ signals in astrocytes can occur spontaneously and mainly rely on Ca²⁺ release from intracellular stores. More importantly, Ca²⁺ diffusion in astrocytes depends on cell morphology. Because of their unique morphology, astrocytes can modulate the functional properties of thousands of synapses over defined anatomical regions. However, the mechanisms involved in functional interplay between astrocyte morphology and Ca²⁺ signalling in astrocytes remain poorly understood.

We aim to elucidate the role of astrocyte morphology and its dynamical changes in shaping Ca²⁺ characteristics in cultured hippocampal astrocytes. We show how to uncover Ca²⁺ transients in astrocytes from GCaMP6s fluorescence signals. From that we quantify and further characterize astrocyte Ca²⁺ signalling. By corresponding analysis of astrocyte morphology, we correlate astrocytes Ca²⁺ characteristic to their geometry. Furthermore, by actively modulating astrocytes morphology by up- or downregulating the activity of small GTPases, we show that astrocytes Ca²⁺ characteristics are directly coupled to their morphology.

Figure: Mean Ca²⁺ response time of a mouse hippocampal astrocyte



The role of PMP22 in CMT disease type 1A

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Duplications of the gene for peripheral myelin protein 22 (PMP22) cause CMT1A, the most common form of human Charcot-Marie-Tooth disease. This slowly progressive neuropathy is characterized by demyelination of peripheral nerves, starting in the lower limbs. PMP22 has been implicated in lipid metabolism and Schwann cell differentiation, but the biological function of this protein in Schwann cells is largely unknown. In Pmp22 transgenic animal models of CMT1A, we found a decrease PI3 kinase activity and the AKT signaling pathway, suggesting a critical role of PMP22 in the regulation of Schwann cell differentiation. However, by what mechanism a 1.5-fold increase of gene dosage turns wildtype PMP22 into a disease gene remains obscure. In Western blot experiments comparing sciatic nerve lysates already young CMT1A rats (P6) show a significant increase of PMP22 protein compared to age-matched wildtype controls. To identify possible interaction partners of PMP22 and signaling pathways that contribute to the CMT disease phenotype, we are employing immunoprecipitation and mass spectroscopy to systematically compare sciatic nerves of CMT1A mice and rats at different developmental time points.

Poster Topic

T10: Aging and Developmental Disorders

- [T10-1A](#) A deepening of actigraphic sleep quality and 24-h activity rhythm in adults with attention-deficit/hyperactivity disorder
Chiara Colombo, Lorenzo Tonetti, Andreas Conca, Giancarlo Giupponi, Vincenzo Natale
- [T10-2A](#) Age-related hearing loss: Auditory plasticity gone too far?
Understanding the relationship between the perineuronal net and glia within the auditory pathway in a mouse model of age-related hearing loss.
Shmma Quraishie, Genevieve Brixton, Bethan Impey, Edward T F Rogers, Carl Verschuur, Tracey A Newman
- [T10-3A](#) Analysis of transcriptomic changes induced by mitochondrial complex II inhibitor in a neuronal cell line: focus on mitochondrial function with implications for neurodegenerative disorders
Ranganayaki Sathyanarayanan, Santosh Narwade, Deepthi Deobagkar, Gayathri Narayanappa, Srinivas Bharath
- [T10-4A](#) Comparison of percentages of neurons immunoreactive for NMDA receptor subunit 2A/B in the fusiform gyrus in people with autism spectrum disorder and a control group
Juliane T. Zimmermann, Steven A. Chance
- [T10-5A](#) Deficiency of Latrophilin-3, a risk factor for Attention Deficit Hyperactivity Disorder, increases locomotor activity and alters learning and memory in mice
Olga Rivero, Niall Mortimer, Sandy Popp, Florian Freudenberg, Klaus Peter Lesch
- [T10-2B](#) GABAergic substrate of abnormal prefrontal-hippocampal communication during development in a gene-environmental model of mental illness.
Mattia Chini, Christoph Lindemann, Henrike Hartung, Sebastian H. Bitzenhofer, Ileana L. Hanganu-Opatz
- [T10-3B](#) Golgi-associated Cohen syndrome protein COH1 regulates neurite outgrowth in vitro
Stefanie Lommatzsch, Jirko Kühnisch, Tanja Maritzen, Denise Horn, Sebastian Bachmann, Volker Haucke, Wenke Seifert
- [T10-4B](#) Identify dysregulated autophagy as cause for changes in synaptic functioning in Koolen de Vries syndrome
Katrin Linda
- [T10-1C](#) Lack of *Shank1* Leads to Cognitive Deficits, Reductions in Cortical Parvalbumin Expression, and Altered Hippocampal BDNF Levels Related to Epigenetic Modifications in Mice
Ayse Özge Sungur, Federica Filice, Magdalena CE Jochner, Karl Jakob Vörckel, Hani Harb, Ayse Kilic, Holger Garn, Rainer KW Schwarting, Beat Schwaller, Markus Wöhr

- [T10-2C](#) Neuronal networks on Micro-Electrode Arrays: a model to study Neurodevelopmental Disorders
Monica Frega, Katrin Linda, Britt Mossink, Jason Keller, Dirk Schubert, Nael Nadif Kasri
- [T10-3C](#) Neuronal redox imbalance in Rett syndrome: a key player in neuronal network dysfunction and altered neurotransmitter responsiveness?
Karolina Can, Karina Festerling, Johan Tolö, Sebastian Kügler, Michael Müller
- [T10-4C](#) Optogenetic studies on dysfunctions of striatal cholinergic interneurons in dystonia
Anne Bauer, Julia Gerstenberger, Franziska Richter, Angelika Richter
- [T10-5C](#) Homozygous *YME1L1* Mutation Causes Mitochondriopathy with Optic Atrophy and Mitochondrial Network Fragmentation
Bianca Hartmann, Timothy Wai, Hao Hu, Thomas MacVicar, Luciana Musante, Björn Fischer-Zirnsak, Werner Stenzel, Ralph Gräf, Lambert van den Heuvel, Hans-Hilger Ropers, Thomas F. Wienker, Christoph Hübner, Thomas Langer, Angela M. Kaindl
- [T10-6C](#) Loss of MeCP2 disrupts cell autonomous and autocrine BDNF signaling in mouse glutamatergic neurons
Charanya Sampathkumar, Yuan-Ju Wu, Mayur Vadhvani, Thorsten Trimbuch, Britta Eickholt, Christian Rosenmund
- [T10-1D](#) Respiratory acidosis induces migration defects of neurons in cerebral cortex and hippocampus
Xuejun Chai, Lingzhen Song, Michael Frotscher
- [T10-2D](#) Sex and Violence – Social phenotypes in *Dnlg2* and 4 deficient *Drosophila*
Robert Kossen, Kristina Corthals, Alina S. Heukamp, Isabel Großhennig, Nina Hahn, Heribert Gras, Ralf Heinrich, Bart R.H. Geurten
- [T10-3D](#) SOMATOSTATIN DISTRIBUTION IN THE ENTORHINAL CORTEX OF DELAYED AND SENESCENCE ACCELERATED MOUSE MODELS
Noemi Villaseca Gonzalez, Joaquin Gonzales-Fuentes, Carmen Moya, Lucia Castro-Vazquez, M. Victoria Lozano, Virginia Rodriguez-Robledo, Manuel J. Santander-Ortega, Pilar Marcos, M. Mar Arroyo-Jimenez
- [T10-4D](#) Studying the long term neuropathological consequences of encephalopathy of prematurity in a small animal model
Bobbi Fleiss, Stephanie Sigaut, Luisa-Sophie Klein, Leslie Schwendimann, Juliette Van Steenwinkel, Dulcie Voerden, Jason P Perch, Anthony C Vernon, Thomas Schmitz, Pierre Gressens
- [T10-5D](#) The hypoxia sensing pVHL-EGLN1-Hif1 pathway is critical for cerebellar granule cell migration
Jan A Kullmann, Niraj Trivedi, Danielle Howell, David J Solecki
- [T10-6D](#) Homozygous *ARHGEF2* gene mutation causes intellectual disability and midbrain-hindbrain malformation
Ethiraj Ravindran

A deepening of actigraphic sleep quality and 24-h activity rhythm in adults with attention-deficit/hyperactivity disorder

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1. Background and aim.

Previous studies have shown an impaired actigraphic sleep quality and an altered 24-h activity rhythm in adults with attention-deficit/hyperactivity disorder (ADHD).

The aim of the present study was to deepen such features in ADHD adult patients through a comparison with other clinical populations – i.e., severely obese (SO) and hypertensive (HT) patients – that also present low sleep quality.

2. Methods.

Four groups of participants were included in the present study: 18 (7 females) ADHD patients (mean age 39.61±12.01 years old), 33 (21 females) SO patients (mean age 47.61±9.70 years old), 31 (15 females) HT patients (mean age 62.97±8.55 years old) and 37 (16 females) healthy controls (HC) (mean age 37±11.90 years old).

Participants wore the Actiwatch AW-64 actigraph (Cambridge Neurotechnology Ltd, Cambridge, UK) around the non-dominant wrist continuously for seven consecutive days. Such device is able to continuously record motor activity through an accelerometer and also allows to indirectly assess sleep quality and quantity. The following actigraphic sleep measures were computed: total sleep time (TST), the sum, in minutes, of all sleep epochs between sleep onset and get-up time; sleep efficiency (SE, %), the ratio of the TST to time in bed multiplied by 100; sleep onset latency (SOL), the interval, in minutes, between bedtime and sleep start; wake bouts (WB), the number of episodes of activity from sleep onset until wake-up time; mean activity score (MAS), the mean value of activity counts in 1-min epoch during the assumed sleep; wake after sleep onset (WASO), the sum, in minutes, of all wake epochs between sleep onset and get-up time. In order to describe the 24-h activity rhythm, the hourly mean activity levels in the 24-h period were extracted for each participant.

At the end of the week of the actigraphic recording, participants filled in the Mini Sleep Questionnaire (MSQ) that allows to assess the perceived quality of the sleep/wake cycle. MSQ is composed of two main factors, i.e. sleep and wake. Higher scores on these two factors correspond to a lower quality of sleep and wake.

3. Results.

The distribution of gender across the four groups of participants was not significantly different, while groups differed by age.

With reference to the actigraphic sleep parameters, we observed significant differences between groups with reference to SE – lower in SO patients than HC – and SOL – longer in SO patients than HC. Regarding the 24-h activity rhythm, it was observed a significant interaction between groups and time of day, with ADHD patients showing highest motor activity at 6:00, 15:00 and 16:00.

Groups did not differ in MSQ sleep, contrary to MSQ wake dimension, with higher scores in HT patients than HC.

4. Conclusions.

Overall the actigraphic data on sleep quality show that the worst sleep quality is observed in SO patients, while the perception of wake quality is impaired in HT patients. Such data seem to point out that sleep alterations – both objective and perceived – are not so marked in ADHD patients when compared with other clinical populations frequently reporting poor sleep. On the contrary the 24-h activity rhythm of ADHD patients seems to be peculiarly characterized by the lack of the typical post-lunch dip, even when compared with other clinical samples.

Age-related hearing loss: Auditory plasticity gone too far? Understanding the relationship between the perineuronal net and glia within the auditory pathway in a mouse model of age- related hearing loss.

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Age-related hearing loss (ARHL) affects over 11 million people in the UK and ~50% of over 75yr olds. Although hearing loss is often perceived as an inconsequential part of aging, there is evidence it is important in general brain health. Adults with ARHL develop significant impairments in their cognitive abilities 3 years sooner and 30-40% more severely than those with normal hearing. The severity of ARHL is associated with a greater risk (2-5 fold) of developing dementia. Hearing aids and cochlear implants improve quality of life for many individuals however there are no approved therapies to prevent or slow ARHL. This is likely due to inadequate understanding of the neurobiological changes underling the progression of this chronic debilitating condition. In a murine model of ARHL, we have observed changes within the auditory pathway in both the perineuronal net (PNN) and glia.

We hypothesise that the loss of sensory input to the auditory nerve in ARHL may induce compensatory changes to the PNN to alter neuronal activity and synaptic plasticity. Such changes may contribute to a pathological glial response making the auditory pathway more vulnerable to inflammation and progression of hearing loss.

The auditory circuit is modulated by synaptic inhibition to maintain temporal precision and process sound localization cues. The majority of fast-spiking interneurons associated with this inhibition are surrounded by a specialized extracellular matrix, the PNN. The PNN is important for synaptic stabilization, protects against glial activation and pathological insults and has restrictive effects on plasticity in the mature CNS.

Here we exploit the well-characterized C57BL/6J mouse model of ARHL, to assess changes in expression and localization of the PNN and glial cells across the life-course and in disease progression. We have found changes in expression of the PNN during progression of hearing loss. We also observe changes in the organization and phenotype of microglia and astrocytes in the auditory pathway.

Gaining a better understanding of the pathological processes involved in progression of ARHL may identify cellular or molecular compartments amenable to modulation. For example tempering the glial response and associated changes in the PNN may slow disease progression and help retain auditory function for longer. ARHL is associated with increased risk of developing dementia and exacerbating cognitive decline. Therapies that modulate ARHL could therefore be significant in the treatment of dementia and related neurodegenerative conditions.

Analysis of transcriptomic changes induced by mitochondrial complex II inhibitor in a neuronal cell line: focus on mitochondrial function with implications for neurodegenerative disorders

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Neurodegenerative disorders like Huntington's disease (HD) and Parkinson's disease (PD) are clinically manifested as motor dysfunction. Although these are multifactorial pathologies, mitochondrial dysfunction is one of the important processes that significantly contribute to degeneration. Consequently, analysis of molecular events following mitochondrial dysfunction could help in delineating the processes entailing neurodegeneration. Several chemical models of neurodegeneration mimic human degenerative etiology via mitochondrial dysfunction. 3-nitropropionic acid (3-NPA) is one such neurotoxin that recapitulates the pathological features of HD *in vivo*. 3-NPA is a mitochondrial complex II inhibitor that induces mitochondrial damage and apoptosis. However, the impact of 3-NPA on mitochondrial and extra-mitochondrial functions including regulation of the nuclear encoded genes in neuronal cells remains largely unknown.

To address this, we exposed N27 dopaminergic neuronal cells to 3-NPA followed by analysis of biochemical, morphological, transcriptomic and epigenetic changes. 3-NPA induced dose and time dependent neuronal death associated with oxidative stress, altered mitochondrial membrane potential and decreased complex II activity. While gross morphology showed flattening of the cell body and loss of processes, ultrastructure showed cytoplasmic vacuolation, altered mitochondrial structure and evidences of autophagy.

Whole genome transcriptome analysis of the 3-NPA cell model revealed over-expression of 2651 genes and down-regulation of 1028 genes. Gene ontology (GO) analysis showed enrichment of genes associated with mitochondrial function, regulation of synaptic activity and autophagy, and this was supported by validation experiments. Interestingly, these genes were not enriched in the 1-methyl-4-phenylpyridinium (MPP+) model (mitochondrial toxin model involving complex I inhibition) and manganese (Mn) model (non-mitochondrial neurotoxin), indicating that these are exclusive to complex II inhibition. Network analysis of shortlisted genes revealed that Nos2 and Bcl2 formed the core of the mitochondrial network; Faslg, Fosl1, Sqstm1 and Rictor formed the core of the autophagy network and Adora2a, Grm4, Rab3a, Bdnf and Stxbp1 were the core genes in the synaptic network. Genome-wide DNA methylation analysis also highlighted the epigenetic regulation in 3-NPA neurotoxicity. In summary, using the 3-NPA model, our study provides evidences for genome-wide transcriptomic and epigenetic changes in neuronal cells exposed to mitochondrial complex II inhibitor and highlights alterations in mitochondrial, synaptic and autophagy networks. Our work emphasizes the complexity of the mitochondria-nuclear cross-talk with implications for neurodegenerative disorders.

Comparison of percentages of neurons immunoreactive for NMDA receptor subunit 2A/B in the fusiform gyrus in people with autism spectrum disorder and a control group

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The developmental and maintaining factors of autism spectrum disorder (ASD) are not yet fully understood resulting in limited therapeutic options. Hyperactivity of the glutamatergic system has been proposed as a possible contributing factor of the disorder. Several findings so far underpin this notion, amongst other studies investigating differences in quantities of glutamatergic receptors. Some studies have found differences in the amount of glutamate receptors within the brains of people with ASD compared to brains of control subjects.

This study investigated the percentage of neurons that showed immunoreactivity for an NMDA receptor subunit within the fusiform gyrus of people with ASD and a control group. Immunohistochemistry with an antibody against NMDA receptor subunit 2A and 2B and a haematoxylin counterstain was performed on the brain sections.

No significant difference was found between the groups. However higher percentages of labelled neurons in the male subgroup of people with autism compared to the male subgroup of the control group were observed. The female subgroup was smaller and not represented in ages younger than 21. While the observations made in this study are in line with the idea of a hyperglutamatergic system only in males, they might hint at differences during early ages in particular.

Increased numbers of immunopositive neurons suggests different modes of action within the glutamatergic system which may be found in a larger sample size. Generally speaking these observations are consistent with the idea of a subtle glutamatergic imbalance in ASD.

Deficiency of Latrophilin-3, a risk factor for Attention Deficit Hyperactivity Disorder, increases locomotor activity and alters learning and memory in mice

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Attention Deficit Hyperactivity Disorder (ADHD) may be defined as a highly heritable chronic neurodevelopmental condition marked by persistent inattention, hyperactivity and sometimes impulsive behavior. Noteworthy, multiple polymorphisms in the *LPHN3* gene, encoding Latrophilin-3, a member of the latrophilin subfamily of secretin G protein coupled receptors (GPCR), have been repeatedly linked with an increased risk of ADHD in several studies. In addition, *LPHN3* variants have also been associated with cognitive abilities as well as behavioral and neurophysiological measures of cognitive response control. All these studies support *LPHN3* as one of the most promising novel ADHD risk genes. However, the mechanisms underlying *LPHN3* dysfunction in ADHD patients are still unclear. *LPHN3* is broadly expressed in the cerebral cortex, among other brain regions, where it plays a role in synapse formation and connectivity. Moreover, *LPHN3* deficiency in mice, zebrafish, and *Drosophila melanogaster* is associated with increased locomotor activity and alterations in the dopaminergic system, phenotypes which resemble some of the alterations found in ADHD patients.

In this study, we aimed to further examine the characteristics of *Lphn3*-deficient mice beyond hyper locomotion, by assessing for the first time other behavioral domains that are also altered in ADHD patients, including emotional behavior, learning and memory as well as aggressive behavior. Specifically, *Lphn3* knockout (-/-) and wildtype (+/+) mice of both genders were tested in the Open Field, the Light Dark Box, the Object Recognition Task, the Barnes Maze and the Resident-Intruder Task.

We observed that *Lphn3*^{-/-} mice weighed significantly less than wildtype mice. Moreover, the Open-Field Test confirmed the increased locomotive activity of *Lphn3*^{-/-} mice that had been previously described. Contrasting this increased horizontal locomotive activity is a drastically reduced rearing behavior. On the other hand, no alterations in anxiety-like behavior were detected in *Lphn3*^{-/-} animals when tested in the Light-Dark Box. Notably, *Lphn3*^{-/-} mice displayed reductions in recognition memory during the Object Recognition Task and altered visuospatial memory in the Barnes Maze. Furthermore, *LPHN3* deficiency is associated with reduced aggressive behavior, since none of the tested *Lphn3*^{-/-} animals attacked the intruders during the Resident-Intruder Task. This interesting novel finding has led us to currently investigate other aspects of social interaction and recognition via the social interaction test, which allows measuring social approach without triggering aggressive responses. Additionally, we are also evaluating the effect of *Lphn3* deficiency in the performance of the 5-Choice Serial Reaction Time Task, which assesses attention and impulsivity, behavioral domains that are also strongly affected in ADHD. Additionally, we plan to further expand our study of *Lphn3*^{-/-} mice by analyzing changes in global gene expression and methylation in several key brain regions, including the striatum, prefrontal cortex and hippocampus.

In summary, we show that *Lphn3* deficiency induces hyper locomotion as well as cognitive alterations that are in coherence with the previously described role of *LPHN3* in cortical synaptic strength. Our novel findings therefore confirm the *Lphn3* knockout mouse as a promising model of ADHD, as well as an excellent tool to improve our knowledge of how *LPHN3* contributes to brain function.

GABAergic substrate of abnormal prefrontal-hippocampal communication during development in a gene-environmental model of mental illness.

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Psychiatric diseases, such as schizophrenia, represent one of the biggest unresolved issues in modern medicine. A comprehensive understanding of the pathophysiology of the disorder lacks, and while advancements have been made in the treatment of positive symptoms, little is known about the disease-related cognitive dysfunction. Using mice models mimicking genetic (Disrupted-In-Schizophrenia 1, DISC1) and environmental (maternal immune activation, MIA) risk factors of disease (dual-hit mice) we previously showed that impaired maturation of functional communication within hippocampal–prefrontal networks switching from hypo- to hyper-coupling may represent a mechanism underlying the pathophysiology of these cognitive deficits. However, the cellular substrate of disturbed long-range coupling in the developing brain is still unknown. To fill this knowledge gap, we combine here morphological assessment with optogenetic manipulation and electrophysiological recordings from the prefrontal cortex (PFC) and hippocampus (HP) of neonatal (postnatal day 8-10) dual-hit GE mice in vivo. We found that the density of GABAergic neurons, especially in upper layers of the prelimbic subdivision of PFC, is significantly increased in dual-hit GE mice when compared with controls. Confocal microscopy-based reconstruction and morphometric analysis of different sub-populations of interneurons revealed disease-related morphological deficits. Correspondingly, the sharp wave-ripple complexes (SPW-R), which critically depend on interneuronal activity, were affected in their power and coupling with prefrontal activity. The contribution of interneurons to abnormal activity patterns within neonatal prefrontal-hippocampal networks of dual-hit GE mice was confirmed by light stimulation of interneurons specifically transfected with high efficiency channelrhodopsins. These data give first insights into the early GABAergic dysfunction in mental illness.

Golgi-associated Cohen syndrome protein COH1 regulates neurite outgrowth in vitro

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Cohen syndrome is an autosomal recessive disorder caused by mutations in the gene VPS13B (COH1). Prominent clinical features are intellectual disability, postnatal microcephaly, pigmentary retinopathy, and intermittent neutropenia. We identified COH1, which encodes a protein of 3997aa, as a peripheral scaffold protein that localizes to the Golgi complex and contributes to its structural maintenance and function. Another study showed that disturbed Golgi complex homeostasis affects glycan maturation and that COH1-deficient cells display a reduced quantity of early endosomes and abnormally enlarged lysosomes, pointing to a role of COH1 in endosomal-lysosomal trafficking. We show that RNAi-mediated knock down of the small GTPase RAB6A/A', which tethers vesicles to the Golgi membrane and controls several trafficking steps, prevents Golgi localization of COH1. Co-immunoprecipitation experiments and mass spectrum analyzes confirmed the physical interaction of COH1 with RAB6, which is in line with studies on yeast Vps13p.

Our ongoing work focusses on Coh1 expression analyses, identification of other COH1 interactors similar to the known yeast Vps13p network, and cortical development studies using RNAi. Depletion of Coh1 by RNAi in primary neurons from the cortex negatively interferes with microtubule dynamics and similar to Rab6 knock down (Schlager et al. 2010) with neurite outgrowth indicating a causal link between the integrity of the Golgi complex, the cytoskeleton and abnormal intracellular trafficking. Due to the lack of an appropriate animal model, we established in utero electroporation to induce Coh1 knock down using RNAi in mice for loss of function studies in the embryonic cerebral cortex. Initial experiments confirmed efficient Coh1 knock down. Currently, we aim to analyze the role of Coh1 for neuronal migration. Together, we conclude that COH1 is a RAB6 effector protein and that reduced brain size in Cohen syndrome patients likely results from impaired COH1 function at the Golgi complex causing decreased neuritogenesis.

Identify dysregulated autophagy as cause for changes in synaptic functioning in Koolen de Vries syndrome

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Autophagy is a process that allows controlled degradation and recycling of proteins, which is essential for maintaining homeostasis. It showed to play an important role in synaptic functioning by reducing the number of surface glutamatergic AMPA receptors (AMPA). Additionally, dysregulation of autophagy has been linked to several neurodevelopmental disorders. Koolen de Vries syndrome (KdVS) is a multisystem disorder characterized, amongst others, by moderate-to-mild intellectual disability, severe speech delay, and epilepsy. Recently, haploinsufficiency of KANSL1 proved to be sufficient to cause the classical KdVS phenotype. Whole transcriptome sequencing data of patient cells showed an enrichment of differentially expressed genes that are involved in neuronal and synaptic functioning, but the exact mechanisms through which KANSL1 affects brain function remain unclear. However, KANSL1 is known to be essential for acetylation of Histone 4 on Lysine 16, an acetylation mark that is implicated in the regulation of autophagy in neurons.

In order to test whether the loss of KANSL1 impairs the regulation of autophagy and thereby affects synaptic functioning, KANSL1 was knocked down (KD) in neuronal cultures. Results indicated that reduced KANSL1 expression increased autophagy in these neurons and thereby affecting the number of surface receptors. Similar investigations are being conducted in neurons derived from induced pluripotent stem cells of Koolen-de Vries syndrome patients in order to examine how autophagy is involved in the regulation of neuronal functioning and differentiation in human cells.

Lack of *Shank1* Leads to Cognitive Deficits, Reductions in Cortical Parvalbumin Expression, and Altered Hippocampal BDNF Levels Related to Epigenetic Modifications in Mice

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Autism spectrum disorders (ASD) are a class of neurodevelopmental disorders characterized by persistent social deficits and repetitive patterns of behavior, often associated with intellectual disability. Among the most promising ASD candidate genes is the *SHANK* gene family, including *SHANK1*. To study the contribution of *SHANK1* mutations to ASD symptoms and potential underlying neurobiological mechanisms throughout development, *Shank1*^{+/+}, *Shank1*^{+/-}, and *Shank1*^{-/-} mice were compared in behavioral assays developed to detect social and cognitive deficits as juveniles and adults. At both developmental stages, mice of both sexes were tested in two social behavior assays, one for assessing social motivation deficits, i.e. social approach, and one for assessing social cognition deficits, i.e. social recognition. In addition, a non-social memory task, i.e. novel object recognition, was conducted. As juveniles, social approach and recognition were evident irrespective of genotype. In adulthood, *Shank1*^{-/-} males and controls displayed normal social approach, but impaired social recognition. Adult *Shank1*^{-/-} females exhibited deficits in social recognition only. However, the most prominent genotype effect was detected in novel object recognition. In juveniles, novel object recognition was clearly affected by *Shank1* deletion, with *Shank1*^{-/-} mice being severely impaired, i.e. not showing a preference for the novel object. Novel object recognition was also impaired in adult *Shank1*^{-/-} males but not females. Object recognition deficits in *Shank1*^{-/-} mice were not due to impairments in object discrimination. Specifically, when exposing mice to three identical and one unique object at the same time, juvenile *Shank1*^{+/+} and *Shank1*^{-/-} mice both displayed a preference for the unique object. Moreover, object recognition deficits in *Shank1*^{-/-} mice were replicated in a separate cohort of juvenile animals. In the same cohort, hippocampal BDNF protein levels were determined and genotype-dependent differences detected. Specifically, hippocampal BDNF protein expression was found to be higher in *Shank1*^{-/-} mice than in *Shank1*^{+/+} littermate controls. No such difference in BDNF protein expression was seen under baseline conditions in mice not exposed to novel object recognition. Epigenetic regulation of BDNF in the hippocampus was also found to be affected by genotype. While H4 acetylation did not differ between genotypes, H3 acetylation was found to be higher in *Shank1*^{-/-} mice, in line with the observed increase in hippocampal BDNF protein levels detected in *Shank1*^{-/-} mice. Finally, the expression of the Ca²⁺-binding protein parvalbumin was studied in the somatosensory cortex using a stereological approach. Parvalbumin expression was found to be clearly lower in juvenile *Shank1*^{-/-} mice. This reduction was not due to a lower number of parvalbumin-expressing GABAergic interneurons but caused by lower protein levels, and is in line with a decrease in parvalbumin immunoreactivity reported for several ASD mouse models. Parvalbumin plays an important role in regulating the excitation/inhibition balance in the brain and its absence leads to a robust ASD-like behavioral phenotype in mice. In summary, the present findings indicate that *Shank1* deletions lead to cognitive deficits, reductions in cortical parvalbumin expression, and altered hippocampal BDNF levels, possibly due to epigenetic modifications. Funding by

the Deutsche Forschungsgemeinschaft (DFG): WO 1732/1-1.

Neuronal networks on Micro-Electrode Arrays: a model to study Neurodevelopmental Disorders

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Motivation

Neurodevelopmental disorders (NDDs), including intellectual disability (ID) and autism spectrum disorders, are phenotypically and genotypically heterogeneous. One increasing group of NDDs is caused by mutations in epigenetic regulators of gene expression. Recent studies have identified Euchromatin Histone Methyltransferase 1 (EHMT1) as a key modulator in cognition. Heterozygous loss of EHMT1 causes Kleefstra syndrome, which is characterized by moderate-to-severe ID, autistic behaviour, microcephaly and dysmorphic features [1].

While the identification of disease genes has great benefits for diagnostic and prognostic purposes, for the vast majority of these genes there is little knowledge about neurobiological mechanisms that they control at the cellular and network level. Recent studies demonstrate that NDDs are “diseases of the synapse” [2]. Synaptic malfunction can severely affect network connectivity and dynamic. Understanding the neural circuit basis of NDDs is therefore imperial for a better understanding of these disorders.

Here, we studied the effect of EHMT1-deficiency on neuronal activity in-vitro. To understand if the disruption of EHMT1 give rise to an impairment of the network maturation, we monitored the electrophysiological activity of neuronal networks coupled to Micro-Electrode Arrays (MEAs) during development. We used three models: (1) rat dissociated cortical neurons; (2) dissociated neuronal cultures from a heterozygous knock-down mouse; and (3) neurons derived from Induced Pluripotent Stem Cells (iPSC) of Kleefstra patients. In all cases, we compared the electrophysiological activity of EHMT1-deficient neurons to wild type controls.

Results

In order to understand if the disruption of EHMT1 gives rise to an impairment of the network maturation we monitored the electrophysiological activity of a dissociated culture from wild-type (WT, n=12) and Ehmt1^{+/-} mice (n=10) on MEAs during development. Figure 1 shows raster plots of the spontaneous activity of a representative experiment performed on WT and EHMT1-deficient networks. EHMT1-deficient network presents less global activity and synchrony compared to the WT condition: the regularity of the network bursting activity is also impaired.

The level of firing rate of the EHMT1-deficient network is statistically lower ($p < 0.01$) than the one exhibited by the control network at DIV 13 (WT 1.1 ± 0.1 spike/s; Ehmt1 0.7 ± 0.1 spike/s), DIV 15 (WT 2.1 ± 0.2 spike/s; Ehmt1 1.1 ± 0.2 spike/s) and DIV 17 (WT 2.1 ± 0.2 spike/s; Ehmt1 1.2 ± 0.2 spike/s). The rate of synchronous events generated by the EHMT1-deficient networks is also statistically lower during early development (DIV 13, WT 4.3 ± 0.9 burst/min; Ehmt1 1.1 ± 0.4 burst/min. DIV 15, WT 12.9 ± 1.8 burst/min; Ehmt1 1.7 ± 0.6 burst/min. DIV 17, WT 7.5 ± 1.1 burst/min; Ehmt1 1.6 ± 0.6 burst/min). Later in development (i.e. DIV 20) the EHMT1-deficient network showed a level of activity similar to the control (WT, 2.0 ± 0.2 spike/s; Ehmt1, 1.6 ± 0.2 spike/s). However the synchronized bursting activity remained

impaired, which resulted in a statistically different bursting rate (WT 8.2 ± 1.1 burst/min; Ehmt1 2.6 ± 0.7 burst/min) and in a lower level of network regularity (i.e. coefficient of variability computed on the interval between two consecutive network burst is statistically higher in the EHMT1-deficient networks).

We also monitored the electrophysiological activity of dissociated cultures from rats (WT, n=16 and Ehmt1, n=13). We show that EHMT1 deficiency affects network maturation. The results are comparable with the ones obtained with dissociated neuronal networks from mice.

Then, we also recorded the electrophysiological activity of neurons derived from healthy subjects and Kleefstra patients. Few days after plating on MEAs, the neurons derived from healthy subject form a neuronal network, showing spontaneous events already during the second week in vitro. Late in development (i.e. fourth week in vitro) the neuronal network shows high level of spontaneous activity and synchronous events are detected. Regarding the neurons derived from Kleefstra patients, spiking activity is present early in development and the neuronal network exhibits synchronous events involving most of the channels of the MEAs. Our preliminary results show that there is a difference between the two conditions in terms of connectivity and network organization.

Discussion

We demonstrated that the emergence of spontaneous network activity was delayed EHMT1-deficient networks compared to control condition. The delay in spontaneous network activity early in development resulted in an increased network burst irregularity in later time points. These results support the notion that early developmental deficits could lead to irreversible changes. This suggests that EHMT1 may play a critical role in a developmental mechanism of gene expression regulation during early brain maturation. The identification of reasons that cause abnormal network activity will be important for the understanding, and ultimately treatment, of Kleefstra syndrome.

References

- [1] Kleefstra, T. et al. (2006). *Am. J. Hum. Genet.* 79: 370–377.
- [2] Pavlowsky, A. et al. (2011). *Mol. Psychiatry* 17: 682–693.
- [3] Zhang, Y. et al. (2013). *Neuron* 78(5): 785-798.

Neuronal redox imbalance in Rett syndrome: a key player in neuronal network dysfunction and altered neurotransmitter responsiveness?

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Rett syndrome (RTT) is a neurodevelopmental disorder with a delayed onset, which occurs almost exclusively in girls. Its main genetic reasons are *de novo* mutations in the methyl-CpG binding protein 2 gene (*MECP2*). After a short time of apparently normal development, a RTT child falls into developmental stagnation, followed by neuronal and autonomic dysfunction, which manifests as mental retardation, erratic breathing, epilepsy, loss of speech, stereotypical hand movements and motor disturbances. Furthermore, RTT is associated with early mitochondrial dysfunction and systemic oxidative stress. We previously showed that mitochondria of MeCP2-deficient (*Mecp2*^{-/-}) mouse hippocampus are partly uncoupled and consume higher rates of oxygen. In concert with less efficient cell-endogenous scavenging systems, this culminates in cellular redox dysregulation. To uncover the very molecular events contributing to this redox impairment, we extended optical redox imaging specifically to neurons and their cytosolic and mitochondrial compartments. Taking advantage of the genetically-encoded redox sensor reduction-oxidation sensitive GFP1 (roGFP1), expressed either in cytosol (cyto-roGFP1) or mitochondrial matrix (mito-roGFP1), we quantified redox conditions in these compartments. Optimized and neuron-specific roGFP1 expression was guaranteed by viral vectors (AAV-6) and the synapsin I promoter. In dissociated cell cultures and organotypic slices of *Mecp2*^{-/-} hippocampus, we confirmed a clear redox imbalance in both cytosol and mitochondria. These changes were especially obvious in organotypic slices, in which redox challenge by H₂O₂ and severe hypoxia elicited intensified oxidizing and reducing transients in *Mecp2*^{-/-} neurons, respectively. More importantly, stimulation by glutamate, dopamine, serotonin, and norepinephrine consistently evoked intensified oxidizing shifts in the cytosol of *Mecp2*^{-/-} neurons, suggesting that even physiological stimuli such as neurotransmitters are sufficient to provoke overshooting redox responses. In mitochondria, the neurotransmitter-evoked redox changes were more moderate than in cytosol, and the genotypic differences between WT and *Mecp2*^{-/-} neurons were less pronounced. This identifies the cytosol as an important ROS source and as less stable redox buffered compartment. Cellular Ca²⁺ overload due to massive Ca²⁺ influx can be excluded as a primary cause for the neurotransmitter-induced redox responses and their overshooting characteristics in *Mecp2*^{-/-} neurons. Instead, by pharmacological intervention we confirmed a leading role of NADPH- and xanthine oxidases in ROS production; the very route of activation of these oxidases apparently differs among *Mecp2*^{-/-} and WT neurons. All of these changes were already evident in tissue of neonatal and presymptomatic mice. Therefore, it will be crucial to map these changes as RTT emerges and slowly progresses in severity. A crucial tool for this future endeavour will be our recently generated redox-indicator mice, which are currently crossbred with Rett mice. Only then it will become clear to what degree early neuronal redox alterations contribute to neuronal network dysfunction and promote the further progression of RTT.

Supported by the DFG Research Center Molecular Physiology of the Brain (CMPB).

Optogenetic studies on dysfunctions of striatal cholinergic interneurons in dystonia

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Dystonias are movement disorders, defined by sustained or intermittent muscle contractions causing twisting movements and postures. It has been hypothesized that an increased activity of cholinergic interneurons in the striatum, resulting in abnormal synaptic plasticity, plays an important role in the pathophysiology of an inherited type of dystonia, which is caused by a mutation in the gene for torsin A (DYT1, GAG) and shows a low penetrance. However, this hypothesis is merely based on ex vivo electrophysiological measurements in brain slices of animal models which do not show a dystonic phenotype. Here we aim to substantiate 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 GAG 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.

First, we ensured that ChR2 is expressed in cholinergic interneurons of ChR2 mice by immunohistochemistry and confocal microscopy and that these neurons respond to blue light exposure in brain slices. Effects of different stimulation parameters (e.g. pulse width, Hz) on motor activity were examined in WT/ChR2 (n=7) and Dyt1/ChR2 mice (n=5). Bilateral and unilateral photostimulation with blue LED light was done in comparison with yellow LED light which does not open the ChR2 (negative control). As expected, the yellow light had no influence on behavior. Effects of bilateral stimulations with blue light were more robust than unilateral stimulations. The chosen stimulation parameters induced significant increases of the general activity (increased distance moved) in Dyt1/ChR2 compared to the period before as well as after stimulation while the WT/ChR2 showed no effect by stimulation with blue LED light. This substantiate the role of striatal cholinergic neurons in motor activity. Preliminary data of an investigation of neuronal activity in the striatum after stimulation indicated an increased number of ChR2 cells positive for c-Fos in Dyt1/ChR2 (n=2) in comparison to WT/ChR2 (n=3) while there was no difference in total number of c-Fos positive cells. Currently we examine the neuronal activity in the striatum in unstimulated WT/ChR2 and Dyt1/ChR2 mice.

Based on the established stimulation protocol we now perform comprehensive studies with short- and long-term stimulations in the Dyt1 mice to clarify the importance of overactive striatal cholinergic interneurons for the manifestation and abnormal striatal plasticity in DYT1 dystonia. Therefore, optogenetics will be combined with tests on sensorimotor behavior and with pharmacological, electrophysiological and molecular examinations.

Homozygous *YME1L1* Mutation Causes Mitochondriopathy with Optic Atrophy and Mitochondrial Network Fragmentation

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Mitochondriopathies often present clinically as multisystemic disorders of primarily high-energy consuming organs. Assembly, turnover, and surveillance of mitochondrial proteins are essential for mitochondrial function and a key task of AAA family members of metalloproteases. We identified a homozygous mutation in the nuclear encoded mitochondrial escape 1-like 1 gene *YME1L1*, member of the AAA protease family, as a cause of a novel mitochondriopathy in a consanguineous pedigree of Saudi Arabian descent. The homozygous missense mutation, located in a highly conserved region in the mitochondrial pre-sequence, inhibits cleavage of *YME1L1* by the mitochondrial processing peptidase, which culminates in the rapid degradation of *YME1L1* precursor protein. Impaired *YME1L1* function causes a proliferation defect and mitochondrial network fragmentation due to abnormal processing of *OPA1*. Our results identify mutations in *YME1L1* as a cause of a mitochondriopathy with optic nerve atrophy highlighting the importance of *YME1L1* for mitochondrial functionality in humans.

Loss of MeCP2 disrupts cell autonomous and autocrine BDNF signaling in mouse glutamatergic neurons

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Methyl CpG-binding protein 2 (MeCP2) is a transcriptional regulator whose loss-of-function mutations result in neurodevelopmental disorders such as Rett syndrome. Prior studies reported that either loss or doubling of MeCP2 results in reduced or increased synaptic output and glutamatergic synapse number, respectively. This study aims to understand the role of MeCP2 in regulating synapse formation in excitatory neurons and the significance of optimal brain-derived neurotrophic factor (BDNF) signaling in maintaining neuronal function in MeCP2 mutant neurons. Techniques including whole-cell voltage patch clamp and imaging by confocal/epifluorescence microscopy were employed to study excitatory postsynaptic currents (EPSCs) and morphology of cultured hippocampal neurons, respectively.

Respiratory acidosis induces migration defects of neurons in cerebral cortex and hippocampus

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Diseases of the nervous system may result from genetic factors or from brain damage during development. Especially during late stages of gestation, hypoxia leading to acidosis may cause severe developmental defects. Generally, two forms of acidosis can be differentiated, metabolic and respiratory acidosis. Maternal acidosis may eventually affect the normal development of the fetus, but detailed studies are missing so far. In order to study the impact of acidosis on fetal brain development, we induced respiratory acidosis in pregnant mice by exposing them to 80% carbon dioxide at E 14.5-E17.5. This was followed by immunostaining of neuronal layers of the cerebral cortex and hippocampus in new-born mice using layer-specific markers. Under normal conditions, cortical neurons born in the ventricular zone migrate radially towards the marginal zone. In the hippocampus, new-born granule cells migrate from the hilus to the granule cell layer. The results of our experiments show that carbon dioxide exposure induced accumulation of late-born neurons in the deep layers of the cerebral cortex and loss of asymmetric polarity of migrating granule cells destined to the granule cell layer in the dentate gyrus. Cofilin is an actin-depolymerizing protein, and phosphorylation of cofilin induces its deactivation. Western blot analysis indicated that maternal acidosis caused an increased phosphorylation of cofilin in migrating neurons of the cerebral cortex and hippocampus. Our results provide evidence that maternal acidosis may induce migration defects of neurons in the cerebral cortex and hippocampus by promoting abnormal cofilin phosphorylation, which results in cytoskeletal stabilization and neuronal arrest. (supported by the DFG: FR 620/12-2)

Sex and Violence – Social phenotypes in *Dnlg2* and *4* deficient *Drosophila*

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Neuroligins (NLGNs) are a group of postsynaptically expressed proteins which contribute to the formation and maturation of synapses in both vertebrates and invertebrates, ensuring stable and correct connections between nerve cells [1, 2]. Neuroligins have been reported to play a role in both mammals and insects [3]. Mutations of neuroligin encoding genes in humans have been associated with the autism spectrum disorders (ASDs) [4, 5]. ASDs are characterized by deficits in social interactions, communication impairments and repetitive behaviours. NLGN mutations have also been implied in behavioural phenotypes of *Drosophila melanogaster*, which possesses four NLGN genes.

In the presented study, we investigate the role of two of these genes, *dnl2* and *dnl4*, and its effect on the behaviour. *Drosophila* normally shows stereotypical courtship and aggression behaviour towards conspecifics. We therefore investigated, whether these behaviours were altered or reduced in Neuroligin-deficient mutants, by analysing video recordings of freely behaving flies and assessing their performance using a custom software. Group assays showed that Neuroligin mutant flies show abnormal group sizes and male chaining behaviour. Aggression and courtship behaviour on the other hand was assessed in a smaller setup, containing 2 male flies and a decapitated female, which allowed for detailed tracking of the flies actions. This experiment revealed altered frequency of aggressive and courtship behaviour in response to external sound stimuli.

Furthermore, we wanted to analyse if any alterations in behaviour were due to an effect on the physiology, resulting in the inability to acoustically perceive courtship songs and aggression sounds or act upon them, or if the effect resulted from a direct effect on the neuronal circuits controlling behaviour. We therefore studied the physiological ability of neuroligin deficient flies to perceive sound using a Laser-Doppler-Vibrometer, combined with electrophysiological recordings of the auditory system and basic locomotion using a Benzer climbing assay. We found no significant impairments in Neuroligin mutant flies.

References

1. Nam CI, Chen L (2005) Postsynaptic assembly induced by neurexin-neuroligin interaction and neurotransmitter. *Proceedings of the National Academy of Sciences USA* 102:6137-6142
2. Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, Zhang W, Südhof TC, Brose N (2006) Neuroligins determine synapse maturation and function. *Neuron* 51:741–754
3. Ichtchenko K, Hata Y, Nguyen T, Ullrich B, Missler M, Moomaw C, Südhof TC (1995) Neuroligin 1: a splice-site specific ligand for -neurexins. *Cell* 81:435–443
4. Jamain S, Quach H, Betancur C, Råstam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B,

Leboyer M, Gillberg C, Bourgeron T (2003) Paris Autism Research International Sibpair Study. Mutations of the X-linked genes NLGN3 and NLGN4 are associated with autism. *Nature Genetics* 34:27-29

5. Pardo CA, Eberhart CG (2007) The neurobiology of autism. *Brain Pathology* 17(4):434-447

SOMATOSTATIN DISTRIBUTION IN THE ENTORRHINAL CORTEX OF DELAYED AND SENESCENCE ACCELERATED MOUSE MODELS

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The hippocampal region has been one of the most studied areas of the brain in the last decades. This region includes the Hippocampal Formation (HF) and the Parahippocampal Region (PHR). On one side, the mouse HF looks like a C-shaped structure and comprises dentate gyrus, Cornu Ammonis fields (CA1-3) and Subiculum. On the other side, the PHR has a cortical cover which is extended in a rostrocaudal and ventrodorsal way. PHR is made up of presubiculum, parasubiculum, medial and lateral entorhinal cortex (MEC and LEC), perirhinal cortex and postrhinal cortex. The EC could be considered the most important area of the PHR regarding to its connections. EC includes six layers, where layers II and III are the main source of the perforant pathway, the cortical inputs to dentate gyrus and the hippocampus proper. According to this projection to the DG, the EC has been divided into MEC and LEC. However, from a cytoarchitectonic and chemoarchitectonic point of view, the EC could be subdivided into 6 areas. By using immunoreactivity (IR) (with specific antibodies), it has been possible to find several populations of neurons in the EC. In the last decades, somatostatin (SOM) has received growing scientific interest as the depletion in SOM-IR in the cortex has been observed not only in normal aging, but also in pathological processes, such as Alzheimer disease. In this disease, loss of the SOM-IR can be related with a poor cognitive function and memory impairment. Since a correct excitation/inhibition equilibrium seems to be crucial for normal brain development, functioning, and controlling cortical plasticity, the aim of this work is the analysis of the pattern of SOM-IR in MEC and LEC during aging in two different mice models.

We analysed the SOM-IR by the immunolabelling of the EC of a delayed age cognitive decline mouse model, polymerase μ knockout. Afterwards, we performed the same assay in the accelerated senescence model, SAMP8 to compare the SOM-IR pattern of both mouse populations. The density and distribution of SOM-IR neurons have been analysed in the EC mice at 4, 12 and 18 months old. Immunohistochemistry allowed the observation of positive neuronal cell bodies and fibers widely distributed along the EC. The results show different expression patterns of SOM-IR profiles in the above-described areas of the EC through age in both models. A better knowledge of the changes in the expression and distribution of SOM in a key cerebral structure like EC could help to establish the morphological basis underlying age-related cognitive decline, not only in the normal but also in pathological situations. This work has been supported by Junta de Comunidades de Castilla-La Mancha, Spain, grant PEII-14-0348-8331.

Studying the long term neuropathological consequences of encephalopathy of prematurity in a small animal model

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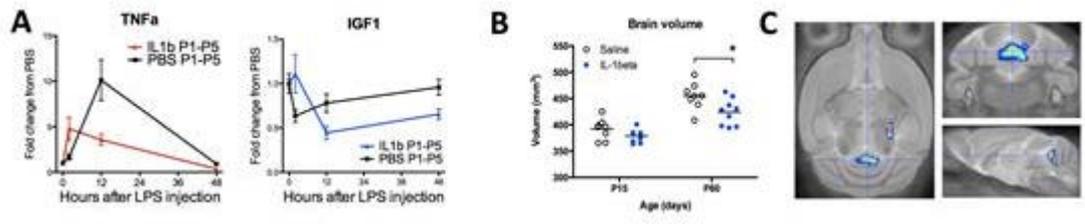
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Introduction: Preterm birth causes more death and disability than any other disease or disorder in children under 5. Rates of preterm birth are at 10% of live births and steadily increasing in the developed world. Significant improvements in clinical care mean that mortality has fallen sharply, but rates of morbidity are still relatively high and we have a growing number of aging adults that were born preterm. It is increasingly appreciated that preterm infants do not just suffer from a static neurological injury, but that they have on going inflammatory changes and are vulnerable to further neurodegeneration with age. The specific neuropathological substrate of this effect is thought to involve microglia but remains to be understood.

Methods & Results: We have a pre-clinical model that recapitulates the early clinical hallmarks of encephalopathy of prematurity, specifically, exposure of mice to interleukin 1B (IL-1B) from postnatal day (P) 1-5. We are now using this model to study the long-term consequences of early life injury on the mouse brain. In this study we examined the systemic and neuroinflammatory milieu of perinatally exposed mice that were exposed to an immune rechallenge as adults. Specifically, we took mice that were exposed to IL-1B or PBS from P1-P5 and injected i.p. lipopolysaccharide (LPS; 0.03mg/kg) at P45 and measured the systemic immune response (multiplex cytokines/chemokine) and the gene expression (n=12 markers) of MACS isolated CD11B+ microglia/macrophage at +2, +14 and +24 hours (examples in Figure panel A). Systemic cytokines/chemokines and microglial activation state markers indicators showed an exaggerated early response, but an overall quicker resolution of the response to LPS. In addition to study how the brain is changing over time from a global standpoint, using gadolinium enhanced T2 weighted MRI we observe a significant reduction in total brain volume at P60 (figure panel B), that is not present at P15. Using voxel-wise deformation based morphometry, decreases in brain volume were found to be localised to the cerebellum (figure panel C) and to the globus pallidus (repeated measures ANOVA significant age x treatment interaction; q=0.05). Preliminary assessment of the pathological substrates of these specific changes reveals that for the cerebellum that although there is an early myelin deficit (decreased MAG protein via western blotting), that there is no difference in proteins levels of MAG, MOG or MBP by P60. As such, persistent myelin changes are unlikely to underpin the age related reduction in volume in the cerebellum. In the deep grey matter, there is a persistent increase in Iba1 positive cell number (130±7%, p=0.032, t-test). Further studies: Additional neuropathological analysis of neuronal numbers, glial numbers, and glial activation states are being undertaken to link these MRI changes to their cellular correlates.



The hypoxia sensing pVHL-EGLN1-Hif1 α pathway is critical for cerebellar granule cell migration

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Approximately 10% of live birth every year in the world are preterm and many infants are born with very low birth weights. These infants often experience cognitive, behavioral, or socialization deficits due to oxygen deprivation during pregnancy or delivery. Associated with these deficits are structural brain sequelae that include regional reduction of brain volume, enlarged ventricles, and in the worst cases, profound white matter hemorrhagic lesions. Intriguingly, these structural abnormalities are due not only to cellular damage but also to profound physical underdevelopment of the brain. During brain development, defective oxygen homeostasis arrests the maturation and polarization of neuronal progenitor cells by previously unknown mechanisms. In the last years we have discovered the link between oxygen homeostasis and cellular control of neuronal differentiation. The EGLN1-pVHL-Hif1 oxygen-sensing pathway is required for cerebellar granule neurons (CGNs) to exit the germinal zone (GZ). Under normoxic conditions Hif1 is continuously targeted for proteasomal degradation through the activity of EGLN1 and pVHL. During hypoxia Hif1 is stabilized and activates the expression of Zeb1, a transcription factor that negatively regulates CGN differentiation and polarization through the repression of members of the Partitioning-defective (PAR) polarity signaling complex and the cell adhesion molecule Chl1.

As a model system to study GZ exit and neuronal migration we made use of mouse organotypic cerebellar ex vivo slices. Deletion of EGLN1 or pVHL via electroporation of a constitutive Cre vector into cerebelli of EGLN1^{fl/fl} or pVHL^{fl/fl}-mice, overexpression of stabilized Hif1 or incubation at low oxygen concentration result in failed CGN exit of the GZ and migration to the IGL. Migration could be rescued in all conditions by coelectroporating EGLN1 or pVHL or deleting Hif1 in cerebelli of Hif1^{fl/fl}-mice.

To screen for Hif1 -downstream targets that mediate the repression of differentiation and migration we purified CGN overexpressed with stabilized Hif1 and extracted total RNA for subsequent microarray and qRT-PCR analysis. Among downregulated Hif1 target genes are several genes that are already known to be necessary for neuronal migration (e.g. NDE1, DCC, NEUROD4), the PAR complex member PAR3 and PAR6 and the cell adhesion molecule Chl1. Significantly upregulated is the transcription factor Zeb1, that was recently shown to prevent CGN GZ exit and downregulate PAR complex member. Thus, silencing Zeb1 with Zeb1-shRNA rescued the failed GZ exit in cerebella overexpressed with stabilized Hif1 or slices incubated at low oxygen concentrations. Similarly, the Zeb1 downstream targets PAR3 and PAR6 and the cell adhesion molecule Chl1 rescue the failed GZ exit.

Taken together, our data reveal a critical role of the oxygen sensing EGLN1-pVHL-Hif1 -Zeb1 signaling pathway for the differentiation and migration of cerebellar granule neurons.

Homozygous ARHGEF2 gene mutation causes intellectual disability and midbrain-hindbrain malformation

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Mid-hindbrain malformations can occur during embryogenesis through a disturbance of transient and localized gene expression patterns within these distinct brain structures. Controlling timing and site of Rho GTPase activation is a key task of Rho guanine nucleotide exchange factor (ARHGEF) family members. We identified, by means of whole exome sequencing, a homozygous frameshift mutation in the ARHGEF2 gene as a cause of intellectual disability, mild microcephaly, and a midbrain-hindbrain defect in a consanguineous pedigree of Kurdish-Turkish descent. We demonstrate that the human brain phenotype of pons and cerebellum hypoplasia as well as microcephaly can be mimicked in Arhgef2 deficient mice. Loss of ARHGEF2 inhibits precursor cell differentiation. This is associated with a shift of mitotic spindle plane orientation, putatively favoring more symmetric divisions, and with reduced activation of the RhoA/ROCK/MLC pathway downstream of Wnt. Our results identify a mutation in ARHGEF2 as a cause of a neurodevelopmental disorder highlighting the importance of ARHGEF2 for mid-hindbrain development in humans.

Poster Topic

T11: Alzheimer's, Parkinson's and other Neurodegenerative Diseases

- [T11-1A](#) Acid sphingomyelinase inhibitor amitriptyline induces angiogenesis of cerebral microvascular cells by mechanisms involving the Notch pathway
Tanja Bergmann, Ayan Yusuf, Nina Hagemann, Dirk M. Hermann
- [T11-2A](#) alpha-Synuclein aggregation and spreading in mouse models of Parkinson's disease
Karina Joppe, Lars Tatenhorst, Karin Giller, Stefan Becker, Markus Zweckstetter, Mathias Bähr, Paul Lingor
- [T11-3A](#) Altered autophagic pathway in TBK1-mutant Motor Neurons derived from human induced pluripotent stem cells
Alberto Catanese, Alena Ehrmann, Maria Demestre, Axel Freischmidt, Jochen Weishaupt, Francesco Roselli, Tobias Boeckers
- [T11-4A](#) Altered hippocampal and cortical EEG frequency characteristics in the APPswePS1dE9 model of Alzheimer's disease
Marco Weiergräber, Julien Soos, Andreas Lundt, Carola Wormuth, Ralf Müller, Christina Henseler, Karl Broich, Dan Ehninger, Britta Haenisch, Anna Papazoglou
- [T11-5A](#) Altered long-term potentiation in the hippocampus of PS19-P301S transgenic mice
Michael Bahr, Eva Harde, Ana Relo, Berthold Behl, Karsten Wicke, Maria Vasileva
- [T11-6A](#) Assessment of The Neuroprotective Effects of Ezetimibe versus Simvastatin in Animal Model of Alzheimer's Induced Dementia
Mohamed Elgamal, Mohamed Salama, Mahmoud KhalafAllah, Mohamed Zalabia, Esraa Elsayed, Wael Mohamed, Mohamed Sobh
- [T11-7A](#) BACE1 modulates synaptic transmission at the hippocampal mossy fiber synapse by regulating K_v3.4 channels.
Stephanie Hartmann, Fang Zheng, Michele Constanze Kyncl, Sandra Lehnert, Carla D'Avanzo, Kerstin Völkl, Christian Alzheimer, Doo Yeon Kim, Tobias Huth
- [T11-8A](#) Characterization of epileptiform activity in hippocampal slices
Maria Vasileva, Tanja Georgi, Karsten Wicke, Eva Harde
- [T11-9A](#) Contextual fear learning and hippocampal plasticity in APP/PS1 mice
Georgia - Ioanna Kartalou, Thomas Endres, Gloria Hölzl, Elke Edelmann, Kurt Gottmann, Volkmar Leßmann

- [T11-10A](#) Contralateral BoNT-A injection in the striatum of 6-OHDA hemilesioned rats give evidence for long lasting effects on basal ganglia circuitry
Alexander Hawlitschka, Andreas Wree
- [T11-11A](#) Could hyperhomocysteinemia affects neurodegeneration after ischemia/reperfusion injury of rat brain?: An experimental model of a possible development of Alzheimer`s disease
Maria Kovalska, Barbara Tothova, Dagmar Kalenska, Marian Adamkov, Jan Lehotsky
- [T11-12A](#) Cuprizone induced de- and remyelination in the spinal cord of transgenic mice
Phillip Rieder, Andreea Pantiru, Babette Fuss, Anja Scheller, Frank Kirchhoff
- [T11-13A](#) Detailed classification of epileptiform activity reveals anti-correlation between severe and mild epileptic bursts.
Katharina Heining, P. Janz, A. Kiliyas, CA. Haas, U. Egert
- [T11-14A](#) Developing an isogenic neuron-astrocyte model to study the isoform-specific effect of APOE in late-onset Alzheimer`s Disease
Shadaan Zulfiqar, Katja Nieweg
- [T11-15A](#) Poly(Propylene Imine)dendrimers inhibit RT-QuIC based in vitro amplification of prion protein
Niccoló Candelise, Susana Margarida da Silva Correia, Dietmar Appelhans, Matthias Schmitz, Inga Zerr
- [T11-1B](#) Development of Parkinsonian pathophysiology on the single unit level: a rat 6-OHDA study
Zifeng Xia, Frank W Ohl, Michael Lippert, Kentaroh Takagaki
- [T11-2B](#) 'Disease Modeling in a dish' for sporadic Amyotrophic Lateral Sclerosis using Human Embryonic Stem Cells derived Motor Neurons.
Sumitha Rajendrarao, Sabitha K.R, Laxmi. T. Rao, Phalguni. A. Alladi, A Nalini, Boris .W Kramer, Sathyaprabha T.N., Raju T.R
- [T11-3B](#) Disturbed GABAergic transmission in the *cln3*^{-/-}-mouse model of Batten disease
Benedikt Grünwald, Maren D. Lange, Christian Werner, Aet O`Leary, Andreas Weishaupt, Sandy Popp, David A. Pearce, Andreas Reif, Hans C. Pape, Klaus V. Toyka, Claudia Sommer, Christian Geis
- [T11-4B](#) Early EEG abnormalities in a model of tauopathy
Peter Veselcic, Maria Vasileva, Eva Harde, Karsten Wicke
- [T11-5B](#) Effect of caffeine and MDMA or methamphetamine combination on DA and 5-HT release and DNA damage in the mouse brain
Krystyna Golembiowska, Anna Gorska, Katarzyna Kaminska, Grzegorz Kreiner
- [T11-6B](#) Effect of galactose-rich diet on neurodegeneration in an animal model of multiple sclerosis
Kristina Kuhbandner, Sarah Hirschberg, Stefanie Jörg, Ralf Gold, Aiden Haghikia, Ralf A. Linker
- [T11-7B](#) Effects of RGFP109, a specific Histone Deacetylase (HDAC) inhibitor, on neuronal health and rescue of transcription in neuronal culture model of Huntington`s disease.
Ashraf Nabil Abdo, Miguel Angelo Lopes, Foteini Paraskevopoulou, Christian Rosenmund,

- [T11-8B](#) Evaluation of chronic nicotine treatment on hippocampal oscillatory activity and sleep pattern analysis of a G72 transgenic mouse model for schizophrenia
Anna Papazoglou, Andreas Lund, Julian Soós, Boris Hamsch, Andreas Zimmer, Karl Broich, Marco Weiergäber
- [T11-9B](#) EXPERIMENTAL PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY
Olga Gevka, Nataliia Luchkiv, Olena Deltsova, Sergii Gerashchenko, Mykola Ostrovskiy
- [T11-10B](#) Functional impairment of cortical astrocytes in ALS-transgenic mice
Maximilian Bauer, Melisa Suljkanovic, Sabine Liebscher
- [T11-11B](#) Inhibition of GABA A Receptor Improved Spatial Memory Impairment in the Local Model of Demyelination in Rat Hippocampus
Forough Ebrahim Tabar, Alireza Mousavi, Arghavan Afghani Afghani, Sahand Ashraf Pour, Mohammad Gol, Fereshteh Pourabdolhossein
- [T11-12B](#) Graph properties of the functional connected brain under the influence of Alzheimer's disease
Claudia Bachmann, Heidi Jacobs, Kim Dillen, Simone Buttler, Gereon R Fink, Juraj Kukolja, Abigail Morrison
- [T11-13B](#) Impaired synaptic plasticity and increased hyperexcitability in a mouse model of Alzheimer's disease
Eva Harde, Karsten Wicke, Maria Vasileva
- [T11-14B](#) Intracellular Ca^{2+} stores affect cortical visual processing in a mouse model of Alzheimer's disease
Nithi Asavapanumas, Bianca Brawek, Olga Garaschuk
- [T11-15B](#) Intracellular transport steps involved in degradation of α -synuclein aggregates
Anna Maria Hilverling, Björn Falkenburger
- [T11-16B](#) The role of cellular prion protein in Alzheimer's disease
Ângela Patricia Silva Correia, Tobias Thom, Matthias Schmitz, Inga Zerr
- [T11-1C](#) Investigation of sleep architecture of G72/G30 Transgenic Mouse Model of Schizophrenia
Julien Soós, Anna Papazoglou, Andreas Lundt, Maheshwar Bakki, Andreas Zimmer, Karl Broich, Marco Weiergräber
- [T11-2C](#) iPSC derived neurons as a human model to study altered AMPA receptor function in Niemann-Pick Type C1
Michael Rabenstein, Franziska Peter, Arndt Rolfs, Moritz J. Frech
- [T11-3C](#) Lipid microdomain modification sustains neuronal viability in models of Alzheimer's disease
Silke Herzer, Sascha Meldner, Hermann-Josef Groene, Viola Nordstroem
- [T11-4C](#) Live-Imaging of calcium-induced axonal degeneration in transgenic mouse models of Parkinson's Disease

- [T11-5C](#) Loss of tubulin-alpha-4a polyglutamylation in mice
Torben Johann Hausrat, Petra Breiden, Sabine Hoffmeister-Ullerich, Louisa Rathgeber, Irm Hermans-Borgmeyer, Matthias Kneussel
- [T11-6C](#) Lower affinity of isradipine for L-type Ca²⁺ channels during *Substantia nigra* dopamine neuron-like activity: implications for neuroprotection in Parkinson's disease
Nadine Jasmin Ortner, Gabriella Bock, Antonios Dougalis, Maria Kharitonova, Johanna Duda, Petronel Tuluc, Thomas Pomberger, Nadia Stefanova, Florian Pitterl, Thomas Ciossek, Herbert Oberacher, Henning J. Draheim, Birgit Liss, Jörg Striessnig
- [T11-7C](#) Modeling of EEG time-series by Conditional Probability Echo State Networks.
Hannes Rapp, Martin Paul Nawrot, Moritz Deger
- [T11-8C](#) Morphological alterations of cerebellar cells in Engrailed-2 transgenic mice: A Quick Golgi study
Nicoletta Czechowska, Jakob Jankowski, Christian Liebig, Michael Hofmann, Stephan L. Baader
- [T11-9C](#) Morphological and molecular changes in mossy fiber – CA2 connectivity in epilepsy
Midori Johnston, Philipp Janz, Carola A. Haas, Ute Häussler
- [T11-10C](#) Neurochemical effect of vitamins C, E and DMSO combinations on the oxidative stress biomarkers and severity of ischemic stroke in albino rats
Nasiru Suleiman, Lawal Suleman Bilbis, Yusuf Saidu, Abdullahi Yahaya Abbas, Salisu Abdullahi Balarabe, Abdullahi Abubakar Ngaski, Salisu Buhari, Bulama Ibrahim, Aishat Ibrahim Danmalle
- [T11-11C](#) Neuroethological and histological evidence for hereditary spastic paraplegia in zebrafish
Selina André, Ralf Heinrich, Roland Dosch, Bart R. H. Geurten
- [T11-12C](#) Non-canonical role of autophagy in neurotrophin signalling and axonal homeostasis
Natalia L. Kononenko, Albert Negrete, Sujoy Bera
- [T11-13C](#) Non-invasive imaging of early tissue damage and subsequent microstructural reorganization predicts the severity of hippocampal sclerosis in mesial temporal lobe epilepsy
Philipp Janz, Niels Schwaderlapp, Katharina Heining, Ute Häussler, Jan Korvink, Dominik von Elverfeldt, Jürgen Hennig, Ulrich Egert, Pierre LeVan, Carola A. Haas
- [T11-14C](#) Overexpression of rAAV-mediated human alpha synuclein in the locus coeruleus (LC) leads to a neuronal loss in the nucleus ambiguus: A novel focal mouse model for prodromal Parkinson's disease?
Bolam Lee, Martin Henrich, Wei-Hua Chiu, Lina A. Matschke, Wolfgang H. Oertel
- [T11-15C](#) Quantification of intracellular protein levels in cationic lipid-mediated siRNA transfected primary neurons
Lenka Hromadkova, Birgitta Wiehager, Susanne Frykman, Lars Tjernberg, Sophia Schedin-Weiss

- [T11-1D](#) Rescue of Gliosis in Niemann-Pick Type C1 Patient-Specific iPSC Derived Glia Cells
Franziska Peter, Michael Rabenstein, Arndt Rolfs, Moritz J Frech
- [T11-2D](#) Reversal of pathologic lipid accumulation in NPC1-deficient neurons by drug-promoted exocytotic release of LAMP1-coated lamellar inclusions
Frank W. Pfrieder, Valerie Demais, Amelie Barthelemy, Martine Perraut, Nicole Ungerer, Celine Keime, Sophie Reibel
- [T11-3D](#) Role of ULK1 in axonal degeneration and regeneration in cortical neurons *in vitro*
Björn Friedhelm Vahsen, Vinicius de Toledo Ribas, Christof Lenz, Uwe Michel, Henning Urlaub, Mathias Bähr, Paul Lingor
- [T11-4D](#) Performing deep brain stimulation and neural recordings at the same target from awake animals: A new bidirectional wireless device
Liana Melo-Thomas, Alexander Engelhardt, Uwe Thomas, Dirk Hoehl, Frank Bremmer, Rainer Schwarting
- [T11-5D](#) SK channels protect locus coeruleus neurons from rotenone induced toxicity: A new target to treat premotor Parkinson's disease
Lina Anita Matschke, Susanne Rinné, Wei-Hua Chiu, Martin Henrich, Carsten Culmsee, Wolfgang H. Oertel, Amalia M. Dolga, Niels Decher
- [T11-6D](#) Source localization based on multi-electrode local field potentials
Robin Pauli, Abigail Morrison, Tom Tetzleff
- [T11-7D](#) Spatial memory impairment and hippocampal cell loss induced by okadaic acid
Mariam Chighladze, Khatuna Rusadze
- T11-8D Retracted
- [T11-9D](#) Targeted overexpression of A53T- α -synuclein induces progressive neurodegeneration and electrophysiological changes of noradrenergic locus coeruleus neurons – a preclinical model of Parkinson's disease
Martin Timo Henrich, Lina Anita Matschke, Annette Stoehr, Wei-Hua Chiu, Bolam Lee, Fanni Fruzsina Geibl, James Koprach, Niels Decher, Wolfgang Hermann Oertel
- [T11-10D](#) The effect of cerebral ischemia-reperfusion injury to the methylation of DNA in homocysteine-treated rats
Barbara Tóthová, Mária Kovalská, Dagmar Kalenská, Ján Lehotský
- [T11-11D](#) The inferior colliculus: An alternative structure for deep brain stimulations in Parkinson's Disease?
Karl-Alexander Engelhardt, Rainer K. W. Schwarting, Liana Melo-Thomas
- [T11-12D](#) Impaired glucose metabolism in the brain depends on the nature of the activation and damage of astroglial cells and dysregulated neurogenesis
Yulia Komleva, Yana Gorina, Olga Lopatina, Anatoly Chernykh, Alla Salmina
- [T11-13D](#) Treatment with probiotics, thiamine and melatonin ameliorates aluminum-induced neurotoxicity in rats

Dmitrii Pavlov, Anna Gorlova, Eugenu Zubkov, Anna Morozova, Tatyana Cherdyntseva, Olga Karpuchina, Anatoly Inozemtsev, Tatyana Strekalova, Vladimir Chekhonin

[T11-14D](#) Trial-by-trial variability: a new marker for visual hallucinations in Parkinson's disease?
Kristina Miloserdov, Carsten Schmidt-Samoa, Holger Sennhenn-Reulen, Christiane Weinrich, Claudia Trenkwalder, Kathrin Bürk, Mathias Bähr, Melanie Wilke

[T11-15D](#) X- ray diffraction and X-ray fluorescence on Parkinson's disease substantia nigra
Eleonora Carboni, Jan-David Nicolas, Tim Salditt, Paul Lingor

Acid sphingomyelinase inhibitor amitriptyline induces angiogenesis of cerebral microvascular cells by mechanisms involving the Notch pathway

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Post-stroke, new microvessels are formed in the peri-infarct brain, which via release of trophic factors contribute to the remodeling of the brain parenchyma. Strong efforts are currently made in the stroke field to promote neurological recovery by enhancing brain remodeling. Following in vivo observations that the acid sphingomyelinase (ASM) inhibitor and anti-depressant amitriptyline promotes post-stroke angiogenesis, we herein evaluated effects of amitriptyline on the proliferation, migration and tube formation of cerebral microvascular HCMEC/ D3 cells in cell culture. HCMEC/ D3 cells were seeded in proliferation, migration and tube formation assays and treated with various concentrations of amitriptyline. Amitriptyline dose-dependently increased the migration and tube formation of cerebral microvascular HCMEC/ D3 cells when administered at doses of 5 - 50 μ M, at the same time reducing the proliferation of HCMEC/ D3 cells. These effects were attenuated by N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), an inhibitor of Notch signaling pathway. Our data suggest that DLL-4/ Notch signaling is involved in the angiogenic actions of amitriptyline.

alpha-Synuclein aggregation and spreading in mouse models of Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by degeneration of dopaminergic neurons in the Substantia nigra pars compacta (SNpc) as well as protein inclusions called Lewy bodies. Available therapies for PD patients have currently only symptomatic effects leading to a partial symptomatic relief, but without cure. Therefore, novel therapeutic approaches for the treatment of PD are urgently needed. One novel molecular target in PD is Rho-associated protein kinase (ROCK). By using cell culture and animal models of PD we could show that pharmacological ROCK inhibition with the clinically approved isoquinoline derivative Fasudil displayed protective properties on neuronal survival and fostered axonal regeneration. In toxin-induced models of PD using MPTP and 6-OHDA, nigral dopaminergic cell loss was attenuated by Fasudil treatment and regeneration was increased. The protein alpha-synuclein, as major component of Lewy bodies, has become an important target in PD due to its ability to adopt different structural conformations within the diseased brain. Alpha-synuclein misfolding results in protein fibrils and aggregates, which act neurotoxic triggering apoptotic mechanisms in neurons.

Here, we investigated the anti-aggregative potential of pharmacological ROCK inhibition with Fasudil. Interestingly, Fasudil treatment affected alpha-synuclein aggregation by direct C-terminal binding in a cell-free aggregation assay. Furthermore, long-term Fasudil treatment in a transgenic mouse model expressing A53T human alpha-synuclein reduced aggregate pathology in the midbrain, leading to improved motor and cognitive behavior, without apparent negative side effects. The intra-striatal injection of pathogenic alpha-synuclein fibrils into mouse and rat brains is an established method to analyze the spreading of alpha-synuclein fibrils through the brain. Based on the anti-aggregative properties of Fasudil, we thus further evaluated the effects of Fasudil on alpha-synuclein propagation. Intra-striatal stereotactic injections of previously sonicated pre-formed alpha-synuclein fibrils (PFFs) were performed into the striatum of mice. We show that PFFs are present within the SNpc of the injected hemisphere already 60 days after the injection indicating a fast uptake and retrograde transport of the injected PFFs. Furthermore, we demonstrate the effects of pharmacological ROCK inhibition with Fasudil on alpha-synuclein propagation.

Our data suggest that Fasudil may be a promising disease-modifying drug for the treatment of PD.

Altered autophagic pathway in TBK1-mutant Motor Neurons derived from human induced pluripotent stem cells

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TBK1 is a protein that plays a fundamental role in selective autophagy and mitophagy, and mutations impairing its kinase function have been described to alter these specific pathways. Recently, mutations in TBK1 gene have been shown to cause Amyotrophic Lateral Sclerosis (ALS), although the pathogenic mechanisms are still poorly understood. Moreover, mutation in other autophagy-related genes such as SQSTM1 (p62), OPTN and C9ORF72 have been shown to cause ALS, suggesting the presence of converging pathways in this disease. Considering these evidences, we investigate the autophagic pathway in human induced pluripotent stem cells (hiPSC) derived from ALS patients carrying TBK1 mutations. After reprogramming and basic characterization of three hiPSC cell lines carrying three different mutations in the TBK1 gene, we investigated autophagy focusing on p62. Our experiments highlighted an increase of p62 cytoplasmic inclusions in the three TBK1 mutants cell lines in respect to healthy patient control. Moreover, immunostaining experiments revealed alterations in the size distribution of those inclusions between patients cell lines and control: TBK1 mutant hiPSC show indeed bigger size of p62 inclusions.

Since ALS affects mainly Motor Neurons (MNs), we investigated the autophagic pathway in this specific cell population. Differentiated MNs from mutant hiPSC cell lines show increased cytoplasmic p62 inclusions when compared to healthy MNs. In addition, as highlighted in hiPSC, TBK1-mutant MNs show aggregates that are bigger in size than in control MNs.

Our data highlight that hiPSC and hiPSC-derived MNs can be a good model to investigate ALS related phenotype. This in-vitro system can be also used to test potential new drugs to tackle the pathology and for better understanding the molecular pathways leading to the disease.

Altered hippocampal and cortical EEG frequency characteristics in the APPswePS1dE9 model of Alzheimer's disease

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Alzheimer's disease (AD) is a complex neurodegenerative disorder leading to progressive cognitive decline, memory loss and eventually death. We analyzed the APPswePS1dE9 AD mouse model using computerized 3D stereotaxic electrode positioning and implantable video-EEG radiotelemetry to perform long-term surface and deep intracerebral EEG recordings from the primary motor cortex M1 and the hippocampal CA1 region in both genders. EEG recordings were analyzed for motor activity, electroencephalographic seizure activity and frequency characteristics using a FFT. Male but not female APPswePS1dE9 AD mice displayed increased motor activity during the dark cycle. Automatic seizure detection unraveled severe electroencephalographic seizure activity in both M1 and CA1 deflection in APPswePS1dE9 mice that turned out to be gender-specific. Seizure activity in APPswePS1dE9 was highly variable as has been reported for other AD mouse models as well. Frequency analysis of both surface and deep EEG recordings elicited complex age, gender, circadian and activity dependent alterations in the theta and gamma range. Females displayed an antithetic decrease in theta () and increase in gamma () power at 18-19 weeks of age whereas related changes in males occurred earlier at 14 weeks of age. In females, theta () and gamma () power alterations were most prominent in the inactive state suggesting an impairment of atropine-sensitive type II theta in APPswePS1dE9 mice. Our results demonstrate a systematic gender-specific evaluation of cortical M1 and hippocampal CA1 hyperexcitability / seizure and frequency analysis in APPswePS1dE9 mice and controls using implantable video-EEG radiotelemetry without restraint. The observed gender-specific network alterations in APPswePS1dE9 are likely to be related to cognitive and behavioral deficits and might serve as early biomarkers / EEG fingerprints for AD in the future.

Altered long-term potentiation in the hippocampus of PS19-P301S transgenic mice

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Neurofibrillary tangles consisting of hyperphosphorylated tau are a pathological hallmark of multiple age-related diseases such as Alzheimer's disease, frontotemporal dementia, progressive supranuclear palsy and primary age-related tauopathy. The disease pathology is characterized by progressive cognitive decline which is thought to be due to the ongoing neurodegenerative processes. Disease progression in both patients and pre-clinical animal models might be preceded by altered synaptic transmission and plasticity in brain regions crucial for memory formation and consolidation like the hippocampus¹. Long-term potentiation (LTP) is a measurement of synaptic plasticity and is regarded as a cellular correlate to hippocampal memory function^{2, 3}. LTP can be easily assessed via extracellular recordings in acute hippocampal slices under strictly controlled conditions allowing a direct comparison between various disease models.

In this study we characterized genotype- and age-related changes in synaptic transmission and plasticity in the hippocampus of PS19-P301S model of tauopathy⁴. This model expresses human tau with the P301S mutation leading to accumulation of insoluble and hyperphosphorylated tau during aging. We used field potential recordings to monitor neuronal activity in the CA1 region of female PS19 of different ages and compared them to age-matched non-transgenic animals. At 8 month of age, LTP evoked by high-frequency stimulation was enhanced in PS19 animals as compared to controls. Interestingly, the initial potentiation early after LTP induction did not attenuate during the recording period of 90 min. Additionally, the elevated potentiation in PS19 mice was accompanied by an increased hyperexcitability of the hippocampal network at this age. At ages beyond 8 months LTP steadily declined below control levels in the PS19 while remaining stable in non-transgenic mice. This decline in plasticity paralleled a trend towards reduced basal synaptic transmission in the transgenic mice, which might reflect the loss of synapses. Changes in short-term plasticity (paired pulse facilitation) were not detected in PS19 mice.

The transiently increased LTP in 8 months old PS19 mice coincided with the occurrence of modest tau pathology evident by a very weak but significant enhancement of insoluble tau (which continued to increase with older age (10 months), the appearance of a small number of AT8 positive neurons, but the absence of thioflavin S staining. Our data suggest that abnormalities in hippocampal LTP might precede the robust increase in insoluble tau and the formation of tau agglomerates in female PS19 mice which may be either specific for the onset of tau pathology or due to the overexpression of tau independent from the mutation. This might reflect a physiological function of tau as a mediator of neuronal plasticity. At older ages accumulated insoluble tau may reduce LTP. Thus, the change of synaptic plasticity with age in PS19 mice might reflect the transition of tau from a physiological form beneficial for neuroplasticity to a pathological form that is detrimental for synaptic plasticity.

Reference List

1. Bakker, A. et al. Reduction of hippocampal hyperactivity improves cognition in amnesic mild cognitive impairment. *Neuron* 74, 467-474 (2012).

2. Gruart,A., Munoz,M.D., & Delgado-Garcia,J.M. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. *J. Neurosci.* 26, 1077-1087 (2006).
3. Whitlock,J.R., Heynen,A.J., Shuler,M.G., & Bear,M.F. Learning induces long-term potentiation in the hippocampus. *Science* 313, 1093-1097 (2006).
4. Yoshiyama,Y. et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* 53, 337-351 (2007).

Assessment of The Neuroprotective Effects of Ezetimibe versus Simvastatin in Animal Model of Alzheimer's Induced Dementia

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Background: Alzheimer Disease (AD) is the most common neurodegenerative disorder all over the world. Among many theories, brain insulin resistance stood out in the last decade as a new approach for AD. Simvastatin and Ezetimibe can provide neuroprotection through their lipid lowering actions. However, their insulin-sensitizing actions can be as important as the former and has not yet been highlighted. **Objective:** To compare the potential neuroprotective effects of Ezetimibe versus Simvastatin in Alzheimer's rat model and to explore how they modify Insulin Growth Factor 1 (IGF-1) signaling in the brain, which play a crucial role in learning and synaptic plasticity. **Methods:** Forty nine female Sprague-Dawley rats were divided into seven equal groups: AD model (single unilateral ICV STZ, 3mg/kg), Sham group (single unilateral ICV 0.9 saline), Simvastatin Treated and Ezetimibe Treated groups received the same regimen as AD model plus simvastatin 10 mg/kg P.O (in the simvastatin-treated group) and Ezetimibe 10 mg/kg P.O (in the Ezetimibe-treated group) for 21 days. Simvastatin and Ezetimibe Control groups received simvastatin 10 mg/kg P.O and Ezetimibe 10 mg/kg P.O respectively for 21 days. Blank group was maintained on DW and standard diet. At 15th day, all animals were evaluated regarding spatial memory and learning through Morris Water Maze (MWM) and novel object recognition tests. On the 21st day, rats were sacrificed and brain sections were stained with Congo red and Golgi-Nissel stains and blotted against Anti-tau and Anti IGF-1 receptor antibodies. **Results:** Both Simvastatin and Ezetimibe showed protective effects through reducing amyloid plaques and tau aggregates and up regulating IGF-1 receptors in the hippocampus and the frontal cortex. Simvastatin treated group showed better performance than Ezetimibe treated group in MWM and novel object recognition tests. **Conclusion:** Both Simvastatin and Ezetimibe could protect against AD-induced dementia, evidenced by reducing amyloid and tau proteins through up regulation of IGF-1 receptors in the hippocampus and cerebral cortex.

BACE1 modulates synaptic transmission at the hippocampal mossy fiber synapse by regulating $K_V3.4$ channels.

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The γ -secretase BACE1 (γ -site APP-cleaving enzyme 1) plays a pivotal role in the generation of amyloid β -peptides, the main component of amyloid plaques, a neuropathological hallmark of Alzheimer's disease (AD). Therefore, BACE1 is a prime drug target, but the protease's physiological functions, important for evaluating the safety of a therapeutic intervention, need to be further understood. Interestingly, we found that BACE1 specifically co-localizes with the voltage-gated potassium channel 3.4 ($K_V3.4$) in the mossy fiber pathway of the hippocampus. This A-type potassium channel with fast activating and inactivating currents is critical for presynaptic action potential repolarization regulating synaptic transmission. Therefore, we investigated the impact of BACE1 on $K_V3.4$ expression and function in BACE1 wild type (WT) and knockout (KO) mice.

Biotinylation experiments of hippocampal slices revealed a significant reduction of channel surface levels in BACE1 KO compared to WT littermates. Also, specifically in the hippocampal synaptic fraction, $K_V3.4$ levels were significantly decreased in BACE1^{-/-} whereas other synaptic potassium channels remained unchanged. At the synapse, inhibition of $K_V3.4$ led to a strong increase in the input-output relation in WT animals in hippocampal slice recordings. To study BACE1 overexpression, which is relevant for AD patients, we performed surface biotinylation experiments in transfected HEK293T cells. The surface levels of $K_V3.4$ were dramatically increased in the presence of BACE1 and also after BACE1 inhibitor treatment. As we reported recently, BACE1 can directly interact with ion channels. We were able to co-immunoprecipitate $K_V3.4$ with BACE1 and vice versa in transfected HEK293T cells.

We herewith show a new mechanism of how BACE1 affects synaptic function at the mossy fiber synapse. The strength of the $K_V3.4$ -mediated inhibition of synaptic transmission is dependent on BACE1 expression. As BACE1 levels are supposed to increase in AD, synaptic transmission could be severely attenuated.

Characterization of epileptiform activity in hippocampal slices

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The risk for epilepsy is elevated in Alzheimer's disease (AD) patients and epileptiform activity has been shown in many AD mouse models. For example, a recent *in vivo* study in Tg2576 mice overexpressing the Swedish mutation of hAPP demonstrated spontaneous interictal spikes in the EEG (Kam et al. 2016). Our own work shows that neuronal hyperexcitability in Tg2576 mice can also be detected in hippocampal slices *ex vivo*. The *ex vivo* approach thereby allows a fast testing of compounds that might affect epileptiform activity on slices before going *in vivo*. Additionally, *ex vivo* recordings using a double-recording chamber offers the advantage to use vehicle-treated slices from the same animal preparation as internal controls and thereby reducing animal-to-animal variability.

Here we present the establishment of a protocol to reliably induce epileptiform activity in acute hippocampal slices of Tg2576 AD mice. A low magnesium protocol was chosen that induces interictal spike-like discharges mainly through NMDA receptors. In long term potentiation (LTP) recordings we previously demonstrated that under normal ACSF, Schaffer-collateral evoked fEPSPs in the CA1 region of Tg2576 slices are polysynaptic suggesting a reduced threshold for epileptiform activity. Epileptiform activity in hippocampal slices is mainly generated in the CA3 region or the entorhinal cortex and propagates to the CA1 region. Therefore, we aimed to show epileptiform activity at its origin and continuously recorded from the CA3 region. We started by perfusion of slices with a zero magnesium (0 mM Mg²⁺) ACSF protocol which leads to the appearance of short, oscillatory large-amplitude discharges, resembling interictal spikes. Here, we systematically analyzed the effect of slice orientation (coronal vs horizontal), slice age (freshly prepared vs older slices), and slice position along the dorsoventral axis of the hippocampus on the development of epileptiform discharges. Furthermore, we investigated whether interictal spike-like discharges are generated intrinsically in the recurrent network of the CA3 region.

Unexpectedly, the perfusion of Tg2576 slices with the zero magnesium (0 mM Mg²⁺) ACSF did not show any difference to wildtype controls. This indicated that the zero magnesium ACSF protocol might be too strong and inducing epileptiform activity in wildtype slices too early. We therefore modified the protocol to a low magnesium (0.3 mM Mg²⁺) ACSF. Under these conditions we observed a difference between slices from Tg2576 and wildtype controls. However, epileptiform discharges appeared in the Tg2576 slices very late when quality of acute slices already declines. Consequently, we slightly further lowered the threshold for epileptic activity by additionally raising the extracellular potassium concentration from 3 to 5 mM. To confirm that the low Mg²⁺/high K⁺ (0.3 mM Mg²⁺, 5 mM K⁺) ACSF protocol still leads to epileptiform activity mainly through NMDA receptors we additionally perfused slices with AP5 to block the receptor and could not detect any epileptiform discharges. With this low Mg²⁺/high K⁺ protocol we could reliably detect epileptiform discharges in Tg2576 slices soon after the solution change, whereas wildtype slices develop those discharges very late. The establishment of this protocol enables further investigation and pharmacological modulation of the underlying molecular and cellular mechanisms that lead to neuronal hyperexcitability in AD mouse models.

References

Kam, K. et al., 2016. Interictal spikes during sleep are an early defect in the Tg2576 mouse model of - amyloid neuropathology. Scientific reports, 6, p.20119. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4730189&tool=pmcentrez&rendertype=abstract> [Accessed September 6, 2016].

Contextual fear learning and hippocampal plasticity in APP/PS1 mice

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One of the most challenging topics in neuroscience research is the identification of novel treatment approaches for Alzheimer's disease (AD). It has been shown by several studies, that in AD patients emotional processing, e.g. the recognition of fearful faces or the learning of fear, is impaired. Interestingly, these impairments occur already at early stages of the AD etiopathology. Thus, an altered emotional processing might be regarded as an early symptom in the development of AD.

In the present study, we analyzed different aspects of hippocampus dependent fear learning, synaptic and structural plasticity in differently aged APP/PS1 mice. This AD mouse model combines the Swedish APP (KM670/671NL) mutation with the PS1-L166P mutation under control of the Thy1 promoter (Radde et al., 2006, EMBO), resulting in a rather mild but constant post-developmental expression of A β and subsequent plaque formation. By testing contextual fear learning, we observed deficits in six months old APP/PS1 animals. Here, animals could not discriminate between the conditioned and a neutral context. However, the subsequent extinction of these contextual fear memories seemed to be unimpaired. To investigate the possible underlying cellular mechanisms contributing to this deficit, we focused on synaptic plasticity in CA1 area of the hippocampus. Parallel to the observed deficit in contextual fear learning, hippocampal long-term potentiation (LTP) was impaired in slices taken from six but not three months old APP/PS1 mice compared to wild type animals. Besides synaptic plasticity we now started to investigate for structural changes in APP/PS1 mice by spine morphology and plaque analysis. In addition, we will analyze the protein level of A β 40/42 in the hippocampus of the tested animals in order to correlate the local occurrence of these toxic A β -species with the observed deficits in functional and structural synaptic plasticity in these animals.

In conclusion, we demonstrate selective impairments in contextual fear learning in middle-aged APP/PS1 mice, which correlate with impaired LTP in CA1 of the hippocampus of similarly aged APP/PS1 mice. With the currently ongoing analysis of the abundance of soluble and insoluble forms of A β protein and characterization of the spine morphology we want to link the observed deficits in learning and functional plasticity in these mice with structural changes at the spine level.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, SFB 779/B6) and by the EU-Joint-Programming Initiative – Neurodegenerative diseases (JPND, BMBF FK: 01ED1616A).

Contralateral BoNT-A injection in the striatum of 6-OHDA hemilesioned rats give evidence for long lasting effects on basal ganglia circuitry

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Parkinson's disease is one of the most frequent neurodegenerative disorders. The loss of dopaminergic neurons in the substantia nigra leads to a disinhibition of cholinergic interneurons in the striatum. The recent pharmacotherapeutical strategies of Parkinson related hypercholinism have numerous adverse side effects. Recently our group injected experimentally Botulinum Neurotoxin-A (BoNT-A) directly into the striatum of hemiparkinsonian rats to prevent acetylcholine release from overactive cholinergic neurons. We showed that intrastriatal injections of 1 ng in unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats inhibit apomorphine induced rotation behaviour significantly up to six months. Thereby, side effects known from current anticholinergic therapies were avoided.

In this study we investigated the impact of intrastriatal BoNT-A injection contralateral to the 6-OHDA lesioned side on the basal ganglia circuitry and motor functions. We used hemiparkinsonian rats and injected 1 ng BoNT-A or vehicle substance into the contralateral striatum one month after 6-OHDA lesion. Spontaneous motor behaviour was examined by cylinder test. To analyze the impact on the direct and indirect basal ganglia circuit apomorphine and amphetamine induced rotation tests were performed. The distinct apomorphine induced rotation behaviour evoked by the unilateral 6-OHDA lesion was significantly increased one month after the contralateral intrastriatal BoNT-A injection. Interestingly, the intrastriatal application of 1 ng BoNT-A contralateral to the lesioned substantia nigra initially led to a significant readjustment of the right and left forepaw usage. However, during the following months the right forepaw preference increased again. The amphetamine induced rotation rate was significantly reduced one month after the BoNT-A treatment and continuously increased thereafter.

The results of the amphetamine rotation test and of the cylinder test were astonishing. In previous experiments we found, that ipsilateral administration of BoNT-A in the right striatum of hemilesioned rats led to a significant reduction of the amphetamine induced rotation rate and a significant readjustment of the forepaw usage. We expected that a contralateral injection of BoNT-A should increase right-sided forepaw preference and clockwise turning rate under amphetamine influence. However, we measured an initial reduction of the amphetamine rotation rate. Interestingly, we observed also an adjustment of the forepaw usage one month after BoNT-A administration, but in the course of the following six months right forepaw preference was reinforced again and more strongly than in the control group. These long-ranging and contrary effects suggest, that intrastriatally applied BoNT-A acts not only as an inhibitor of acetylcholine release but also has long lasting impact on transmitter expression and thereby on the basal ganglia circuitry. These transmitter receptor changes are subject of ongoing studies of our work group.

Could hyperhomocysteinemia affects neurodegeneration after ischemia/reperfusion injury of rat brain?: An experimental model of a possible development of Alzheimer`s disease

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Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder that results in massive neuronal loss, mainly in the hippocampus and associated regions of the neocortex. These conditions lead to dementia and death. The exact cause of Alzheimer's disease is unknown, although a number of factors are thought to increase the risk of developing this disease. Hyperhomocysteinemia (hHcy) presents one of these factors. hHcy comprises a high circulating concentration of homocysteine (Hcy) in blood and it is considered to be a strong, independent risk factor for stroke, dementia and AD. Why study combination of stroke and AD?

- The combination of "silent strokes" and low level Alzheimer's results in dementia.
- Both share the same risk factors.
- Strokes damaged areas exhibit pathological precursors of AD.
- Inflammation is a dominant force in neurodegenerative properties of stroke and AD.
- Clinical stroke and dementia are not treatable.
- Vascular cognitive impairment preceding stroke and AD is treatable, i.e. during the "brain at risk stage".
- The interactions between small strokes and AD.

Disease during the "brain at risk stage" (preceding clinical stroke and dementia) may suggest targets for therapy. However, the molecular mechanisms underlying these mechanisms are not fully understood.

Global forebrain ischemia was induced by 4-vessels occlusion. Concretely, 15 min of ischemia followed with reperfusion period of 72h and 7 days. hHcy was induced by subcutaneous injection 0,45 $\mu\text{mol/g}$ of Hcy in duration of 14 days. The result showed that Ischemia/reperfusion (IR) after induced hHcy may attenuate the neural cell death in the forebrain cortex. We demonstrated occurrence of degeneration of selectively vulnerable neurons after induced IR as well as after hHcy. Further western blot study as well as immunohistochemical analysis of MAPKs (Mitogen-Activated Protein Kinases) suggested that IR and hHcy affect number and level of MAPK/ERK and MAPK/p38 proteins, which are associated with survival/death of neural cells. Immunohistochemistry of neurofibrillary tangle as well as β -amyloid plaques showed that hHcy might lead to progression of AD-like pathological features after IR insult. These findings suggest that IR injury after induced hHcy have a neurodegenerative role in global brain ischemia on rats. Our results also indicate that this model of compound insults could lead to development of AD.

This study was supported by Grant VEGA 1/0229/15 and by project "Identification of novel markers in diagnostic panel of neurological diseases", co-financed from EU sources and European Regional Development Fund.

Cuprizone induced de- and remyelination in the spinal cord of transgenic mice

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While neuronal network functions are largely encoded by distinct patterns of action potential firing, the physiology of glial cells is determined by a complex temporal and spatial arrangement of intracellular calcium signals (Ca^{2+}). Dysregulation of Ca^{2+} homeostasis is a common phenomenon of neuroinflammatory diseases, such as stroke or multiple sclerosis (MS). And indeed, changes of glial Ca^{2+} contribute to the initiation and progression of brain pathologies, but they are also important for regeneration as well. Influx of Ca^{2+} makes oligodendrocytes more vulnerable and causes demyelination, which subsequently triggers activation of scar-forming microglia and astrocytes as well as axonal degeneration. Simultaneously, Ca^{2+} signals can trigger also neuroprotective functions to limit neuronal damage and cell death.

Here, we investigated the cuprizone (bis-cyclohexanone-oxaldihydrazone) model, a non-inflammatory experimental animal model of MS to reveal the molecular and cellular mechanisms of de- and remyelination.

Our analysis of oligodendroglial gene expression after cuprizone treatment revealed a significant reduction at mRNA and protein levels. At the cellular level, we identified microglia as first responders that could be detected after two weeks of cuprizone treatment, at a time point before obvious demyelination. Three weeks later, not only the number of microglia was clearly increased, but the activated microglia also cleared the CNS of myelin debris. After induction of the demyelination process we could also observe activated astrocytes.

We further employed *in vivo* two-photon laser-scanning microscopy (2P-LSM) to follow morphological glial cell responses over time as well as cytosolic Ca^{2+} changes during and after cuprizone treatment.

Detailed classification of epileptiform activity reveals anti-correlation between severe and mild epileptic bursts.

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The most common form of epilepsy is mesial temporal lobe epilepsy (MTLE), where a wide range of epileptiform activity (EA) emerges from hippocampal structures. Typically, a set of distinctive anatomical alterations, such as granule cell dispersion (GCD) and cell death, are the histopathological substrate for MTLE.

Up to now research on EA mostly focused on behavioral seizures and single epileptic spikes. Whether single epileptic events are promoting or suppressing seizures is a subject of debate. To better understand seizure susceptibility, we describe and classify the whole range of EA and try to uncover the relationships between different event-types. Working with intracranial EEG-data from a mouse model of mesial temporal lobe epilepsy, we developed methods to detect bursts of EA and to classify them according to severity. We provide evidence that EA can be decomposed into two main categories: mild and severe bursts. A higher background rate of mild bursts was associated with fewer severe bursts. Within recording sessions, mild and severe events tended to occur in clusters, suggesting the existence of less susceptible states (dominated by epilepsy free periods, single discharges and mild bursts) and more susceptible states (dominated by severe events). Regarding anatomy, the degree of GCD was anti-correlated to the rate of severe bursts.

Our results suggest that brains with high GCD have a higher threshold for generating severe EA and that mild epileptic bursts might possibly suppress seizure-like activity.

Developing an isogenic neuron-astrocyte model to study the isoform-specific effect of *APOE* in late-onset Alzheimer's Disease

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APOE4 is a known genetic risk factor that accelerates onset of dementia and neurodegeneration. The *APOE* locus is triallelic and its isoforms *APOE2*, 3 and 4 differ from each other by a single amino acid. Although several pathways underlying the risk associated with *APOE4*-linked AD have been studied through in vitro and in vivo studies, the exact mechanisms are not completely understood. The cellular and animal models used for most of these studies have been generated by replacing mouse *APOE* with human *APOE4* in combination with overexpression of early onset AD genes. Accordingly, these models do not reflect the situation in the late onset AD (LOAD) form of the disease, which accounts for 90-95% of AD cases.

Thus, we aimed to study the isoform-specific effects of *APOE* using LOAD patient-derived neurons, which enable a more accurate representation of the affected human system. Since AD is a polygenic disorder, in order to avoid confounding effects of the genetic background, we are using the CRISPR-Cas9 nickase editing system to generate isogenic patient lines that differ from each other only at the *APOE* locus. A double nickase approach would also reduce potential off target modification, frequently reported with WT Cas9. To improve co-transfection of both guide RNAs, we modified the PX461 plasmid (Addgene) to develop a single GFP-tagged plasmid encoding Cas9 nickase and both guide RNAs.

We have generated induced pluripotent stem cells (hiPSC) from LCLs and fibroblasts derived from 3 *APOE4/4* individuals affected with sporadic AD, using an episomal plasmid-based system. These lines have been characterized by staining for markers of pluripotency, differentiating to cell types of all 3 germ layers and karyotyping to ensure chromosomal integrity. iPSCs were then differentiated to neural stem cells (NSCs), which are highly amenable to clonal expansion, a prerequisite for genome editing.

AD patient *APOE4*-NSCs were transfected with the nickase plasmid in combination with single stranded oligonucleotides (ssODN) to introduce the *APOE3* sequence. RS1 and SCR7 were added to increase the rate of homologous recombination (HR). GFP positive cells were then sorted and grown for clonal selection. By sequence-specific-primer PCR, we could confirm successful genome editing and generation of the *APOE3* allele in the transfected cells. Once *APOE3* positive clones are expanded, these will be differentiated to astrocytes and neurons using standardized protocols to generate a co-culture system for studying effects of cell-type specific *APOE* in disease progression.

Poly(Propylene Imine)dendrimers inhibit RT-QuIC based in vitro amplification of prion protein

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Prion diseases are a group of fatal neurodegenerative disease caused by the misfolding of the cellular prion protein (PrP^C) into its aggregating-prone scrapie isoform (PrP^{Sc}), characterized by a higher content of beta sheets. No therapies thus far have been proven effective in preventing or fighting the disease. In our laboratory, we recently demonstrated the ability of doxycycline to inhibit the conversion of PrP^C to PrP^{Sc} when tested by Real Time Quaking Induced Conversion (RT-QuIC), an in vitro assay that monitor the formation of PrP^{Sc} by means of Thioflavin T, a molecule that intercalates through beta sheets, resulting in a proportional increase in fluorescence signal. Here we tested poly(propylene imine) dendrimers (PPI), a class of synthetic anti-amyloidogenic molecules which have been proven effective in inhibiting aberrant prion conversion in both in vitro and intracellular settings. Different generations of PPI, defined by the amount of maltose units in the outermost shell, have been tested by RT-QuIC, showing a generation-dependent anti-prionic activity. These results, comparable with our previous results obtained with doxycycline, point to a potential role as pre-screening molecules for potential therapeutic target for spongiform encephalopathies.

Development of Parkinsonian pathophysiology on the single unit level: a rat 6-OHDA study

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Parkinson's disease is a central nervous system disorder which mainly affects the motor system. Symptoms develop slowly over years. The neurotoxin 6-hydroxydopamine (6-OHDA) is able to target and destroy the dopaminergic neurons when injected into the medial forebrain bundle (MFB), and thereby models the central pathogenesis of this disorder. While this is one of the most established translational animal models in experimental neurology, it is yet unclear how single units change their firing over time as the pathological dynamics develop. In our study, we implanted carbon nanotube coated tetrodes into regions of the basal ganglia, such as dorsal striatum, STN, GPe, etc. While recording stable single units over 3+ weeks, we injected 6-OHDA with a microinjector and demonstrate the evolution of single unit firing patterns as the Parkinsonian brain dynamic develops.

'Disease Modeling in a dish' for sporadic Amyotrophic Lateral Sclerosis using Human Embryonic Stem Cells derived Motor Neurons.

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Amyotrophic Lateral Sclerosis (ALS) is an adult onset disorder selectively affecting the motor neurons of the cortex, brainstem and spinal cord. Mean survival rate is 3- 5 years post diagnosis, and death is mainly due to respiratory failure. Most of the research on ALS encompasses the use of familial transgenic mouse and rat models (both in-vivo and in-vitro) following exposure to Cerebro Spinal Fluid (CSF) from Sporadic ALS patients. Although these models aid us in understanding the disease pathogenesis and its progression, they may not reproduce the pathophysiology as seen in humans, mainly due to species differences. Thus, there is a critical need for disease modeling using cells from human source. Human Embryonic Stem Cells (hESC) offers a renewable source for generating sufficient quantities of motor neurons which could be successfully grown in culture.

This study was aimed at deriving motor neurons from hES Cell line- BJNHem20, which could be a potential cellular model for sporadic ALS. BJNHem20 is a stem cell line derived from the blastocyst stage embryo of Indian origin by Inamdar et al., in 2009. These hESC's were grown on Mouse Embryonic Fibroblasts and characterized with pluripotency markers. Embryoid bodies (EBs) were formed when hESCs were grown on feeder free, low adherent dishes. EBs were induced with neural induction media consisting of retinoic acid and purmorphamine for motor neuron differentiation. Neuritic processes appeared from day23 of differentiation and the complexity of the network increased with consecutive days. hESC's/ cells at different stages of differentiation were analyzed for the expression of stage specific markers using quantitative RT-PCR, Immuno-cytochemistry and Western Blot. Differentiated cells were positive for a panel of motor neuron specific markers such as Olig2, HB9, Islet1, FOXP1 and Choline Acetyl Transferase which confirmed the presence of progenitors and post mitotic motor neurons in the heterogeneous population. The expression of FOXP1 confirmed the derivation of limb innervating motor neurons majorly affected in ALS. In addition, these neurons exhibited spontaneous action potentials from Day27 of differentiation that were recorded using Multi Electrode Array system and data analyzed using spike2 software. Majority of the neurons (84%) fired at a frequency <5Hz indicating the population enriched for excitatory neurons; a very few neurons fired at a frequency >5Hz indicating putative interneurons. These stem cell derived motor neurons were susceptible to toxicity induced by CSF from sporadic ALS patients. This preclinical study with human motor neurons could provide a stronger basis to be translated into clinical trials, as it would serve as an excellent bioassay system of human origin to evaluate novel therapeutic agents.

Disturbed GABAergic transmission in the *cln3*^{-/-}-mouse model of Batten disease

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Batten disease (also juvenile neuronal ceroid lipofuscinosis, JNCL) is a neurodegenerative lysosomal storage disorder with the key symptoms of loss of vision, motor and cognitive decline, ataxia, progressive seizures, psychiatric abnormalities, and death in the third or fourth decade of life. The syndrome is caused by mutations in the *CLN3*-gene and it is the most prevalent inherited neurodegenerative disease in childhood. To date there is only incomplete understanding of the neurophysiological consequences of loss of function of CLN3-Protein and how this may cause characteristic disease symptoms.

In a multidimensional approach we analyzed behavior, neurodegeneration, and neuronal function in *cln3*^{-/-}-mice. The knockout mice developed learning deficits, increased anxiety-related behavior, and motor coordination abnormalities in an age-dependent manner. In hippocampus and amygdala, we found reduced GABAergic inhibition in principal neurons using whole-cell patch-clamp analysis. In the cerebellum we found evidence for reduced inhibition onto cerebellar Purkinje cells. In contrast spinal inhibitory transmission was unaffected as measured *in-vivo*. Histological studies further revealed a reduced number of distinct interneuron subtypes in the hippocampus and the amygdala.

Taken together we found behavioral and functional abnormalities in the *cln3*^{-/-}-mice that are caused by reduced GABAergic synaptic transmission and degeneration of inhibitory interneurons.

Early EEG abnormalities in a model of tauopathy

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Abnormalities in brain activity (oscillations) and sleep architecture are a common feature for most neurodegenerative conditions and are supposed to contribute to disease onset and progression. Sleep and brain activity are conserved across different species and disturbances in these physiological processes can be detected both in patients and in preclinical disease models. Therefore, brain activity can serve as a translational biomarker during the development of therapeutic agents ¹.

The formation of pathological tau aggregates is characteristic for multiple age-related diseases such as primary age-related tauopathy, progressive supranuclear palsy, frontotemporal dementia, Alzheimer's disease. The underlying disease pathology is associated with altered neuronal function, neurodegeneration and cognitive decline. The cause of sleep disturbances in tauopathies might be attributed to several factors – age-related modifications, symptoms of the disease, comorbid conditions and the neurodegenerative process itself. PS19 is an animal model of tauopathy in which overexpression of P301S mutated human tau leads to tau hyperphosphorylation², starting from the spinal cord and extending to the forebrain. In an exploratory study we investigated whether sleep/wake pattern and brain wave activity are affected by the presence of human p-tau. For this purpose we used longitudinal EEG recordings. We performed continuous 24-hour recordings from the same animals starting at 3 months of age and followed these animals during their aging (last recording was at 12 months of age). We analyzed sleep structure in terms of total sleep time, time spent in different vigilance states (Wake, NREM, REM) and the number of rapid eye movement (REM) episodes were analyzed. Additionally, we performed a detailed analysis of brain wave spectra, concentrating on delta and theta bands.

Sleep abnormalities were detected from 3 months of age. PS19 animals were more awake with less time spent in NREM and REM. No further decrease in sleep time could be detected at higher ages.

Power spectral analysis revealed changes in 2 regions – frontal cortex and hippocampus. Analysis demonstrated a shift in the delta and theta wave range in PS19 mice when compared to age-matched littermates. Both, delta and theta oscillations in the frontal cortex of PS19 mice were higher in power throughout ageing compared to non-transgenic animals. Hippocampal theta power declines as compared to control littermates from slightly higher at 3-to 4-month-old PS19 to reduced levels at higher ages (10 and 12 months).

Our data suggests that PS19 mice undergo desynchronization of the underlying hippocampal network along a conditional hypersynchronization of cortical network when compared to non-transgenic, age-matched littermates. Desynchronization in the hippocampal theta range could indicate cognitive decline. Taken together, we suggest that changes in neuronal function detected in continuous EEG recordings can be identified early during the life of PS19 mice and might serve as a biomarker for disease progression. These encouraging results enable further studies with this mouse model.

References

1. Platt B et al., *Biochem Soc Trans.* 2011 Aug;39(4):874-80. doi: 10.1042/BST0390874
2. Yoshiyama Y et al., *Neuron.* 2007 Feb 1;53(3):337-51.

Effect of caffeine and MDMA or methamphetamine combination on DA and 5-HT release and DNA damage in the mouse brain

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Combination of psychostimulants such as caffeine and amphetamine derivatives is often found in ecstasy tablets. Caffeine (CAF), adenosine A1/A2A receptor antagonist and 3,4-methylenedioxymethamphetamine (MDMA) or methamphetamine (MTH) affect dopamine (DA) and serotonin (5-HT) neuronal terminals and cause development of oxidative stress which may damage brain tissue. We aimed to investigate whether DA and 5-HT release induced by combined chronic treatment with CAF and MDMA or MTH may damage presynaptic monoamine terminals and postsynaptic cell bodies in the mouse brain. CAF (2x5 mg/kg) and MDMA (4x10 mg/kg) or MTH (3x5 mg/kg) were given in a “binge” mode of administration during two days/week for three weeks. DA and 5-HT release in the mouse striatum was studied using microdialysis in freely moving animals while alkaline comet assay was used to determine oxidative DNA damage. In addition, the striatal content of DA, 5-HT and their metabolites was determined. The DA, 5-HT, DOPAC, HVA and 5-HIAA levels were measured by HPLC with electrochemical detection. MDMA and MTH potently increased DA and 5-HT release in response to challenging doses of 20 or 5 mg/kg, respectively. CAF (10 mg/kg) markedly enhanced MDMA or MTH-induced release of DA, but lowered MDMA or MTH-evoked release of 5-HT in animals treated chronically with amphetamine derivatives. Combination of CAF and MDMA increased DA and 5-HT synthesis/metabolism while combination of CAF and MTH increased only 5-HT synthesis. CAF potentiated MDMA and MTH-induced DNA damage observed as single and double-strand breaks in comet assay. This data suggest that overproduction of free radicals resulting from persistent overflow of DA and 5-HT from neuronal terminals causes damage of DNA in postsynaptic cells, but spares the presynaptic terminals. CAF seems to potentiate effects of MDMA and MTH on DA but diminishes their effect on 5-HT neuronal terminals. CAF also enhances damage in nuclear DNA produced by MDMA and MTH. Thus, combination of various psychostimulants found in ecstasy tablets may be harmful for central nervous system, especially when they are taken repeatedly.

Effect of galactose-rich diet on neurodegeneration in an animal model of multiple sclerosis

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Considering the typical geographical distribution of multiple sclerosis (MS) and the rising disease incidence in industrialized nations and developing countries, the role of environmental factors in disease pathogenesis has become a topic of major interest. In the last decades, a marked increase in the consumption of milk and dairy products has been recognized, especially in developing countries. Several epidemiological studies provided evidence for a relation between consumption of dairy products and the prevalence of MS. Furthermore, galactose, a constituent of the milk sugar lactose, was implicated in the induction of neurological impairment in a rodent aging model.

Here we investigate the effect of galactose on peripheral immune cells and the CNS *in vitro* and *ex vivo* in experimental autoimmune encephalomyelitis (EAE) as a model mimicking many aspects of MS. Mice fed a galactose-rich diet, but not fructose displayed a more severe form of EAE and had a significantly higher disease incidence compared to the control group (galactose-diet: 67%, control: 30%, n=20 per group, p 0.05). However, FACS analyses did not display any alterations in immune cell frequencies in the spleen or spinal cord. Matching these findings, we did not detect any relevant effect of galactose on different peripheral immune cells *in vitro* as assessed by T cell differentiation and proliferation assays and cell culture experiments with bone marrow-derived dendritic cells and macrophages. Interestingly, axonal densities were significantly reduced in spinal cord lesions of mice receiving a galactose-rich diet (profiles \pm SEM: galactose-diet: 11.58 ± 0.57 , control: 13.50 ± 0.43 , n=4 per group, p 0.05). These observations may indicate a direct impact of galactose on neuronal integrity. Currently, experiments to test the direct effect of galactose on stem cell derived neurons from MS patients are under way.

In summary, our data may help to better inform about the role of milk and dairy products in the pathogenesis of neurodegeneration and to identify further dietary risk factors for MS.

Effects of RGFP109, a specific Histone Deacetylase (HDAC) inhibitor, on neuronal health and rescue of transcription in neuronal culture model of Huntington's disease.

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Huntington's disease (HD) is a progressive, fatal, autosomal dominant hereditary, neurodegenerative disorder which affects the central nervous system. It is associated with motor choreic movements, mood imbalance, depression, and dementia. The genetic mutation leading to Huntington's disease has been identified as abnormal CAG repeat expansions in Huntingtin gene (Htt) exon 1. It is considered one of the most common polyglutamine disorders (PolyQ). HD is associated with 39 repetitions or more of glutamine in huntingtin protein. Previous studies showed massive downregulation of many key genes in HD models. Here we investigated the molecular and the morphological effects of epigenetic drug RGFP109 acting as histone deacetylase inhibitor (HDACi). We also investigated any potential beneficial effects on HD cell culture model and evaluated its effects on downregulated key genes and morphology parameters (Soma size, dendritic length and synapse number). We used Lentivirus-mediated mutant-(Mut-Htt) and wild-type-Huntingtin (WT-Htt) cell culture as an in vitro model of HD. After 24 hour incubation with RGFP109, several different cytotoxicity and viability tests were performed and reverse transcriptase quantitative real time PCR was carried out to assess levels of expression of five key genes (BDNF, Egr1, Arc, C-fos and synaptophysin). We also checked the differences in morphology and synaptic density between the mutant cultures upon RGFP109 treatment via staining with neuron specific marker MAP2 and VGAT. We detected a significant difference in the expression levels of the tested genes as they play a crucial role in growth and differentiation of both cortical and striatal neurons. We found that RGFP109 has beneficial effects where it increases the cell viability and decreases cell toxicity in RGFP109-treated mutant Htt cultures compared with untreated mutant Htt cultures. We also detected significant beneficial effects of RGFP109 on the morphology of the neurons and the expression of VGAT, comparing the mutant cultures versus mutant RGFP109-treated cultures, suggesting its promotion of synaptic connectivity. We are currently conducting further assays to fully assess the neuroprotective potentials of RGFP109 in HD models.

Evaluation of chronic nicotine treatment on hippocampal oscillatory activity and sleep pattern analysis of a G72 transgenic mouse model for schizophrenia

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The primate specific gene locus G72/G30 encoding the 153aa protein LG72 is highly associated with the cognitive and behavioral symptoms of schizophrenia. Transgenic mice expressing the human G72/G30 gene locus show similar cognitive deficits as seen in schizophrenia patients which are often related to hippocampus dysrhythmia.

Several studies suggest that nicotine treatment can reduce cognitive impairment in schizophrenia patients. G72 transgenic mice chronically treated with nicotine present improvement on cognitive functions on prepulse inhibition, working memory and social recognition behavior tests. Furthermore, in the same mice, nicotine treatment resulted an increase of 7-nAChR density in the dentate gyrus. Hence, we investigated the impact of chronic nicotine treatment on hippocampal oscillation activity and the hippocampus/motor cortex interaction. We performed radiotelemetric intrahippocampal (dentate gyrus) and motor cortex recordings in nicotine treated G72 mice and wild type littermates. Radiofrequency transmitters were implanted into a subcutaneous pouch on the back of the animal. Surface and deep, intracerebral brain electrodes were placed using a 3-dimensional stereotaxic set-up and a neurosurgical high-speed drill. Once the animals have recovered from the implantation procedure, the technique allows for physiological EEG recordings under unrestrained conditions. Chronic nicotine (24mg/kg/day) or 0.9% NaCl treatment was delivered by subcutaneous implanted osmotic mini-pumps for 10 days. The effect of nicotine on EEG recordings was evaluated by time-frequency analysis using the Neuroscore 3.2.0 software (DSI).

Preliminary results show that nicotine effects beta (12-30 Hz) and low gamma (30-50 Hz) frequencies in the mouse brain in a similar manner as in the patients treated with nicotine.

EXPERIMENTAL PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY

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Paclitaxel (P) is an antitumor drug commonly used as a first line treatment for patients with ovarian cancer, metastatic breast cancer and other tumors. But its adverse effects on the nervous system (especially peripheral neuropathy) occur in majority of patients. In our study P was administered intraperitoneally to random bred male-rats at a dose of 2 mg/kg 4 times every other day. Sciatic nerves (SN) and dorsal root ganglia (DRG) samples were taken from animals on certain days during 120 days of experiment and were examined by means of light microscopy with morphometric analysis and with an electron microscopy.

We've come to conclusion that P-induced damage of SN is mosaic and can be divided into three periods. The first period (1-7th day) is a primary response to a toxic effect of the drug: swelling of axial cylinders and hypertrophy of myelin sheath on the background of endoneurial edema, destruction of mitochondria in axial cylinders and neurolemmocytes, disorders of microtubules and neurofilaments architectonic in axons. Next period (15th to 60th day) manifests itself in swelling and delamination of myelin sheath, severe degeneration of axial cylinders, demyelination and destruction of myelinated nerve fibers (MNF), dystrophy of the neurolemmocytes. In the third period (90th - 120th day) regeneration of nerve fibers dominated, but full recovery of their structure was not observed. Using morphometric analysis, we determined the wavy-like changes on histograms of the basic parameters of MNF. This was significantly different from the results in control group. The mean value of the area profiles of MNF on the 1st day of the experiment was equal to $(80,03 \pm 2,57) \mu\text{m}^2$, on the 7th - $(74,03 \pm 2,70) \mu\text{m}^2$, on the 15th - $(75,45 \pm 2,29) \mu\text{m}^2$, on the 27th - $(78,27 \pm 3,15) \mu\text{m}^2$, on the 60th - $(92,98 \pm 2,67) \mu\text{m}^2$, on the 90th - $(82,80 \pm 2,19) \mu\text{m}^2$, on the 120th - $(60,55 \pm 2,53) \mu\text{m}^2$; in control - $(44,00 \pm 1,26) \mu\text{m}^2$; ($p < 0,05$).

Paclitaxel also causes pronounced toxic effects on the DRG. Disruption of endoplasmic reticulum, profound structural disturbances of mitochondria, activation of autophagy were noticed within 60 days of experiment. Severity of degenerative changes that developed in gliocytes correlates with the degree of neuronal damage. Within 90th to 120th days in neurons and gliocytes were determined intracellular processes of regeneration. Morphometric analysis revealed undulating nature of changes of the mean values of area profiles of sensory neuron perikarya. On the 1st day its value increased to $(522,8 \pm 18,03)$, on the 7th – it was $(473,05 \pm 17,64) \mu\text{m}^2$, on the 15th - reduced to $(369,56 \pm 9,40) \mu\text{m}^2$, on the 27th - $(450,82 \pm 12,57) \mu\text{m}^2$, on the 60th - $(902,55 \pm 42,09) \mu\text{m}^2$, on the 90th it reduced to $(447,51 \pm 8,82) \mu\text{m}^2$, on the 120th - $(443,43 \pm 9,77) \mu\text{m}^2$; in control - $(410,58 \pm 11,81) \mu\text{m}^2$; ($p < 0,05$). In the capillaries we noticed morphological features of increased transendotelial transport, degenerative changes in the endothelial cells and pericytes, their swelling, dissociation and focal basement membrane fusion on the 90th day of the study. On the 120th day the tendency towards normalization of ultrastructure of the endothelial cells with moderate abnormalities of the basement membrane was observed.

Functional impairment of cortical astrocytes in ALS-transgenic mice

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Amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disease, is a fatal disorder caused by the progressive degeneration of upper and lower motor neurons. Intriguingly, there is *in vitro* and *in vivo* evidence that dysfunction of astrocytes bearing ALS-associated mutations alone can be sufficient to cause selective motor neuron death. We hypothesized that astrocytes are compromised in their physiological function during the course of the disease, which might fuel or even trigger motor neuron degeneration.

Despite being electrically silent, astrocytes can display calcium signals of varying forms, ranging from subcellular microdomain activity to global waves spreading over long distances. Moreover, one recently discovered functional response property of cortical astrocytes, are large calcium signals driven by the locomotion of the animal.

In order to test whether physiological response properties of cortical astrocytes are impaired during the course of the disease, we recorded calcium signals in behaving ALS-transgenic mice (expressing the human superoxide dismutase 1 (SOD1) gene containing the G93A mutation) by means of two-photon imaging. We employed the genetically encoded calcium indicator GCaMP6, selectively expressed in astrocytes, while mice were free to run on a spherical treadmill.

We found that the marked locomotion-associated calcium responses, typically seen in astrocytes in the motor cortex (M1) of WT mice were disturbed in ALS-transgenic mice in multiple ways: First, astrocytes responded less vigorously, as seen e.g. in a reduced population response. Furthermore, astrocytes responded less reliable to running events, as shown by an increase in the fraction of non-responded running epochs (failure rate) and an overall weaker correlation of the astrocytic calcium signal to the running velocity. To probe how well the astrocyte population encodes information on the actual running velocity, we implemented a machine learning algorithm to decode running speed based on calcium activity. Corroborating our results, we found a poorer coding accuracy in ALS-transgenic mice. All above mentioned effects were observed both in layer 1 and layer2/3 of M1.

Interestingly, we witnessed functional impairments of astrocytes not only in the motor cortex (M1), the site typically affected in ALS, but moreover we found evidence for similar alterations within the primary visual cortex (V1), a non-affected control area, indicating that astrocytes might be globally affected in the disease.

Taken together, our results add further credence to the notion of pronounced functional alterations of cortical astrocytes in ALS. Future studies are needed to unravel the underlying molecular mechanisms, the time course, regional specificity and importantly the impact of such functional alterations seen in astrocytes on neuronal health.

Inhibition of GABA A Receptor Improved Spatial Memory Impairment in the Local Model of Demyelination in Rat Hippocampus

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Introduction: Demyelination is the pathological hallmark of Multiple sclerosis (MS) which occurs in both white and grey matter resulting in sensory, motor and visual disabilities in young adult. Cognitive impairment is a common feature in MS affecting ~43–72% of patients, out of which over 40% have memory dysfunctions. The mechanism of memory dysfunction in MS is unknown, but neuroimaging studies suggest that hippocampal demyelination is involved. Several lines of evidence indicate that γ -aminobutyric acid type A (GABA_A) receptors impair memory formation. The main purpose of this study was to determine the role of GABA A receptors on spatial memory in the local model of hippocampal demyelination.

Methods: Local model of demyelination was induced in male Wistar rats weighing 200-250 g. Single dose of lysophosphatidyl choline (LPC) 1% (1.5 μ l) was bilaterally injected into the CA1 regions of hippocampus and an equivalent volume of saline for control animals. The treatment groups were received daily intraventricular injection of Bicuculline (25, 50 ng/animal) or Muscimol (100, 200 ng/animal) 5 days after LPC injection. All animals underwent the Morris Water Maze test for 5 days to assess learning and memory processes. At the end, animals were perfused and their brains were processed for histological studies. Loxul fast blue staining and cresyl violet were performed to evaluate demyelination extent.

Results: Behavioral study revealed that LPC injection in CA1 region of hippocampus impaired memory function. Animals treated with both doses of bicuculline improved spatial memory impairment and in probe test, animals spent more time in target quadrant zone ($p < 0.001$, ANOVA, $n = 8$). However muscimol treatment has no effect on memory function in behavioral test. Histological study by Luxol fast blue staining confirmed that demyelination extent in LPC group was maximal, bicuculline treatment significantly reduced demyelination extension ($p < 0.01$) but we did not find any effect in muscimol treated animals.

Conclusion: Our results support that bicuculline improves learning and memory function and the myelin repair slightly in the local model of hippocampal demyelination. We conclude that disruption of GABAergic homeostasis in hippocampal demyelination context causes memory impairment with the implication for both pathophysiology and therapeutic approaches.

Graph properties of the functional connected brain under the influence of Alzheimer's disease

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Diagnosing Alzheimer's Disease (AD), especially in the early stage, is costly and burdensome for the patients, since it comprises a battery of psychological tests and an extraction of disease specific biomarkers from the cerebrospinal fluid. A more convenient procedure would be a diagnosis based on images obtained through fMRI. Based on previous polymodal studies demonstrating disrupted inter- and intra-cortical connectivity in AD [1], we argue that the functional connectivity of the whole cortex might be a good predictor for the cause of the disease. In resting state fMRI, previous attempts to analyze graph properties of whole brain networks contradict each other [2]. In our opinion there are two general critical points in the methodology of these studies that are likely to contribute to the variability of the results.

First, we criticize that the activities of the brain areas (graph nodes) that are used to calculate the functional connectivities (weights of the graph edges) are composed of functionally inhomogeneous signals, as individual brains are often mapped onto a standard atlas brain of known functional coherent areas [2,3]. The second problem consists in converting the resulting weighted graphs into simple graphs, by setting weights above an arbitrary threshold w_{\min} to 1, and those below it to 0 [2]. The drawback here is that there is no validation for an optimal threshold, and information that might be relevant in AD may be lost.

In this work we address the first problem by applying an activity-driven, region-growing clustering algorithm derived from image processing [4]. In order to guarantee functionally homogeneous clusters, the threshold for inclusion of a voxel in a region is regulated by a heterogeneity criterion [3]. Applying this algorithm, we end up with undirected weighted graphs with varying numbers of nodes for three sets of data: healthy elderly controls, mild cognitive impairment and Alzheimer's disease. Targeting the second problem, we analyze the dependence of graph theoretic measures (shortest path length, in- and out-degree distribution, clustering coefficient, modularity and minimal spanning tree [5]) on w_{\min} . Finally, we investigate the distribution of these measures for each data set to determine candidates for a predictive measure.

Acknowledgements

We acknowledge partial support by the Helmholtz Alliance through the Initiative and Networking Fund of the Helmholtz Association and the Helmholtz Portfolio theme "Supercomputing and Modeling for the Human Brain".

References

1. Bokde AL, Ewers M, Hampel H.: Assessing neuronal networks: understanding Alzheimer's disease. *Prog Neurobiol.* 2009, 89: 125-33.
2. Tijms BM, Wink AM, de Haan W, van der Flier WM, Stam CJ, Scheltens P, Barkhof F.: Alzheimer's disease: connecting findings from graph theoretical studies of brain networks. *Neurobiol Aging* 2013, 34: 2023-36.
3. Marrelec G, Fransson P.: Assessing the influence of different ROI selection strategies on functional connectivity analyses of fMRI data acquired during steady-state conditions. *PLoS One* 2011, 6(4): e14788.
4. Lu Y, Jiang T, Zang Y.: Region growing method for the analysis of functional MRI data. *Neuroimage.* 2003, 20(1): 455-65.
5. Wang J, Zuo X, Dai Z, Xia M, Zhao Z, Zhao X, Jia J, Han Y, He Y.: Disrupted functional brain connectome in individuals at risk for Alzheimer's disease. *Biol Psychiatry* 2013, 73(5):472-81

Impaired synaptic plasticity and increased hyperexcitability in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) results in a gradient loss of synaptic connections, cell death and a progressive decline in cognition. In animal models, memory impairment is often accompanied by changes in synaptic plasticity, as long term potentiation (LTP) (Chapman et al. 1999). Recent evidence from AD mouse models and human AD patients indicate that neuronal dysfunction during disease progression involves both, synaptic plasticity changes and increased aberrant network excitability. Several AD mouse models show altered network activity, hyperexcitability and an increased susceptibility to spontaneous or pharmacological-induced seizures (Palop & Mucke 2009). Furthermore, AD patients display hippocampal hyperactivity and are at higher risk to develop seizures (Noebels 2011; Friedman et al. 2012).

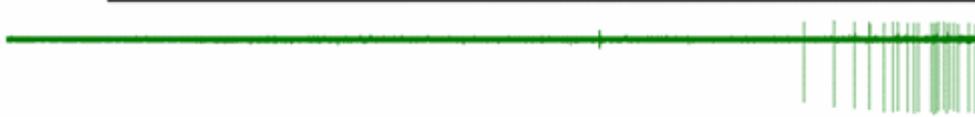
Altered calcium homeostasis has been shown in many AD mouse models and is proposed as a potential cause of neuronal dysfunction in AD. Calcium dysregulation, resulting in imbalanced calcium levels between cytosol and intracellular compartments, might lead to neuronal hyperexcitability and impaired synaptic plasticity due to sustained elevated cytoplasmic calcium concentration. Ryanodine receptors, that control calcium-induced calcium release from the ER, are associated with neuronal dysfunction in AD pathogenesis (Del Prete et al. 2014) and therefore present a potential target for normalizing calcium homeostasis in AD.

Here, we describe the phenotypic electrophysiological characterization of the AD mouse model Tg2576 that overexpresses the Swedish mutation of hAPP. Our results show that Tg2576 mice (females, @ 8-9 month) exhibit impaired LTP and reduced synaptic transmission in hippocampal CA1 region. Interestingly, these results were obtained at a stage when amyloid plaques were not yet formed, but A β oligomers are present in the hippocampus. Moreover, despite decreases synaptic transmission, these mice show increased network excitability. Our recordings demonstrate that evoked fEPSP in slices of Tg2576 are often polysynaptic, an effect that was rarely observed in wildtype controls. To further analyze the hippocampal network excitability in Tg2576, we perfused acute hippocampal slices with low magnesium (0.3 mM)/high potassium (5 mM) ACSF leading to spontaneous epileptiform discharges in the CA3 region. Indeed, slices from Tg2576 mice show i) a reduced threshold and ii) possess a shorter latency to epileptiform activity. The absence of Mg²⁺ activates NMDA receptors and leads to calcium entering into the cell. We therefore proposed that the increased hyperexcitability seen in slices of Tg2576 might arise from imbalanced calcium homeostasis, i.e. elevated basal cytosolic calcium levels. To gain a better understanding of this process, we are currently pharmacologically modulating ryanodine receptors in an attempt to rescue defects in neuronal function.

Figure 1: Hippocampal slices from Tg2576 mice are more susceptible to epileptiform activity as compared to WT. Acute hippocampal slices were prepared from Tg2576 mice and wildtype controls. The extracellular field potential was continuously recorded in the CA3 pyramidal cell layer. After lowering the ACSF [Mg⁺⁺] from 1 to 0.3 mM and increasing the [K⁺] from 3 to 5 mM, the slices develop spontaneous epileptiform activity. Those discharges appear earlier in Tg2576, representing a reduced threshold for network excitability. (Artifacts were removed from the traces.)

Wildtype

Low Mg²⁺, high K⁺ ACSF



Tg2576

Low Mg²⁺, high K⁺ ACSF



Intracellular Ca²⁺ stores affect cortical visual processing in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes a significant impairment of cognition, memory and sensory processing. Neuronal hyperactivity is an emerging functional hallmark of AD, associated with the impairment of visual tuning properties. Here we tested whether acute in vivo blockade of neuronal hyperactivity is able to rescue the disease-mediated visual impairment in a mouse model of AD (APP_{Swe}PS_{G384A} = AD mice). Layer 2/3 neurons in the primary visual cortex of AD, PS_{G384A} and wild type (WT) mice were labeled using multi cell bolus loading technique and their spontaneous as well as visually-evoked activity was measured in vivo using two-photon Ca²⁺ imaging. Based on the frequency of spontaneous Ca²⁺ transients, cells were classified as normal, silent or hyperactive and visually evoked responses were compared between these cell types in different mouse strains. AD mice showed a significant increase in the population of hyperactive neurons accompanied by an increase in the general visual responsiveness, while PS_{G384A} mice exhibited an intermediate phenotype between AD and WT mice. Neurons in AD mice showed a profound decline in visual tuning properties and the degree of the impairment of the orientation but not direction selectivity correlated with an increase in spontaneous activity within the neuronal population. Moreover, the inhibition of spontaneous activity, typically occurring during presentation of the visual stimuli, was impaired in AD mice.

Next, we examined whether the visual tuning properties in AD and PS_{G384A} mice could be rescued by reducing network hyperactivity. To do so we depleted the intracellular Ca²⁺ stores by Cyclopiazonic acid (CPA), a reversible inhibitor of endoplasmic/sarcoplasmic reticulum Ca²⁺-ATPase. Neuronal hyperactivity was effectively reduced in the presence of CPA both in PS_{G384A} and AD mice. Although the impairment of orientation/direction selectivity could not be reversed by store depletion, the enhanced overall responsiveness to visual stimuli, which was observed in PS_{G384A} and AD mice, was improved.

Taken together, our data provide thorough analyses of the visual response properties of neurons in the mouse occipital cortex and describe in detail the impairment caused by the AD-associated mutations. They also show that reduction of network hyperactivity by store depletion is able to ameliorate some (e.g. the pathological increase of responsiveness to visual stimuli) but not all of the impairments observed.

Intracellular transport steps involved in degradation of α -synuclein aggregates

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Autophagy is the main homeostatic pathway guiding cytosolic materials for lysosomal degradation. Aggregates of α -synuclein, the pathological hallmark of sporadic Parkinson disease, are degraded by the autolysosomal pathway. Autophagosomes envelop and sequester intracellular components, including aggregates, and fuse with lysosomes to form autolysosomes. The acidic environment of autolysosomes activates enzymes essential to degrade their contents. Autophagosomes form throughout the cytosol and move towards the perinuclear region to fuse with lysosomes.

In order to improve clearance of α -synuclein aggregates in Parkinson disease, we studied the intracellular transport processes involved in autolysosomal degradation. We transfected HEK293T cells with fluorescent proteins tagged to the lysosomal marker LAMP1, the autophagosomal marker LC3 or the pathogenic α -synuclein mutant A53T. We then performed time-lapse microscopy with 2 images per second and tracked intracellular movement of fluorescent particles. Particles showed Brownian motion, slow "drifting" displacement, and episodes of fast, directed transport we called "leaps". Frequency and length of these "leaps" differed between the different fluorescently tagged particles. The distance to the nucleus at the onset of transport also differed between particle species. Tagging aggregates or autophagosomes with RFP-GFP tandem fluorescence allowed us to observe in addition differences between neutral autophagosomes and acidic autolysosomes because GFP fluorescence is quenched in the acidic environment of autolysosomes whereas the RFP fluorescence is more resistant.

We used these observations to reconstruct the intracellular transport processes involved in the degradation of α -synuclein aggregates. In particular, we observed significant differences between movement of constitutive or starvation-induced autophagy and autophagic degradation of α -synuclein aggregates.

The role of cellular prion protein in Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent age-related neurodegenerative disease that leads to cognitive impairment and dementia. The major defined pathological hallmark of AD is the accumulation of amyloid beta (A β), a neurotoxic peptide, derived from beta and gamma-secretase cleavage of the amyloid precursor protein (APP). It has been described that cellular prion protein (PrPC) plays a role in the pathogenesis of Alzheimer disease. Although, the role of PrPC is still unclear, previous studies showed contradictious results. To elucidate this issue, the main objective of the present study is to investigate the influence of a knockout of the PRNP gene in 5xFAD mice, 5xFAD mice exhibited 5 mutations related to familial Alzheimer disease. These mice show an A β 1-42 accumulation and an increased neuronal loss during aging. To create a bi-transgenic mouse line, 5xFAD mice were crossed with Prnp0/0 Zurich 1 mice (prion protein knockout mice). We subjected two transgenic mice (5xFAD and Prnp0/05xFAD) at different ages (3, 9 and 12 months of age) to a battery of behaviour task to evaluate cognitive and motoric deficits and a biochemical analysis (ELISA, western blot and immunohistochemistry) to investigate the regulation and potential involvement of downstream signaling proteins in the A β induced toxicity process dependent of the PrPC concentration. The study revealed that the deficits induced by A β mediated toxicity appeared earlier in 5xFAD mice (9 months of age) than in Prnp0/05xFAD (12 months of age). Investigating the amount of amyloid beta in 5xFAD mice we observed a PrPC dependent regulation in 9 month-old animals of A β -40 but not of the toxic form A β -42. We did not found in Prnp0/05xFAD mice the up-regulation of P-Fyn, Fyn or Cav-1 as we found in 5xFAD mice. This suggests an important role of PrPC in Alzheimer's disease as a promoter of toxic effect of A β oligomers. Our results may suggest the loss of PrPC delays the toxicity of amyloid beta. In conclusion, our data support a role of PrPC as a mediator of A β toxicity in AD by promoting early onset of disease.

Investigation of sleep architecture of G72/G30 Transgenic Mouse Model of Schizophrenia

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Schizophrenia is a psychological disorder affecting 1% of the world's population. Its origin is not completely known. However, certain factors such as genetic predisposition and environmental stressors during the early development may lead to schizophrenia. Depending on the type, schizophrenia presents a complex symptomology, such as hallucinations, delusions, and sleep disorders. Disturbed sleep can be found in 30 - 80% of schizophrenic patients, depending on the degree of psychotic symptomatology. It appears to be an important and common part of the pathophysiology of this disorder.

The primate specific gene G72/G30 is highly associated with schizophrenia. Humanized BAC transgenic mice expressing the G72/G30 gene locus exhibit cognitive and behavioral deficits related to schizophrenia symptoms.

The sleep pattern of G72 transgenic mice and their wild type controls littermates from both genders were analysed in spontaneous sleep, sleep deprivation and artificial urethane induced sleep using implantable video-EEG radiotelemetry. Therefore, the investigated mice had to undergo surgery in which a two-channel transmitter was placed subcutaneously and its electrodes were positioned at the SB1 (primary somatosensory barrel field cortex) for electroencephalogram (EEG) recordings and the nuchal muscle for electromyogram (EMG) recordings. This method provides monitoring and collecting data from conscious, freely moving laboratory animals under experimental regimes. EEG and EMG recordings were analyzed using Neuroscore 3.2.0 automated sleep scoring module (DSI) for sleep patterns and frequency analysis.

Preliminary results indicate that sleep architecture alters in TG G72 animals in comparison to their wild type littermates in a similar manner as in schizophrenic patients. Therefore with this method sleep organization in a schizophrenic mouse model can be characterized and evaluated in relationship to sleep parameters, clinical symptoms and medication. It is a simple and easy experimental design to study sleep architecture on mouse models for schizophrenia in correlation to patients.

iPSC derived neurons as a human model to study altered AMPA receptor function in Niemann-Pick Type C1

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NPC1 disease 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 the late endosome and lysosome. The pathogenic mechanisms ultimately leading to neurological manifestations caused by neuronal dysfunction and cell death are not exactly understood. Besides a described variety of morphological alterations of neurons, a detailed knowledge about the pathophysiological processes in neurons is still missing. Of special interest are studies about synaptic transmission and plasticity, as a disturbance of this functionality may be causative for clinical symptoms. In former studies, an increased excitatory synaptic transmission was observed in cultured hippocampal neurons from NPC1^{-/-} mice and in hippocampal slices. Additionally disturbed synaptic plasticity was found in the hippocampus as well as in the neocortex and cerebellum. Based on this we used our recently developed neuronal differentiated cells (NDC) derived from three NPC1 patient-specific induced pluripotent stem cell (iPSC) lines to check the expression and function of excitatory AMPA receptors (AMPA-Rs) in neurons. By means of patch clamp recordings and microfluorimetric measurements of calcium (Ca²⁺), we verified the expression of AMPA-Rs in neurons. Cells of the three used cell lines carrying the c.1836A>C/c.1628delC, the c.1180T>C or the c.3182T>C mutation demonstrated a significantly reduced AMPA-induced Ca²⁺ influx and for the c.1180T>C or the c.3182T>C mutation a reduced AMPA mediated current. This suggests an altered expression profile of these receptors. RT-qPCR revealed a significant upregulation of mRNA for the AMPA-R subunits GluA1 and GluA2, but western blot analysis showed comparable protein levels. Thus, the observed reduced Ca²⁺ influx is presumably not based on a lower expression of AMPA-Rs or an increase of GluA2 containing Ca²⁺-impermeable AMPA-Rs, but indicates a hampered function or localization of these receptors.

Synaptic plasticity depends partly on AMPA-R activation. AMPA-R activation can affect the intracellular Ca²⁺ as central second messenger of synaptic plasticity either through its own Ca²⁺ conductivity or through release of the Mg²⁺ block of NMDA receptors. This alters the number and the composition of AMPA-Rs in the subsynapse. Therefore an altered AMPA-R function can be the result of an altered basal synaptic plasticity as well as the cause for an impaired activity dependent synaptic plasticity. Since such alterations are also present in the murine model of the NPC1 disease, iPSC derived NDC as a human model can be used to study the underlying pathophysiological processes and the effects of pharmacological interventions.

Lipid microdomain modification sustains neuronal viability in models of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by the progressive loss of neurons, accompanied by the occurrence of extracellular amyloid beta (A β) plaques and intraneuronal fibrillary tangles. Specifically soluble oligomeric forms of A β have been shown to bind to synaptic connections and disturb neuronal communication, leading to synaptic failure and ultimately to neuronal death. However, binding of A β oligomers does not occur randomly, but rather at specific subcellular entities, the lipid microdomains. Especially plasma membrane gangliosides, sialic acid-containing glycosphingolipids highly expressed in neurons by the enzyme glucosylceramide synthase (GCS), have been proposed to bind A β and promote its aggregation. Gangliosides are involved in a variety of functions including the modulation of signal transduction via neuronal receptors. Neuronal receptors at the synaptic surface are regarded as target sites for A β oligomer-mediated neurotoxicity. Specifically, an impairment of neuronal insulin receptor (IR) signaling has recently been associated with AD pathology, as binding of oligomeric A β to neuronal processes reduces synaptic surface insulin receptor levels and insulin-dependent memory formation.

The current study shows that inhibition of ganglioside biosynthesis in hippocampal and cortical neurons leads to increased neuronal resistance towards oligomeric A β stress in vivo and in vitro. The neuroprotective effect is achieved when GCS is either deleted genetically in forebrain neurons of an AD mouse model, or when GCS is inhibited pharmacologically by the ceramide analogue Genz-123346 in neuronal cell culture models of AD. The results indicate that reduction of neuronal gangliosides stabilizes dendritic surface IR. Thus, insulin sensitivity and insulin-dependent signal transduction are maintained upon oligomeric A β exposure, which in turn protects the neurons. Furthermore, increased levels of surface IR are accompanied by a decrease in the endocytosis protein caveolin-1. The results suggest that down-regulation of caveolin-1-mediated IR endocytosis as a consequence of ganglioside reduction contributes to the observed neuroprotective effects.

Thus, the current work proposes a novel molecular mechanism showing that neuronal ganglioside reduction protects A β -exposed neurons by stabilizing functional surface IR. These results highlight neuronal ganglioside biosynthesis as a potential novel therapeutic target against AD.

Live-Imaging of calcium-induced axonal degeneration in transgenic mouse models of Parkinson's Disease

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Introduction: In Parkinson's Disease (PD), damage to axonal terminals precedes the loss of somata, which follows later in a “dying-back” pattern (axonal degeneration). The protein alpha-synuclein and its mutant forms A30P and A53T are known to be involved in the degenerative process. Here, we use a live-imaging technique to investigate in vivo, whether aSyn (A30P, and A53T) affects calcium-induced axonal degeneration.

Method: Intravitreal injection of adeno-associated viral vectors encoding for EGFP was used to fluorescently label axons in the optic nerve of wild type (WT) or transgenic aSyn.A30P- and aSyn.A53T-mice. The calcium ionophore A23187 was topically applied on the optic nerve to induce axonal degeneration. Subsequently, time course and extent (quantified by the number of axonal bulbs) of axonal degeneration were studied in vivo. WT and transgenic mice were imaged at the age of 3-5 and 7-9 months, respectively.

Results: First signs of axonal degeneration occurred 90 min after application of the calcium ionophore in all groups. Both, young and old A53T-mice showed a higher number of axonal bulbs in the early stages of axonal degeneration compared to age-matched WT controls. Interestingly, this difference was not apparent in aSyn.A30P-animals.

Conclusion: Live-imaging of the optic nerve is a unique method to study axonal degeneration of the central nervous system in vivo. Our results suggest that presence of the aSyn.A53T mutation increases the vulnerability of CNS axons to calcium-induced axonal degeneration. Different effects of aSyn.A53T and aSyn.A30P could be explained by altered membrane binding of aSyn.A30P to intracellular calcium stores. Further research in this field could reveal targets for innovative therapeutic approaches, which are able to delay the onset or attenuate progression of PD.

Loss of tubulin-alpha-4a polyglutamylation in mice

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Synaptic plasticity, a molecular correlate for learning and memory, describes the ability of a synapse to change in strength. This process requires the delivery and removal of plasticity-related proteins to and from synapses. The transport of neurotransmitter receptors is facilitated by motor proteins, which travel along microtubule filaments (MT), one of the core components of the cytoskeleton. However, the molecular mechanisms responsible for the regulation of these transport processes remain elusive. In previous studies, it has been shown, that tubulin, the monomeric element of MTs, undergoes different forms of posttranslational modifications (PTMs), which generates - beside the differential use of alpha and beta tubulin isoforms- a high diversity in MTs. The "MT-Code Hypothesis" suggests that different PTMs encode for specific transport signals, which might steer cargoes in one or the other direction, thereby modulating synaptic function and/or plasticity in neurons. Interestingly, we could show, that changes in neuronal activity alter tubulin PTMs, suggesting a physiological relevance for these modifications. To further test this hypothesis, we generated a conditional tubulin-alpha-4a (tuba4a) knock-in mouse, in which polyglutamylation, a prominent PTM at the C-terminal tail of tubulin, is prevented. Molecular and biochemical validation of the mouse line revealed a loss of Tuba4a polyglutamylation, independent of the expression and integration of this mutant isoform into MT filaments. Thus, the mouse line provides a new model to investigate the physiological functions of a particular tubulin PTM in the nervous system.

Lower affinity of isradipine for L-type Ca^{2+} channels during *Substantia nigra* dopamine neuron-like activity: implications for neuroprotection in Parkinson's disease

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The selective cell death of midbrain dopaminergic (DA) neurons of the *Substantia nigra* (SN) in Parkinson's disease (PD) causes striatal DA depletion and thereby the primary motor symptoms of PD. Voltage-gated L-type Ca^{2+} channel (LTCCs, Cav1)-mediated oscillatory Ca^{2+} -influx, in particular through Cav1.3 splice variants, increases mitochondrial oxidative stress levels in SN DA neurons and their vulnerability to neurodegeneration. LTCC inhibitors, such as antihypertensives of the dihydropyridine (DHP) class, reduced the risk for developing PD in human epidemiological studies, but their neuroprotective effects in animal models of PD are controversial. Nevertheless, a clinical phase-III trial (NCT02168842, www.clinicaltrials.com) is currently assessing the potential neuroprotective effects of the DHP isradipine (ISR) in early PD.

Here we show that neither Cav1.3 knockout nor chronic treatment with therapeutic plasma levels of the DHP ISR are neuroprotective in a 6-OHDA-induced PD mouse model. Compensatory upregulation of other ion channels could explain the lack of Cav1.3-deficiency-induced neuroprotection, while ISR may fail to rescue SN DA neurons due to weaker state-dependent block of neuronal LTCCs compared to Cav1.2 in arterial smooth muscle (aSM). To test the latter hypothesis, we stably expressed human Cav1.2 and long and short Cav1.3 (Cav1.3_L, Cav1.3_S) 1 subunits in HEK293 cells (+ 3, 2 1) and investigated Ca^{2+} current (I_{Ca}) and pharmacological properties during SN DA- and aSM-like activity patterns using the whole-cell patch-clamp technique (2 mM Ca^{2+}).

Simulated SN DA burst activity resulted in increased integrated I_{Ca} during the 3-spike burst (Cav1.2 + Cav1.3) and action potentials following the burst (only Cav1.3 splice variants). During simulated SN DA regular pacemaking Cav1.3 channels conducted the main fraction of total Cav1.3- I_{Ca} at subthreshold potentials between action potentials, while Cav1.2 only mediated Ca^{2+} -influx in response to the action potential spike. Moreover, all three LTCC constructs underwent pronounced channel inactivation (Cav1.3_S < Cav1.3_L < Cav1.2, with Cav1.3_S inactivating fastest) and only ~20 % of LTCCs were available in steady-state. ISR inhibition of steady-state I_{Ca} was highly splice variant- and isoform-dependent: IC_{50} in nM (95% CI): Cav1.2: 2.9 (2.2-3.9), Cav1.3_L: 6.9 (5.8-8.3); Cav1.3_S: 16.8 (14.1-19.9). In contrast, inhibition of Cav1.2 channels during aSM-like activity, responsible for the dose-limiting vasodilatory effect, required 2-fold lower ISR concentrations than during SN DA-like activity (IC_{50} in nM (95% CI): 1.5 (1.2-1.7)).

Our data predict that Cav1.2 and Cav1.3 LTCC isoforms differentially participate in SN DA neuron Ca^{2+} influx, with profound I_{Ca} through Cav1.3 splice variants during the interspike interval and action potentials following post-burst hyperpolarization. The weaker state-dependent block of LTCCs during SN DA-like activity, compared to the antihypertensive drug target - α SM Cav1.2 channels- , could explain the lack of ISR-mediated neuroprotection in our 6-OHDA mouse model. In conclusion, ISR plasma levels exceeding the tolerable therapeutic range in humans would be required for Cav1.3-mediated neuroprotection in PD – unlikely to be tolerated during long-term treatment.

Modeling of EEG time-series by Conditional Probability Echo State Networks.

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Electroencephalography (EEG) is a common method to record voltage fluctuations on the surface of the brain. Although EEG is often used to assess brain dysfunction like epileptic seizures in clinical contexts, the biophysical origins of the EEG signal are still poorly understood. Therefore only very few predictive or generative models (see e.g. [1,2]) of EEG exist. Here we introduce Conditional Probability Echo State Neural Networks (CPESN) as a new means of modeling EEG and similar highly variable neurophysiological data.

Previous work [3] has shown that neural networks can be used to learn conditional probability distributions of random variables. Additionally, to be able to model complex EEG time series, we employ an Echo State Network (ESN) [4] which generates high-dimensional and multi-timescale features from the raw time series data. Given the temporal memory of the ESN, we train a neural network to represent the conditional probability (CP) density function of the next value of the times series for each step in time. Once the resulting CPESN is trained it can be used as a generative model by presenting predicted samples as inputs to the CPESN iteratively.

Here we train a CPESN on EEG time series recorded from epileptic dogs [5] and demonstrate that the CPESN can help to identify interictal and preictal EEG segments. We can further detect unusual episodes of the EEG signal by evaluating the likelihood of signal occurrence under the trained CPESN model. Therefore we expect our CPESN model to be useful to analyze brain dysfunctions like epileptic seizures or other neurological events.

In contrast to common machine learning approaches which learn to predict the most likely future value of a time series from samples of its past, the CPESN provides an estimate of the entire conditional probability density function, and thus has an intrinsic representation of the process' stochasticity. This aspect might be crucial for modeling highly variable neurophysiological time series such as EEG or other neurological and neurophysiological data.

1. Wulsin, D. F.; Gupta, J. R.; Mani, R.; Blanco, J. A.; Litt, B. (2011). Modeling EEG Waveforms with Semi-Supervised Deep Belief Nets: Fast Classification and Anomaly Measurement. *Journal of Neural Engineering*, 8(3), 036015.
2. Häusler, C.; Susemihl, A.; Nawrot, M. (2012) Temporal Autoencoding Restricted Boltzmann Machine. arXiv:1210.8353
3. Feindt M. (2004) A Neural Bayesian Estimator for Conditional Probability Densities. arXiv:physics/0402093
4. Jaeger, H (2001) The "echo state" approach to analysing and training recurrent neural networks. Technical Report 148, German National Research Center for Computer Science
5. American Epilepsy Society Seizure Prediction Challenge, <https://www.kaggle.com/c/seizure-prediction/data>

Morphological alterations of cerebellar cells in Engrailed-2 transgenic mice: A Quick Golgi study

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Engrailed-2 (En2) encodes for a transcription factor and paracrine regulator of axon growth and guidance. It is also involved in many aspects of cerebellar development, including foliation, neuronal development and regulation of the spatial and temporal development of Purkinje cells (PCs). Genetic variations in the En2 gene have been linked to Autistic Spectrum disorders (ASD) in several studies. However, the molecular and cellular mechanisms underpinning this special condition are still unknown. A major question also remains, whether the genetic variation found in humans is causing a positive or negative effect on En2 expression.

Previous studies of transgenic mice which specifically overexpressed En-2 in cerebellar PCs (L7En-2) demonstrated that these neurons display a temporary reduction in dendritic outgrowth and a disorganization of the highly specialized spatiotemporal axonal wiring of the cerebellar cortex, features which have been described in ASD patients. In En2 knockout mice (KO), behavioral conspicuities were detected which resemble symptoms found in ASD patients. The morphology of adult PCs has however not been described in detail in these mice. We here present a detailed analysis of the morphology of PCs in adult En2 knockout and overexpressing mice, using a variation of the Golgi method called Quick Golgi method. This method is a highly efficient technique for preferenced staining of different neurons and glial cells of the mouse cerebellum. It is a reproducible protocol which is not only very fast and economic to do but also provides reliable and cell type specific results.

By using the Quick Golgi method and analyzing adult neurons of En2 knockout mice, and L7En-2 overexpressing mice, we now can show that distinct morphological characteristics of PCs are altered in both mouse models demonstrating that both, overexpressed and reduced expression of En2 has marked, and not complementary effects on neuronal morphology. Our data suggest that En2 is important for cerebellar development at distinct developmental stages for different cellular processes and support the view that an overexpression of En2 is likely to be caused by the human En2 variation.

Morphological and molecular changes in mossy fiber – CA2 connectivity in epilepsy

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The morphological, structural and functional changes taking place in mesial temporal lobe epilepsy (mTLE) prominently manifest, amongst other features, in extensive mossy fiber (MF) sprouting into the granule cell layer and the molecular layer of the dentate gyrus.

Interestingly, the recently described MF input to the hippocampal CA2 region (Kohara et al., 2013, Nature Neuroscience) undergoes a similar transformation, as shown by our previous work (Häussler et al., 2016 Hippocampus): Using an animal model of mTLE we observed an overall reorganization of MFs and aberrant MF synapses extending into the CA2 pyramidal cell layer.

However, an in depth characterization of the molecular identity of these aberrant MF synapses connecting to either the dendrites or the somata of CA2 pyramidal cells in mTLE is still lacking.

To assess changes in the morphology of MF synapses in the CA2 area (stratum lucidum, radiatum and pyramidale) we used the intrahippocampal kainate mouse model of mTLE in transgenic Thy1-eGFP mice which express enhanced green fluorescent protein (eGFP) in adult granule cells and mossy fibers.

Mice were intrahippocampally injected with the glutamate-receptor agonist kainate and perfused 14 or 21 days later, a time point when epileptogenesis has substantially evolved. We then performed immunocytochemistry with CA2-specific antibodies (regulator of G-protein signaling 14 and Purkinje-cell protein 4) and synaptic markers (synaptopodin, zink transporter-3, bassoon, glutamic acid decarboxylase 2) in order to determine the shares of MF synapses as well as all functioning synapses, including GABA-ergic synapses in the regions of CA2 pyramidal cell somata and dendrites, respectively. Subsequent quantification was based on 3D-reconstructions of MF and putative synapses with Imaris software.

We found that eGFP-positive MF synapses in the injected hippocampus considerably increase in number as well as in individual volume (particularly around CA2 pyramidal cell somata) over the observed time course of 21 days after injection. However, ZnT3 immunoreactivity decreased continuously throughout the time course indicating that functionality of MF might be compromised. The amount of bassoon- and synaptopodin-labelled synapses showed a transient slight increase at 14 days followed by a decrease at 21 days in the dendritic as well as in the somatic regions of the CA2 area. Interestingly, despite the loss of interneurons that we have shown earlier (Marx et al., 2013, Frontiers in Cell. Neurosci.), the number of GAD2-positive synapses stayed almost constant at 14 and 21 days in somatic and dendritic areas, and to some extent overlapped with eGFP-positive structures, indicating a functional shift in MF function. Altogether these results suggest a comprehensive change in the synaptic connectivity in CA2 which might underlie its altered network activity during epileptogenesis.

Support: DFG (grant HA7597), Excellence Cluster 'BrainLinks-BrainTools' (DFG-grant EXC1086), BMBF ERA-Net NEURONII CIPRESS

Neurochemical effect of vitamins C, E and DMSO combinations on the oxidative stress biomarkers and severity of ischemic stroke in albino rats

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Oxidative stress is a major participant that contributes to brain reperfusion injury following ischemic stroke (IS). Well-established sources of reactive oxygen species (ROS) generation in the brain following injury include intracellular organelles especially mitochondria, invading neutrophils, activated macrophages and cerebral blood vessels. Ischemic stroke is caused by a blood clot or occluding arteries in the brain which reduces oxygen and other nutrient supply to specific area of the brain, resulting in rapid cell death in the core of the affected region. Excessive generation of reactive oxygen species (ROS) and impairment of endogenous antioxidant defense mechanism begins immediately after the onset of IS, resulting in secondary events leading to neuronal dysfunction and death. This study reports the role of low molecular weight antioxidants in the management of surgically induced IS in albino rats. Twenty five albino rats were subdivided into five groups of five rats each. Ischemic stroke was induced in albino rats using middle cerebral artery occlusion (MCAO), 45mg/kg body weight of the combinations (Vitamin C, Vitamin E and DMSO) were orally administered to the rats for two weeks, antioxidant enzyme biomarkers (catalase (CAT) superoxide dismutase (SOD) and glutathione peroxide (GPX) activities and oxidative stress biomarkers (thiobarbituric acid reactive species (TBARS) concentration, were assessed. Ischemic stroke caused significant ($p < 0.05$) decrease in the activity of the enzymes and significant increase ($P > 0.05$) in the concentration of TBARS. Treatment with the 45mg/kg BW of the antioxidant combinations resulted in the significant increase ($P < 0.05$) of the activities of CAT, SOD and GPX. Also, there was significant ($p < 0.05$) decrease in the concentration of TBARS. The study concluded that antioxidants reduces oxidative stress and its biomarkers in ischemic rats and underscores the relevance of antioxidants in the management of IS, this might open a new therapeutic possibilities for stroke treatment.

Neuroethological and histological evidence for hereditary spastic paraplegia in zebrafish

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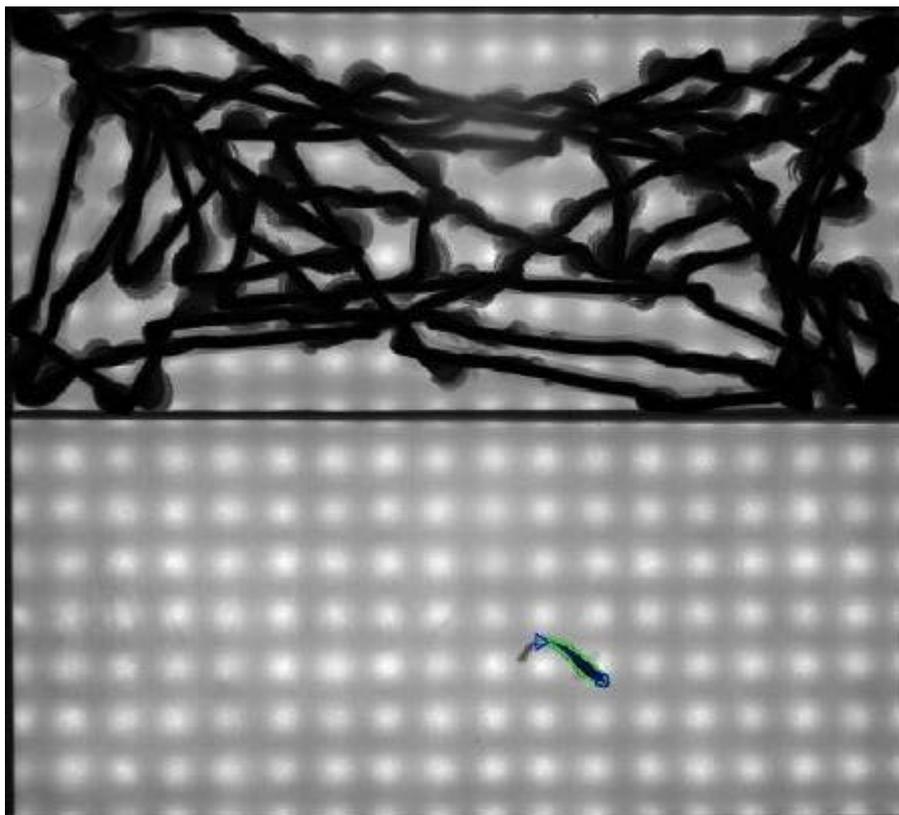
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Spastizin causes a form of hereditary spastic paraplegia (HSP) in humans. HSP symptoms are induced by retrograde degeneration of the longest descending corticospinal tract axons. Currently, there is no therapy available for HSP patients and the number of animal models is limited. In a pilot study, adult zebrafish who lack the *soufflé* gene (their homologue of *spastizin*) revealed locomotion defects. We quantified locomotion phenotypes in *soufflé* mutants, revealing regions of spastic paralysis along the fish's spine. We identified thereby, the most cranial region at which neurons degenerate (paralytic front) by comparing the bendability of *soufflé* mutant and wildtype fish along the head to tail body axis. We will analyze the CNS of mutant and wildtype zebrafish with histological approaches, employing tubulin/ HRP stainings and electron microscopy. Quantifying the number and myelination of large diameter axons in transverse sections of the spinal cord at identified spastic regions will enable us to localize the causative neurodegeneration and gain new insights into its cellular process.

Figure 1 Fully automated tracking of zebrafish.

(a) Trajectory collage of a fish calculated as minimum of every pixel of every frame. (b) Exemplary frame with blue line in the middle of the fish. Blue circle indicates head, blue triangle tail. Green line around the fish shape.



Non-canonical role of autophagy in neurotrophin signalling and axonal homeostasis

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Macroautophagy is thought to provide nutrients during starvation and to eliminate defective proteins and organelles to protect cells from damage. Although originally described as a non-selective bulk process induced in response to starvation, autophagy can selectively degrade an organelle that is damaged. Such a housekeeping role of autophagy in selective protein degradation is especially important in postmitotic cells like neurons, because these cells are not able to dilute the detrimental proteins and organelles by cell division. Accumulation of autophagosomes hallmarks the pathology of neurodegenerative disorders, while neuronal-confined knockout (KO) of several AuTophagy (ATG)- related genes causes neurodegeneration. However, in spite of the critical importance of autophagy for brain function the precise physiological role of ATG proteins in promoting neuronal survival is currently unknown.

A crucial pathway that promotes neuronal survival and protects from neurodegeneration is the brain-derived neurotrophic factor (BDNF) signalling pathway. BDNF and its receptor TrkB require retrograde dynein-dependent axonal transport to the cell body to initiate signaling cascades that control a host of critical functions, including neuronal survival. Neuronal autophagosomes are also formed locally in distal axons and, unlike in non-neuronal cells, trafficked retrogradely to the cell body using dynein motors. Here we describe that autophagosomes carry BDNF/TrkB signals to mediate the neuronal survival. BDNF/TrkB signalling is severely impaired in neurons lacking the crucial autophagy protein ATG5, a phenotype accompanied by the surprising overall loss of TrkB receptors. These alterations coincide with protein inclusions-independent neuroaxonal dystrophy in ATG5 KO neurons, suggesting that autophagy maintains axonal homeostasis by regulating BDNF/TrkB signalling.

Collectively, our results indicate a heretofore-unknown role for ATG5 in neurotrophin signaling and axonal homeostasis and lay the groundwork for understanding neuropathological conditions in which neurotrophin signaling malfunctions and facilitate the identification of new therapeutic targets in neurodegeneration.

Non-invasive imaging of early tissue damage and subsequent microstructural reorganization predicts the severity of hippocampal sclerosis in mesial temporal lobe epilepsy

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Hippocampal sclerosis (HS), which is characterized by pronounced cell loss, reactive gliosis, mossy fiber sprouting and granule cell dispersion, is the most common etiology of intractable mesial temporal lobe epilepsy (mTLE). In acquired forms of mTLE, HS is thought to develop secondarily to an initial precipitating insult [e.g. status epilepticus (SE), head trauma or febrile seizures] and resection of the sclerotic hippocampus often represents the only therapeutic intervention. So far, HS can only be identified by MRI in chronic stages of epilepsy, and there is no way to predict the emergence of HS before its first clinical manifestations. Therefore, we tested the hypothesis whether pathological events occurring during epileptogenesis at anatomical and molecular levels may translate to the subsequent severity of HS.

We did so by performing longitudinal multi-modal MRI during disease progression in the intrahippocampal mouse model for mTLE. Retrospective correlation with histopathological changes and electrophysiological validation of corresponding epilepsy features, allowed us to evaluate the prognostic value of MRI biomarkers.

T2-weighted imaging revealed a transient increase of signal intensity in the ipsilateral hippocampus one day following SE, reflecting early hippocampal damage. Concomitantly, 1H-MR spectroscopy identified substantial changes in the molecular markers for neurons. Concentrations of N-acetyl aspartate and glutamate declined rapidly following SE and stayed low throughout epileptogenesis. Conversely, GABA levels first decreased but recovered within two weeks. Importantly, early T2- and 1H-MR measures were correlated with the extent of cell death-associated microgliosis in chronic epilepsy, suggesting that early neuronal loss predicts the later severity of HS. Using diffusion-weighted imaging (DWI) we monitored the microstructural reorganization in the ipsilateral hippocampus focusing on the dentate gyrus which is severely reorganized during epileptogenesis due to granule cell dispersion. Starting at four days following SE, all DWI metrics increased continuously within the dentate gyrus until chronic stages of epilepsy. Among these measures axial and dorso-ventral diffusivity showed the highest correlation with the degree of HS-associated granule cell dispersion. In a parallel set of experiments we identified radial gliosis, which tightly accompanies granule cell dispersion, as a cellular substrate for the observed DWI changes. Moreover, we corroborated this relationship in resected hippocampi from mTLE patients applying ex vivo DWI and subsequent histology, clearly suggesting its translational value in clinical applications.

In conclusion, our results demonstrate that the severity of HS in chronic mTLE strongly depends on the initial hippocampal damage. Therefore, non-invasive imaging might help to identify patients at risk to develop mTLE, thereby expanding the therapeutic window.

Supported by the Cluster-of-Excellence „BrainLinks-BrainTools“ (DFGgrant EXC1086)

Overexpression of rAAV-mediated human alpha synuclein in the locus coeruleus (LC) leads to a neuronal loss in the nucleus ambiguus: A novel focal mouse model for prodromal Parkinson's disease?

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Background: In the Braak staging model of Parkinson's disease (PD), Lewy pathology progresses in a caudo-rostral pattern from the locus coeruleus (LC) to the substantia nigra (SN). The impact and temporal aspects of the neuronal loss in the LC neurons induced by alpha-synucleinopathy has neither been reported nor used as a prodromal animal model of PD.

Methods: We have performed a unilateral microinjection of recombinant adeno-associated viral vectors (rAAV) carrying human WT-aSYN, A53T-aSYN or luciferase as a control reporter in male C57Bl/6 mice. In total 1.25 µl volumes of rAAV vectors were delivered in the right hemisphere of the LC at coordinates (from dura) AP -5.4 mm, ML -0.9 mm, DV -3.7 mm. Eight animals in each group were sacrificed 3 weeks post the injection by a transcardial perfusion of PBS followed by ice-cold 4% paraformaldehyde. Immunohistochemistry for tyrosine hydroxylase (TH) for the LC and choline acetyltransferase (ChAT) for the medulla oblongata was performed to quantify neurons using stereological analysis.

Result: 3 weeks post aSYN overexpression we have found an approximate 16% neuronal loss of the LC in the injected hemisphere of rAAV WT- SYN and A53T- SYN compared to the non-injected side. The relative numbers of LC neurons in each hemisphere of WT- SYN or A53T- SYN group were significantly decreased when compared to that of rAAV-luciferase injected. In addition, we discovered that the number of ChAT positive neurons in the rostral nucleus ambiguus also significantly decreased in both rAAV WT- SYN and A53T- SYN injected animals, when compared to that of rAAV-luciferase injected.

Conclusion: rAAV WT- SYN or A53T- SYN overexpression in the LC caused a significant neuronal loss in the LC as early as 3 weeks post injection. Moreover, SYN seems to play a role in the cell viability of neurons in the nucleus ambiguus. This result may imply a potential direct or indirect role of noradrenergic neurons in modulating motor innervation of the upper gastrointestinal system.

Disclaimer: B. Lee is a fellow of Deutscher Akademischer Austauschdienst (DAAD), WH. Chiu and LA. Matschke have received a grant by the intramural research fund of the Rhön-AG. W.H. Oertel is supported by the Charitable Hertie Foundation, Frankfurt/Main, Germany.

Quantification of intracellular protein levels in cationic lipid-mediated siRNA transfected primary neurons

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Objectives: Post-mitotic primary neurons have been considered the most resistant to siRNA transfection in comparison with other cell types. The limitation of siRNA transfection is its low efficiency in neurons, even when expressed as a fraction of cells surviving transfection. Thus, siRNA-based studies of neurons should rely on single-cell experiments rather than population-based studies, biochemical experiments, and measurement of mRNA levels.

Aim: Single-cell fluorescence quantitative analysis of studied proteins silenced by cationic lipid-mediated siRNA transfection in primary neurons and detected by confocal microscope was the main goal. We applied the siRNA transfection to knock-down monoamine oxidase B (MAO-B) protein expression and studied the silencing effect on intracellular peptide A β 42 (A 42). The corrected total cell fluorescence (CTCF) was used to quantify the fluorescent signal of both intracellular MAO-B and A 42 in individual neurons by ImageJ 1.47 software (NIMH).

Results: We developed quantification method that allows us to evaluate the MAO-B and A 42 protein expression levels in a single-cell analysis. We were able to quantitatively show a significant reduction of MAO-B protein expression in MAO-B-siRNA transfected neurons in comparison with negative control-siRNA transfected neurons. Moreover, the correlation between MAO-B and A 42 fluorescence signals was demonstrated.

Conclusion: Several parameters of siRNA lipofection with primary neurons to knock-down protein expression involved in A formation have been optimized. Moreover, the developed method for quantification of intracellular protein levels on a single cell basis, in immunocytochemical samples analyzed by confocal microscopy, is a powerful method to investigate effects of siRNA silencing. We readily apply the method to quantify MAO-B and A 42 staining in primary cortex neurons.

Acknowledgments: the research project GACR P304/12/G069; the project Nr. LO1611 with a financial support from the MEYS under the NPU I program and the Boehringer Ingelheim Fonds.

Rescue of Gliosis in Niemann-Pick Type C1 Patient-Specific iPSC Derived Glia Cells

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Niemann-Pick type C1 (NPC1) is a rare progressive neurodegenerative disease, which is caused by a mutation in the NPC1 gene and is inherited in an autosomal recessive manner. In this lysosomal storage disorder the intracellular transport and sequestration of several lipids like cholesterol is severely impaired resulting in their accumulation in the late endosomes and lysosomes. The neurological manifestation of the disease is caused by dysfunction and degeneration of neurons. Regarding glial cells, gliosis was described in a commonly used NPC1 mouse model and reactive astrocytes are discussed to contribute to the neurodegeneration, but less is known for human in vitro cell models. Thus, we analysed two iPSC control cell line and three independent patient-specific iPSC cell lines, carrying different mutations in the NPC1 gene, in regards of gliosis. Terminal differentiation of neural progenitor cells, derived from patient-specific iPSCs resulted in a mixed culture of neurons and glia cells. Flow cytometry revealed an increased amount of cells positive for GFAP and KI67, indicating reactive astrocytes in cell lines with NPC1 mutation. Quantitative realtime PCR, showed an upregulation of GFAP expression and semi-quantitative western blot analysis revealed an increased amount of GFAP in cells with a NPC1 mutation. As another hallmark of gliosis, upregulation of intermediate filaments (IF) vimentin and nestin in NPC1 glial cells could also be observed. Taken together, these results evidence a disease-caused gliosis in NPC1 patient-specific iPSC derived glia cells. Furthermore, we observed changes in cytoskeleton proteins of the intermediate filament type III family, GFAP and vimentin. Immunocytochemical data elucidated changes in the aggregation of IFs, where NPC1-deficient cells displayed an aggregation of longer disorganised bundles of vimentin as well GFAP. In addition, western blot analysis revealed an altered phosphorylation status by a decreased amount of phosphorylated IFs indicating an IF phenotype in NPC1 mutational cell lines.

As the phosphorylation status of IFs can be modulated by PKC we speculated that the PKC activator Phorbol-12 myristate 13-acetate (PMA) can rescue the phosphorylation status of the IFs as well as the observed gliosis. PKC activation altered IF phosphorylation status towards an increased soluble fraction, induced a decrease of glial cells, and ameliorated the proliferation of glial cells, indicating a rescue of gliosis in NPC1 mutational cell lines.

The contribution of glial cells to the pathogenic mechanism of NPC1 disease is still controversially discussed but the here presented data merit further investigations of the role of glial cells. Furthermore investigations of alterations of structural components like intermediate filaments are necessary to understand the impact on NPC1 pathogenic mechanism on a cellular level.

Reversal of pathologic lipid accumulation in NPC1-deficient neurons by drug-promoted exocytotic release of LAMP1-coated lamellar inclusions

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Aging and pathologic conditions cause intracellular aggregation of macromolecules and the dysfunction and degeneration of neurons, but the mechanisms are largely unknown. Prime examples are lysosomal storage disorders such as Niemann-Pick type C (NPC) disease, where defects in the endosomal-lysosomal proteins NPC1 or NPC2 cause intracellular accumulation of unesterified cholesterol and other lipids leading to neurodegeneration and fatal neurovisceral symptoms. Here, we investigated the impact of NPC1 deficiency on rodent neurons using pharmacologic and genetic models of the disease. Improved ultrastructural detection of lipids and correlative light and electron microscopy identified lamellar inclusions as the subcellular site of cholesterol accumulation in neurons with impaired NPC1 activity. Immunogold labeling combined with transmission electron microscopy revealed the presence of CD63 on internal lamellae and of LAMP1 on the membrane surrounding the inclusions indicating their origins from intraluminal vesicles of late endosomes and of a lysosomal compartment, respectively. Lamellar inclusions contained cell-intrinsic cholesterol and surface-labeled GM1 indicating the incorporation of plasma membrane components. Scanning electron microscopy revealed that the therapeutic drug candidate beta-cyclodextrin induces the subplasmalemmal location of lamellar inclusions and their subsequent release to the extracellular space. In parallel, beta-cyclodextrin mediated the NPC1-independent redistribution of cholesterol within neurons and thereby abolished a deleterious cycle of enhanced cholesterol synthesis and its intracellular accumulation that was indicated by neuron-specific transcript analysis. Our study provides new mechanistic insight in the pathologic aggregation of macromolecules in neurons and suggests exocytosis as cellular target for its therapeutic reversal.

Role of ULK1 in axonal degeneration and regeneration in cortical neurons *in vitro*

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Axonal degeneration is a key pathological feature in both traumatic and neurodegenerative diseases of the central nervous system (CNS). It occurs early in disease development and often leads to irreversible defects in neuron function causing progressive disability. As there is only very limited regenerative capacity, the understanding of mechanisms involved in axonal degeneration and regeneration in the CNS is pivotal for the development of novel therapeutic strategies. Autophagy is a regulated cellular degradation process responsible for the turnover of long-lived proteins and organelles. In several previous studies, it has been shown that autophagy plays an important role in different processes involved in neurological disorders, in particular in axonal degeneration. Unc-51 like autophagy activating kinase 1 (ULK1) is a key protein involved in initiation and regulation of autophagy, and has previously been shown to be implicated in neurite outgrowth. Whether a modulation of ULK1 could be beneficial in blocking axonal degeneration and enhance axonal regeneration has not been evaluated so far. We generated adeno-associated viral (AAV) vectors expressing a dominant-negative form of ULK1 (ULK1.DN) in order to decrease autophagy in rat cortical neurons. First, we could show significant changes in levels of autophagy-associated proteins sequestosome 1 (SQSTM1/p62) and microtubule-associated protein 1 light chain 3 type 2 (LC3-II) by AAV.ULK1.DN. Hereafter, we investigated its influence on axonal degeneration and regeneration using microfluidic culture platforms at different time points after axotomy and measured neurite outgrowth on permissive as well as non-permissive substrate. After transduction with AAV.ULK1.DN, we observed a significant decrease in axonal degeneration up to 6 hours after lesion as well as a significant increase in axonal regeneration up to 96 hours after axotomy. Quantitative proteome profiling revealed 122 significantly regulated proteins following AAV.ULK1.DN treatment, several of which are considered to be pro-regenerative. Protein analysis by immunoblot additionally identified several significantly regulated proteins that are known to modulate axonal outgrowth. Our study thus demonstrates that ULK1 is a promising therapeutic target in neurode- and -regeneration and delineates new molecular downstream signaling pathways of ULK1.

PERFORMING DEEP BRAIN STIMULATION AND NEURAL RECORDINGS AT THE SAME TARGET FROM AWAKE ANIMALS: A NEW BIDIRECTIONAL WIRELESS DEVICE

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Although deep brain stimulation (DBS) has been proven to be an effective treatment for several neurological and psychiatric disorders, including Parkinson's disease (PD), dystonia, epilepsy, depression, and obsessive-compulsive disorder, the underlying mechanisms are still unknown. This lack of knowledge could be surmounted e.g. by employment of a suitable micro-stimulation system adapted for chronic DBS in small laboratory animals. Conventional neural recording systems restrict behavioral experiments to a flat indoor environment compatible with the cable that tethers the subject to the recording instruments. To overcome these constraints, we developed a wireless multi-channel system for brain stimulation and neuronal activity recording in freely behaving small animals. This device, which has a size and weight compatible for use in freely moving rats, can be clipped on the animal's head to a previously implanted electrode. This easy "removal" property is crucial because it enables removing or even switching the stimulator/recorder during the experiments without having to anaesthetize or to operate the animal, thus minimizing stress. The device takes extracellular recorded signals from implanted electrodes, amplifies the signals with a programmable gain main amplifier by a factor of 200 to 12800 and transmits the output by radio frequency to a transceiver up to 4m distance. Comparable to tethered recording systems, our device is working without connection cables, and has a weight of 3.9g (without accumulator). Stimulation electronics consists essentially of two assemblies: constant-current source and power supply. The power supply of the constant current (20 to 200 μ A) source is biphasic, so that a voltage of 6 V is required. This allows effective stimulation and recording of neuronal signals in freely behaving animals. The present study describes the validation and in-vivo implementation of this device. Our testing showed that recorded neuronal activities and stimulation of the rat inferior colliculus yields similar results as previously shown by conventional wired devices. Furthermore, the bidirectional wireless device does not restrict the animal's mobility providing a flexible method to control stimulation and neural recording under circumstances where other approaches would be difficult or impossible.

Financial support: AIF

Grant number: KF2780403JL3

SK channels protect locus coeruleus neurons from rotenone induced toxicity: A new target to treat premotor Parkinson's disease

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Background: Dysfunction of the locus coeruleus (LC) noradrenergic system is involved in psychiatric and neurodegenerative diseases and is an early hallmark of Parkinson's disease (PD). The extensive loss of noradrenergic LC neurons in PD is responsible for a large fraction of the non-motor symptoms that occur in early stages of the disease. However, the reasons why LC neurons are selectively vulnerable during the pathogenesis of PD are only poorly understood. To ensure a permanent release of noradrenaline, LC neurons own an intrinsic pacemaking mechanism, which is ultimately coupled to calcium homeostasis and cell survival signaling pathways.

Objective: Calcium activated potassium channels (SK channels) are important regulators of neuronal calcium household and pacemaking. In addition, several studies propose a neuroprotective effect of SK channel activation on dopaminergic neurons. However, the molecular identity as well as the physiological and pathophysiological function of SK channels in noradrenergic LC neurons was not yet investigated.

Methods: In our present study, we utilized RT-PCR expression analysis, patch-clamp recordings of *in vitro* brainstem slices, calcium imaging and stereological approaches to characterize the function of SK channels in LC neurons of wild-type C57Bl/6 mice under normal and under pathological conditions.

Results: LC neurons express a distinct set of SK channel subtypes. Using slice patch clamp recordings, in combination with a selective SK channel agonist and antagonist, we revealed that SK channels conduct potassium currents that modulate the afterhyperpolarization of LC neurons. Recordings of spike trains elucidated that inhibition of SK channels leads to decreased afterhyperpolarizations and an increased firing frequency, whereas activation of these channels results in augmented afterhyperpolarizations and a decelerated firing rate. Hence, SK channels can be considered as important regulators of LC neuron pacemaking. Using calcium imaging experiments in an *in vitro* model of rotenone toxicity we visualized that the pharmacological SK channel activation prevents a dysregulation of the intracellular calcium homeostasis. Additionally, our patch clamp experiments demonstrated for the first time that acute rotenone exposure induced a significant depolarisation and an increase of firing frequency in LC neurons. Strikingly, these effects could be prevented by SK channel activation. In addition stereological analyses showed that SK channel activation significantly counteracts the degeneration of LC neurons induced by toxic concentrations of rotenone *in vitro*.

Conclusion: We propose SK channels as promising drug targets to protect LC neurons in early stages of the PD pathophysiology.

WHC and LAM have received a grant by the intramural research fund of the Rhön-AG. WHO is supported by the Charitable Hertie Foundation, Frankfurt/Main, Germany.

Source localization based on multi-electrode local field potentials

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In Parkinson's disease, deep-brain stimulation (DBS) of the subthalamic nucleus (STN) can suppress pathological oscillations and reduce motor symptoms [1]. The efficacy and the extent of side effects of DBS, however, depend critically on the position of the stimulation electrode [2]. In particular with the increased use of directional DBS, it is becoming increasingly difficult to find optimal stimulation parameters.

A major challenge during the positioning of DBS electrodes is the detection of hotspots associated with the generation of pathological oscillatory (coherent) activity. A precise localization of such sources would significantly speed up the DBS-electrode implantation process and the adjustment of stimulus parameters.

In the framework of a simplified computational model, we develop and test a method aiming at localizing populations of coherently active neurons ("hotspots") based on local field potentials (LFPs) recorded with multiple electrodes. The model consists of an ensemble of hypothetical LFP senders ("neurons") dis-

tributed in 3D space. Subpopulations of coherently active senders ("hotspots") are embedded into a bath of uncorrelated senders ("background noise"). For each electrode contact, the compound LFP is given by the linear superposition of the individual signals weighted by the sender-electrode distances. To reconstruct the hotspot positions, we decompose the compound LFPs measured at the individual electrode

contacts into independent components (ICA). We then determine the spatial origin of these components by a triangulation procedure which accounts for the dependency of the LFP amplitude on the cell-electrode

distance. We investigate how the precision of the hotspot localization depends on parameters such as the hotspot-electrode distance, the hotspot size, as well as the signal-to-noise ratio (power of the hotspot signals relative to the background noise).

References

1. Deep brain stimulation for Parkinson's disease. Benabid, A. L. (2003)
2. Beta-Coupled High-Frequency Activity and Beta-Locked Neuronal Spiking in the Subthalamic Nucleus of Parkinson's Disease, Yang et al. (2014)

Spatial memory impairment and hippocampal cell loss induced by okadaic acid

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In the present study, we evaluated and compared effect of intracerebroventricular (ICV) and intrahippocampal bilateral microinjection of okadaic acid (OA) on spatial memory function assessed in one day water maze paradigm and hippocampal structure in rats. Rats were divided in following groups: Control(icv) - rats injected ICV with aCSF; Control(hipp) - rats injected intrahippocampally with aCSF; OAicv - rats injected ICV with OA; OAhipp - rats injected intrahippocampally with OA. At the end of the behavioral experiments OA treated and control rats were deeply anesthetized with pentobarbital and perfused through the ascending aorta with 300 ml saline followed by 600 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4)). The surviving pyramidal cells in the hippocampus of rats were visualized by Nissl staining. The number of the hippocampal pyramidal cells in Nissl staining sections was counted at X 400 magnification. Nissl staining of hippocampal sections showed that the pyramidal cell loss in OAhipp group is significantly higher than that in the OAicv . The results of our behavioral experiments showed that all rats exhibited a decreased latency to find the hidden platform across the eight training trials and OA treatment did not affect probe-test performance 30s after training. In marked contrast, the present experiments indicate that OA treatment affects probe-test performance 24 h after training. These findings suggest that OA treatment did not affect learning process and short-term spatial memory but induced impairment in spatial long-term memory. OA-induced spatial memory impairment may be attributed to the hippocampal cell death. Based on these results OA induced memory deficit and hippocampal cell loss in rat may be considered as a potential animal model for preclinical evaluation of antidementic drug activity

Targeted overexpression of A53T- α -synuclein induces progressive neurodegeneration and electrophysiological changes of noradrenergic locus coeruleus neurons – a preclinical model of Parkinson's disease

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Introduction: Neurodegeneration of Locus coeruleus (LC) neurons is a common feature of the early prodromal phase of Parkinson's disease (PD) and occurs at Braak's stage 2, actually years before the substantia nigra is affected. However, the mechanisms underlying α -synuclein accumulation and neurodegeneration in LC neurons are still unclear. In our present study we have developed a new mouse model to study the time dependent effects of cellular A53T- α -synuclein overexpression in the LC, regarding α -synuclein aggregation, changes in electrophysiological properties and noradrenergic cell loss.

Methods: Serotype 1/2 recombinant AAV vectors carrying the genetic information of A53T- α -synuclein or luciferase were unilaterally injected in the right LC of C57Bl/6 wild-type mice to induce continuous overexpression of A53T- α -synuclein in LC neurons for 1, 3, 6 or 9 weeks. At each time point eight animals per group were sacrificed for immunohistochemical analysis, whereas four animals per group and time point were used for patch-clamp recordings of LC neurons in acute brainstem slices.

Results: We found that targeted overexpression of A53T- α -synuclein in the LC of wild-type mice caused progressive α -synuclein aggregation and significant loss of noradrenergic LC neurons in a time dependent manner, starting three weeks post-injection. Accumulation of α -synuclein in the LC was accompanied by transport of α -synuclein to various interconnected brain regions observed even after one week of A53T- α -synuclein overexpression. In our model, neurodegeneration of the LC was associated with significant changes in the electrophysiological properties of the noradrenergic neurons. Time dependently, A53T- α -synuclein overexpression induced alterations in action potential shape and increase in the pacemaking frequency.

Conclusions: Our data indicate that overexpressed A53T- α -synuclein accumulates steadily in LC neurons while simultaneously inducing major changes in electrophysiological properties of these noradrenergic cells which might ultimately result in cell death.

BL is a DAAD fellow. WHC and LAM have received a grant by the intramural research fund of the Rhön-AG. WHO is supported by the Charitable Hertie Foundation, Frankfurt/Main, Germany.

The effect of cerebral ischemia-reperfusion injury to the methylation of DNA in homocysteine-treated rats

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Epigenetic processes are involved in the molecular and cellular mechanisms underlying stroke pathogenesis and recovery. One of the most studied epigenetic modification is DNA methylation, the effect of which on CNS development and function is assumed through its regulatory role in neuronal gene expression. The main consequence of methyl modification is that a variety of transcription factors cannot recognize the DNA and thus induce transcriptional repression. Methyl-CpG-binding protein 2 (MeCP2) interact specifically with methylated DNA and mediates gene silencing. Ischemic stroke, one of the leading cause of mortality and long-term disability worldwide, is a heterogeneous multifactorial disorder with still largely unknown epigenetic involvement. Elevated level of Hcy, a sulphur-containing amino acid derived from methionine, is known as hyperhomocysteinemia (hHcy). Among other cerebrovascular diseases, hHcy is also found to be associated with increased risk of stroke. Hcy metabolism plays an important role in DNA methylation. High levels of Hcy lead to increase in the levels of S-adenosylmethionine and higher activity of DNA-methyltransferases. This results in hypermethylation of DNA and gene silencing. Male Wistar rats were used in the experiment. To induce the hHcy, animals were treated with homocysteine subcutaneously (1,2 µmol/g Hcy) for 3 weeks. After this period, global forebrain ischemia was induced by 4-vessels occlusion followed by various times of reperfusion. Plasma Hcy levels were determined. Using specific antibodies, MeCP2 protein levels in brain tissue homogenates were evaluated by western blot analysis. ELISA assay was used for detection of global 5-methylcytosine in DNA. Our first results do not indicate considerable changes in our target protein and methylation levels. Further research will be needed to clarify these findings.

This work was supported by grant VEGA 1/0229/15, from the Ministry of Education of the Slovak Republic and by the project "Identification of Novel Markers in Diagnostic Panel of Neurological Diseases", code: 26220220114 co-financed from EC sources and European Regional Development Fund.

THE INFERIOR COLLICULUS: AN ALTERNATIVE STRUCTURE FOR DEEP BRAIN STIMULATION IN PARKINSON'S DISEASE?

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The inferior colliculus (IC) is widely known as a midbrain auditory relay station. Additionally, IC has also been implicated in processing sensory-motor responses as demonstrated in the animal model of haloperidol-induced catalepsy. High-frequency (830Hz) deep brain stimulation (DBS) of the rat IC reduces haloperidol-induced catalepsy, which models akinesia of Parkinson's disease, but clinical implication of this DBS type is limited since it is aversive. However, typical DBS stimulation frequencies range between 30-130Hz. We therefore asked whether low-frequency (30Hz) DBS of the IC can improve catalepsy without aversive side effects. Young adult rats were implanted with a stimulation electrode unilaterally into the central nucleus of the IC. We determined individual escape threshold intensities during 830Hz DBS of the IC and then assessed the effects of 5min sub-chronic 30Hz DBS at individual escape thresholds on haloperidol-induced catalepsy (0.5mg/kg, i.p.) compared to a sham-stimulated control. We further assessed possible aversive side effects of our stimulation protocol in a conditioned place preference (CPP) test, using 4 conditioning trials. Sub-chronic 30Hz DBS of the IC strongly ameliorated haloperidol-induced catalepsy without any evidence of aversive behavior induced by the stimulation. In fact, in the CPP test we found that the preference for the 30Hz DBS-paired side increased after conditioning, indicating that our stimulation protocol was appetitive. The results show that the IC can serve as an alternative target for DBS in Parkinson's disease. DBS targeted at the IC might be even effective in reducing comorbid depression-related symptoms.

Impaired glucose metabolism in the brain depends on the nature of the activation and damage of astroglial cells and dysregulated neurogenesis

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The concept of "cognitive-metabolic syndrome" has been linking the development of metabolic syndrome and cognitive dysfunction in the pathogenesis of neurodegeneration. Clinical studies suggest a link between type 2 diabetes mellitus and insulin resistance and cognitive dysfunction, but there are significant gaps in our knowledge of the mechanisms underlying this relationship [Moreira RO, 2013]. Although the brain has been considered an insulin-insensitive organ, recent reports on the location of insulin and its receptors in the brain have introduced new ways of considering this hormone responsible for several functions. Alterations of the functional activities may contribute to the manifestation of several clinical entities, such as central insulin resistance, type 2 diabetes mellitus, and Alzheimer's disease (AD) [Blázquez E, 2014]. It is proposed that insulin-regulated aminopeptidase (IRAP) is the site of action of two peptides, angiotensin IV and LVV-hemorphin 7, which have facilitatory effects on learning and memory. One of the possible mechanisms of IRAP action concerns modulation of glucose uptake by influencing intracellular GLUT4 vesicular trafficking (Fernando et al., 2008).

The objective of our study was to examine impaired glucose metabolism in the brain, depending on the nature of the activation and damage of astroglial cells and dysregulated neurogenesis.

Material and methods. We used CD1 male mice, aged 4 months. We tested learning and memory with radial arm maze, fear-condition system. The animal model of AD was induced by injections of beta-amyloid into CA1 area. [Li X. et al., 2011]. Immunohistochemistry staining was used to determine the following markers: GFAP, GLUT4.

Results: By studying the level of expression of insulin-dependent glucose transporter GLUT 4 on astrocytes, we found a statistically significant decrease in the expression of GLUT4 in subgranular layer of the dentate gyrus of the hippocampus in animals with AD ($17,72 \pm 0,58\%$) in comparison with the sham-operated animals ($38,64 \pm 0,52\%$) ($p < 0.05$). Similarly, we have observed in the olfactory bulb. Significantly reduced expression of GLUT4 ($p < 0.05$) in AD ($24,81 \pm 0,78\%$) compared to sham-operated animals ($53,67 \pm 1,18\%$).

Conclusion: Therefore, reduction of GLUT4 expression in astroglial cells in the olfactory bulb and the hippocampus in AD may be due to impaired insulin synthesis, which can lead to contributing to cerebral hypometabolism of glucose in AD.

During performing this work the authors were supported by the grant given by the President Council of the Russian Foundation to support Leading Scientific Teams (project N 10241.2016.7).

Treatment with probiotics, thiamine and melatonin ameliorates aluminum-induced neurotoxicity in rats

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Environmental toxicity is one of the important factors of neurodegeneration, including Alzheimer disease (AD). Yet, little knowledge is available regarding prevention measures to be applied for those subjects who are exposed to this risk factor. An exposure of experimental animals to aluminum chloride (AlCl₃) was shown to generate oxidative damage, mitochondrial dysfunction and neurotoxicity that therefore is used to model environmental toxicity. Recent research showed the anti-inflammatory and antioxidant properties of gut microbiota that can have beneficial effects in animal models of various pathologies. Also, some factors of endogenous origin, e.g., thiamine (vitamin B1) and melatonin, can reduce oxidative stress and attenuate environmental toxicity. We aimed to probe the effects of probiotic treatment with *Bifidobacterium* spp. and *Lactobacillus* spp. (in equimolar concentrations) at the dose of 1×10^9 CFU mL⁻¹ for 30 days, alone or in a combination with thiamine or melatonin, on toxic effects induced by aluminum chloride. Rats were exposed to chronic administration of AlCl₃ at a dose of 100 mg/kg alone, or co-treated with probiotics, alone or in a combination with thiamine (25 mg/kg) or melatonin (10 mg/kg). Thereafter animals were tested in the open field, forced swim test, Barnes maze and a passive avoidance learning model. Subsequently, RT-PCR analysis of 5-HT_{1A} receptor, BDNF and endocannabinoid receptors CB1 and CB2 was carried out. We found that rats chronically exposed to AlCl₃ had profound memory impairment, increased measures of anxiety- and depressive-like behaviours, as well as a reduced expression of BDNF and 5-HT_{1A} and CB1 receptors and increased expression of CB2 receptor in the amygdala and hippocampus. Probiotic treatment, so as administration of either pharmaca ameliorated behavioural changes of chronically intoxicated rats in the tests for anxiety and memory and did not alter gene expression; thiamine and probiotic-treated group that showed a normalized expression of genes encoding CB1 and CB2 receptors. Rats exposed to AlCl₃ treated with probiotics alone or with thiamine alone displayed improved cognitive parameters, which were not found in melatonin-treated animals. While the mechanisms of ameliorative effects of probiotics and combinations with thiamine or melatonin remain to be investigated, it is remarkable that employed here interventions with probiotics can be potent remedies against cognitive and emotional disturbances that result from chronic aluminum toxicity. Thus, a modification of a diet and vitamin supplementation could be effective ways of prevention of chronic environmental intoxication and neurodegenerative disorders that worth to be investigated further.

Trial-by-trial variability: a new marker for visual hallucinations in Parkinson's disease?

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Patients with Parkinson's disease (PD) frequently suffer from visual misperceptions and hallucinations (VH) 1. In the current study we asked whether misperceptions in PD rely on consciously accessed visual information and aimed to develop an objective perceptual measure that can discriminate between hallucinating and non-hallucinating PD patients. Twenty-five non-demented patients with PD (11 PD-VH and 14 PD-non-VH) and 19 age-matched healthy controls underwent psychophysical testing with an image recognition task. Conscious image perception was manipulated by a continuous flash suppression paradigm (CFS) that renders visual stimuli perceptually invisible for prolonged periods of time 2. Images (faces, cars, scrambled) with slowly increasing contrast were presented to one eye, while dynamic Mondrian patterns were flashed into the other eye. In the non-CFS, 'visible' condition, the same images were shown without the rivaling pattern. Subjects were instructed to press a pre-assigned button when they recognized a face or a car. PD-VH patients exhibited a higher proportion of image categorization errors (26%), and reported faces or objects in scrambled images more frequently as compared to PD-nonVH (2%) and controls (4%). Most strikingly, PD-VH patients showed pronounced intra-individual trial-by-trial variability (SD/Mean = 0.37) in recognition times as compared to controls (0.24). All effects were more pronounced in the non-CFS condition and could not be explained by differences in motor performance, medication or lower-level vision impairments. Our findings suggest that misperceptions in PD are triggered by consciously accessed visual information. In addition, fluctuating visual performance appears to be a signature of PD patients with visual hallucinations.

1. Diederich, N.J., Fenelon, G., Stebbins, G. & Goetz, C.G. Hallucinations in Parkinson disease. *Nature reviews. Neurology* 5, 331-342 (2009).
2. Tsuchiya, N. & Koch, C. Continuous flash suppression reduces negative afterimages. *Nature Neuroscience* 8, 1096-1101 (2005).

X-ray diffraction and X-ray fluorescence on Parkinson's disease substantia nigra

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Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. One of the pathological hallmarks of the disease is the presence of protein aggregates called Lewy Bodies (LB) whose major component is a protein called α -synuclein. One of the brain regions most affected by the disease is the substantia nigra (SN). Here we studied histological sections from this area by synchrotron radiation using X-ray diffraction (XRD) and X-ray fluorescence (XRF).

The XRF analysis showed an increased amount of iron in the SN of the PD samples compared to the sample derived from a non-parkinsonian control patient. Iron deposits were mainly located in the extracellular space. Conversely, copper was decreased in the PD samples compared to controls.

In addition to that, the XRD data pointed out the presence of crystallites that produce a specific pattern in the wide-angle scattering range. Moreover, these structures are quasi-exclusively present in the PD samples and there is no co-localization between the spatial distribution of the highly ordered structures and the metal distribution.

Our study confirms increased iron levels in PD brains, but adds an improved spatial resolution and identifies the extracellular space as most affected compartment. Although the precise nature of the highly ordered molecular structures could not be revealed in this study, XRD visualized tissue properties, which are inaccessible to conventional microscopy. Whether these diffraction patterns can be used as a biomarker will have to be determined in further studies

Poster Topic

T12: Neuroimmunology, Inflammation, and Neuroprotection

- [T12-1A](#) Adolescent mouse offspring show microglial changes after prenatal immune activation in an animal model of schizophrenia
Manuela Eßlinger, Marie-Piere Manitz, Simone Wachholz, Jennifer Plümper, Georg Juckel, Astrid Friebe
- [T12-2A](#) Altered ion currents in cerebellar granule cells in an *in vitro* model of neuronal injury.
Lubica Lacinova, Katarina Ondacova, Dana Jurkovicova
- [T12-3A](#) Biomarker screening by an improved immunoblotting technique: Targeting autoantibodies of a peripheral neuropathy
Christian Peter Moritz, Juliette Svahn, Evelyne Federspiel, Jean-Philippe Camdessanché, Jean-Christophe Antoine
- [T12-4A](#) Chronic neuroinflammation induced by influenza A virus infection and the role for hippocampal neuron morphology and function
Shirin Hosseini, Kristin Michaelsen-Preusse, Esther Wilk, Klaus Schughart, Martin Korte
- [T12-5A](#) Differential interaction patterns of antisera to *Neisseria gonorrhoeae* and meningitidis and *Chlamydia trachomatis* with a human first trimester fetal brain multiprotein array
Abdullah Almamy, Christian Schwerk, Horst Schrotten, Hiroshi Ishikawa, Abdul Rahman Asif, Bernhard Reuss
- [T12-6A](#) ECTO-5'-NUCLEOTIDASE MEDIATES MIGRATION OF RAT CORTICAL ASTROCYTES IN SCRATCH WOUND ASSAY *IN VITRO*
Marija Adzic, Nadezda Nedeljkovic
- [T12-7A](#) Effect of microglia depletion on neuronal survival and axon regeneration
Alexander Hilla, Dietmar Fischer
- [T12-8A](#) Cross-talk between mitochondrial permeability transition and K_{ATP} ion channels in mediating neuroprotection
Suhel Parvez, Mohd. Waseem, Heena Tabassum
- [T12-9A](#) Spinal versus brain microglial and macrophage activation traits determine the differential neuroinflammatory responses and analgesic effect of minocycline in chronic neuropathic pain
Li Tian, Zhilin Li, Hong Wei, Sami Piirainen, Antti Pertovaara
- [T12-1B](#) Effects of *Nymphaea lotus* Linn on Structure of Hippocampal Neurons of Rats in Chronic Stress
Kameni Poumeni Mireille, Dzeufiet Djomeni Paul Désiré, Bilanda Claude Danielle, Mengue

Ngadena Yolande Sandrine, Ngoungoure Madeleine Chantal, Mbolang Nguegan Lohik, Tchoupou Tchinda Huguette, Femoe Membe Ulrich, Kamtchouing Pierre

- [T12-2B](#) Engineered hNCSs for Targeting Spinal Cord Gliomas: A Neurobiology-based Therapeutic Approach
Yang D. Teng
- [T12-3B](#) ErbB2 inhibition as a novel treatment option for Traumatic Brain Injury
Akila Chandrasekar, Florian olde Heuvel, Komali Valishetti, Albert C Ludolph, Tobias M Böckers, Markus Huber-Lang, Francesco Roselli
- [T12-4B](#) Erythropoietin dampens injury-induced microglial activity
Liane Wüstefeld, Hana Janova, Miso Mitkovski, Hong Pan, Umer Javed Butt, Debia Wakhloo, Klaus-Armin Nave, Hannelore Ehrenreich
- [T12-5B](#) EXPRESSION OF CD73, CD39 AND CD39L1 IN THE LUMBAR SPINAL CORD DURING THE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS
Nadezda Nedeljkovic, Danijela Laketa, Marija Jovanovic, Ivana Bjelobaba, Irena Lavrnja
- [T12-6B](#) Expression of NKG2D ligands in glioma stem cells in situ and *in vitro*
Charlotte Flüh, Vivian Adamski, Kirsten Hattermann, Guranda Chitadze, Michael Synowitz, Dieter Kabelitz, Janka Held-Feindt
- [T12-7B](#) Ferritin in Microglia
Melanie Schürz, Nikolaus Bresgen, Clara Lipfert, Karin Oberascher, Hubert Kerschbaum
- [T12-8B](#) Genetic ablation of CB2 receptors enhances neuropathic pain development via boosted leptin signaling in peripheral nerves
Chihiro Nozaki, Elisa Nent, Astrid Markert, Andreas Zimmer
- [T12-1C](#) Immunization with S100 leads to increased complement activation in an experimental autoimmune glaucoma model
Sabrina Reinehr, Marcel Gandej, Jacqueline Reinhard, Gesa Stute, H Burkhard Dick, Andreas Faissner, Stephanie C Joachim
- [T12-2C](#) Influence of acid sphingomyelinase deficiency on brain damage after mild focal ischemia in mice
Ayan Mohamud Yusuf, Nina Hagemann, Carlotta Martiny, Erich Gulbins, Richard Kolesnick, Dirk M. Hermann
- [T12-3C](#) Microglia activation in the Interferon- mouse model of depression
Alexandra Knorr, Simone Wachholz, Georg Juckel, Astrid Friebe
- [T12-4C](#) Molecular pathophysiology of human anti-glutamate receptor 2 autoantibodies on AMPA-receptor mediated synaptic transmission
Christian Geis, Holger Haselmann, Christian Werner, Benedikt Grünewald, Sören Doose, Stefan Hallerman
- [T12-5C](#) NO/cGMP signaling via Guanylyl Cyclase isoform 1 (NO-GC1) affects neuronal networks and blood-brain barrier integrity after traumatic brain injury in somatosensory cortex of mice

- [T12-6C](#) Occurrence of tau-reactive antibodies in plasma of cognitively normal individuals
Michala Kolarova, Lenka Hromadkova, Zuzana Bilkova, Ales Bartos, Urmi Sengupta, Rakez Kayed, Jan Ricny
- [T12-7C](#) Progranulin protects against exaggerated secondary consequences of experimental traumatic brain injury in mice
Regina Hummel, Lutz Menzel, Lisa Kleber, Carina Friedrich, Larissa Dangel, Katja Schmitz, Irmgard Tegeder, Michael K.E. Schaefer
- [T12-8C](#) Atorvastatin mitigates neuroinflammation through downregulating cytokine and NF- κ B activity in PTZ-kindled mice
Nouroz Sehar, Sheikh Raisuddin, Nidhi B Agarwal
- [T12-1D](#) Proteome profile of IL-17 and IL-18 in blood serum, cerebrospinal fluid and conditioned media of BM-MSC culture of ALS patients A
Joanna Magdalena Czarzasta, Mariusz Dziekonski, Anna Tutas, Joanna Wojtkiewicz, Wojciech Maksymowicz
- [T12-2D](#) REGULATION OF NOD-LIKE RECEPTORS AND INFLAMMASOME ACTIVATION IN CEREBRAL ENDOTHELIAL CELLS
István A. Krizbai, Mihály Kozma, Kinga Molnár, Csilla Fazakas, Attila E. Farkas, János Haskó, Imola Wilhelm, Péter Nagyoszi, Ádám Nyúl-Tóth
- [T12-3D](#) Removed perineuronal nets and damaged, but persisting GABAergic neurons in the ischaemia-affected nucleus reticularis thalami of wildtype and 3xTg mice
Wolfgang Härtig, Simon Appel, Anne Suttkus, Jens Grosche, Dominik Michalski
- [T12-4D](#) Role of dopamine agonists in mitochondrial dysfunction mediated focal cerebral ischemia in rodents
Heena Tabassum, Syed Suhail Andrabi, Suhel Parvez
- [T12-5D](#) Role of glial NF- κ B signalling in IL-1 mediated central effects
Mareike Bernau, Helge Müller-Fielitz, Markus Schwaninger
- [T12-6D](#) Role of the anti-inflammatory cytokine IL-37 in the brain
Niklas Lonnemann, Gayane Grigoryan, Charles A Dinarello, Martin Korte, Andreas Holz
- [T12-7D](#) The comparison of glucose, lipid and nitric oxide metabolism parameters between schizophrenic patients with metabolic syndrome and internal medicine patients with metabolic syndrome
Nikolai Fattakhov, Ludmila Smirnova, Daria Parshukova, Daria Skuratovskaia, Larisa Litvinova, Arkadiy Semke, Svetlana Ivanova
- [T12-8D](#) The orphan cytokine receptor CRLF3 is involved in erythropoietin induced neuroprotection in *Tribolium castaneum*
Nina Hahn, Debbra Y. Knorr, Johannes Liebig, Liane Wüstefeld, Marita Büscher, Gregor Bucher, Hannelore Ehrenreich, Ralf Heinrich

Adolescent mouse offspring show microglial changes after prenatal immune activation in an animal model of schizophrenia

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Background: Epidemiological studies indicate that maternal infection during pregnancy is associated with a higher risk of the offspring to develop schizophrenia in later life. It is hypothesized that the inflammatory immune response of the mother interferes with normal neurodevelopment of the maturing fetus. Long-lasting changes of this developmental disruption might provide a neural basis for enhanced vulnerability which might manifest in schizophrenia in the adult descendants. The underlying mechanisms of prenatal immune activation affecting the central nervous system substrates and leading to schizophrenia onset are the topic of current research. We suggest microglia as possible initiator for this enhanced vulnerability.

Aims: The effect of prenatal immune challenge on microglia will be examined as they represent an important part of the immune system in the CNS and might serve as a significant link in the etiopathogenesis of schizophrenia.

Methods: We used the Poly(I:C) mouse model of maternal immune activation and investigated descendants from Poly(I:C) vs. saline exposed BALB/c mice at postnatal days 30 (puberty) and 100 (adulthood). Sensory motor gating deficits were determined by testing prepulse inhibition of the acoustic startle reflex. Microglia were analyzed immunohistochemically in different brain areas by Iba1 staining. Furthermore, we screened microglia for pro- (M1) and anti-inflammatory (M2) surface proteins by flow cytometric analysis.

Results: PPI deficits were detectable only in female adult descendants after prenatal immune activation. A significant higher number of microglia cells was found in several brain areas in prenatal Poly(I:C) treated 30 days old mice. Furthermore, FACS analysis revealed a more pro-inflammatory M1 pattern in microglia populations from female adolescent mice after prenatal immune challenge characterized by increased M1 markers and decreased M2 markers. The microglial M1 pattern in females was not present in adulthood and was totally absent in behaviorally unaffected male descendants. Therefore, it could be speculated that PPI deficits in adult females were preceded by a M1-type microglia polarization pattern during adolescence.

Conclusion: It can be concluded that prenatal immune activation with Poly(I:C) induces schizophrenia associated deficits represented by disturbed sensory gating. PPI deficits do not occur before adulthood which mirrors the course of schizophrenia in humans where the first psychotic episode occurs in late adolescence or early adulthood. The deficits probably were preceded by increased microglia numbers and a pro-inflammatory activation pattern of microglia during puberty as this pro-inflammatory polarization was also only detectable in females. This suggests prenatally induced microglial long term effects leading to an altered microglial reaction in adolescence due to unknown processes. This indicates the impact of the immune system in the etiopathogenesis of schizophrenia. Transferring the findings by the rodent model to humans, it could be speculated that activation of microglia during adolescence is correlated with disease onset. Furthermore, M1 polarization could be correlated with recurring psychotic episodes after stressful life events due to prenatally induced long term changes in microglia as they are known to react to stress. Therefore, regulating microglia functions seem to represent a good target for future therapeutic strategies.

Altered ion currents in cerebellar granule cells in an *in vitro* model of neuronal injury.

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Acute injury of central nervous system (CNS) starts a cascade of morphological, molecular, and functional changes including formation of a fibrotic scar, expression of transforming growth factor beta 1 (TGF- β 1), and expression of extracellular matrix proteins leading to arrested neurite outgrowth and failed regeneration. We assessed alteration of electrophysiological properties of cerebellar granule cells (CGCs) in two *in vitro* models of neuronal injury: i) model of fibrotic scar created from co-culture of meningeal fibroblasts and cerebral astrocytes with addition of TGF- β 1; ii) a simplified model based on administration of TGF- β 1 to CGCs culture. Both models reproduced suppression of neurite outgrowth caused by neuronal injury, which was equally restored by chondroitinase ABC (ChABC), a key disruptor of fibrotic scar formation. Voltage-dependent calcium current was not affected in either injury model. However, intracellular calcium concentration could be altered as an expression of inositol trisphosphate receptor type 1 was suppressed by TGF- β 1 and restored by ChABC. Voltage-dependent sodium current was significantly suppressed in CGCs cultured on a model of fibrotic scar and was only partly restored by ChABC. Administration of TGF- β 1 significantly shifted current-voltage relation of sodium current towards more positive membrane potential without change to maximal current amplitude. Both transient and sustained potassium currents were significantly suppressed on a fibrotic scar and restored by ChABC to their control amplitudes. In contrast, TGF- β 1 itself significantly upregulated transient and did not change sustained potassium current. Observed changes of voltage-dependent ion currents may contribute to known morphological and functional changes in injured CNS.

Biomarker screening by an improved immunoblotting technique: Targeting autoantibodies of a peripheral neuropathy

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Chronic inflammatory demyelinating polyneuropathy (CIDP) is considered a neuronal autoimmune disease. Patients with this disease suffer from progressively increasing limb weakness, lack of voluntary coordination of muscle movements (ataxia), absent or diminished tendon reflexes, and sensation of prickling or burning (paresthesia). Identifying the autoantigens of this disease will improve its diagnosis, treatment, and understanding. Recent studies failed to identify an antigen for the majority of patients, since the researchers either used a restricted targeted approach finding their protein-of-interest only in a rare subgroup of patients, or lost interesting but difficult-to-handle proteins during their preparation. To overcome these issues, we applied approved untargeted approaches aiming at novel serum antigens. Further, we performed preliminary experiments to adapt the sample preparation and electrophoresis to the project needs. As a result, **we significantly improved the accessibility and resolution of intractable high-molecular-weight proteins**, as they can be found at one of the interesting target regions, namely the node of Ranvier. The identification step included (1) immunoprecipitation using human serum antibodies to capture corresponding antigens of nerve tissue of rats and cows and (2) protein electrophoresis of nerve tissue proteins, followed by serum incubation. Highly sensitive mass spectrometry identified the bound antigens. **We isolated biomarker candidates that appeared in the patient group, but not in the control group** (both healthy controls and other neurological diseases). Our project will help to understand the role of autoantibodies in CIDP's pathophysiology and will provide new biomarkers improving the diagnosis and treatment of autoimmune disorders.

Chronic neuroinflammation induced by influenza A virus infection and the role for hippocampal neuron morphology and function

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While influenza viruses still today are a leading cause of worldwide severe pandemics, the long-term effects of an influenza infection on the central nervous system (CNS) remain rather elusive. In this study, two months old C57BL/6J mice were infected intranasally with different influenza-A virus strains in order to investigate possible chronic effects on hippocampal structure and function. Neurotropic as well as non-neurotropic subtypes of influenza-A viruses were used. The hippocampal formation was chosen since it is especially vulnerable for neurodegenerative diseases.

Analysis of spine density in the different sub-regions of the hippocampus by Golgi-Cox staining revealed a severe loss 30 days post infection with the neurotrophic H7N7 (SC35M) subtype but also following infection with non-neurotropic H3N2 (maHK68). In contrast, H1N1 (PR8) infection had no chronic effect on hippocampal spine number. A detailed analysis of microglia density, activity and morphology as well as astrocyte density revealed influenza virus induced neuroinflammation only in the hippocampus of H7N7 and H3N2 infected animals. 60 and 120 days post infection a partial, respectively a full recovery of spine number and symptoms of neuroinflammation was found. In line with these findings, we observed a severe impairment in spatial memory formation in the Morris water maze task as well as impaired synaptic plasticity for H7N7 infected animals indicating compromised hippocampal function 30 days post influenza-A infection.

Taken together our results clearly show that influenza-A virus infection can lead to chronic neuroinflammation and synapse loss which have a severe impact on hippocampal function in young animals. The severity of this effect depends on the viral strain used and its capacity to activate the immune response of the CNS. While these animals fully recover future experiments will show whether this is also the case for aged individuals.

Differential interaction patterns of antisera to *Neisseria gonorrhoeae* and meningitidis and *Chlamydia trachomatis* with a human first trimester fetal brain multiprotein array

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Bacterial infections, due to molecular mimicry can cause post-infectious autoimmune disorders and contribute to the pathology of several neurological disorders, including rheumatoid arthritis, lupus erythematosus, and Sydenham's chorea. In this context, contribution of bacterial infections in the development of schizophrenia is under debate, since postnatal infections with *Neisseria gonorrhoeae* have been shown to correlate with an increased lifetime disease risk for the offspring. We have identified now cross-reactive candidate proteins for this, by incubating a human first trimester fetal brain multiprotein array (hEXselect) with antisera to *Neisseria gonorrhoeae* (-NG), *Neisseria meningitidis* (-NM) and *Chlamydia trachomatis* (-CT). Using this approach, we could detect bacteria specific patterns of interaction, with almost no overlap between the different antisera. Thus for -NG, we found distinct crossreactivity with 30 proteins including several known schizophrenia candidate proteins such as Snap23, selenoprotein H, synaptotagmin 1, and tetraspanin 7. In case of -NM, a comparably large number of 29 crossreactive proteins could be identified, however with only a single schizophrenia candidate protein (Notch4) being included. The smallest number of only three interactive proteins were found for -CT and those included no known schizophrenia candidate proteins, however with Rps27a, a putative candidate for systemic lupus erythematosus, associated with neuropsychiatric symptoms. Taken together, our results demonstrate for the first time specificity of interaction patterns of antibacterial antisera with proteins of the human fetal brain, functions of which suggested them to be probably of importance for a better understanding of the neurodevelopmental pathology of neuropsychiatric disorders.

ECTO-5'-NUCLEOTIDASE MEDIATES MIGRATION OF RAT CORTICAL ASTROCYTES IN SCRATCH WOUND ASSAY *IN VITRO*

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Purinergic signaling is a universal intercellular signaling system broadly used in all tissues and cell types. It comprises signaling molecules, like ATP and adenosine, their highly specific membrane receptors, P2 and P1, and ectonucleotidase enzymes, which terminate signaling actions by sequential hydrolysis of the nucleotide. ATP is normally found in the extracellular space in nanomolar concentration. However, its level massively increases in response to danger signals, such as metabolic injury, hypoxia and inflammation, when all other components of the signaling network dynamically change as well. Particularly, in several animal models of human neuropathologies, such as multiple sclerosis and amyotrophic lateral sclerosis, significant upregulation of ectonucleotidases has been observed, including marked upregulation of ectonucleoside triphosphate diphosphohydrolase 1 (NTPDase1/CD39) in activated microglia and ecto-5'-nucleotidase (eN/CD73) in activated astrocytes. Since microglia and astrocytes are the main cellular effectors of neuroinflammation, it is important to understand (patho)physiological meaning of the prominent glial CD39/CD73 upregulation in the neuropathological conditions.

It is well known that ATP and adenosine have significant roles in the immunity. ATP potentiates inflammatory actions of astrocytes and microglia, while adenosine acts as an anti-inflammatory and immunosuppressive factor, with role in cell proliferation, motility and survival. Therefore, ATP to adenosine ratio determines the inflammatory status of extracellular environment. CD73, which catalyzes the conversion of AMP to adenosine, functions also as cell adhesion molecule (CAM), interacting with several extracellular matrix molecules, including tenascin-c. The large body of evidence from cancer biology (including glioma tumors) suggests that adenosine produced by catalytic action of CD73 suppresses immune surveillance and promotes tumor growth. Additionally, CD73 is apparently involved in the process of tumor cell migration, through its interaction with ECM. Therefore, CD73 appears to affect two aspects of tumor cell functioning - its migratory capacity and adenosine-mediated ability to escape immune system surveillance.

Given the fact that CD73 is strongly upregulated at reactive astrocytes in the neuroinflammatory conditions, present study aimed to investigate the role of CD73 in astrocyte migration, which is one of the crucial aspects of the reactive phenotype. We used a scratch wound assay in astrocyte monolayer and applied an inhibitor of CD73 activity (, -metADP) or anti-CD73 specific antibodies, in the presence of additional adenosine. The migration capacity of astrocytes was quantified by measuring the surface areas remaining after defined time. We also evaluated CD73 hydrolyzing activity in the treatment conditions, to elucidate which aspect of CD73 functioning, production of adenosine and/or interaction with ECM, affects the process of astrocyte migration. Our data convincingly show that CD73 is involved in the process of astrocytes migration, in both ways. We suggest that CD73 represent potential target for neuroinflammation therapy, which should be investigated further.

Effect of microglia depletion on neuronal survival and axon regeneration

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Microglia cells are tissue-resident macrophages in the central nervous system (CNS), constantly scavenging for plaques, damaged or unnecessary neurons and synapses as well as infectious agents. They therefore represent the main immune defense in the brain and spinal cord. Thus, under physiological conditions, microglia cells constantly scan their environment and become activated upon injury or disease. This activation induces dramatic changes in morphology, gene expression and cytokine release. As the retina and the optic nerve are part of the brain, microglia cells are activated in respective tissues upon optic nerve injury even before axotomized retinal ganglion cells (RGCs) undergo apoptotic cell death and eventually phagocytose degenerated RGCs. However, the detailed role of microglia in this context is still controversially discussed. Some previous reports suggested that activated microglia are rather detrimental to axotomized RGCs as they would actively induce neurotoxicity. On the contrary, others proposed that microglia are more neuroprotective. To unequivocally determine the overall role of microglia in degenerative and regenerative processes of acutely injured RGCs, we took advantage of a recently established method to efficiently deplete microglia, which is based on inhibition of colony stimulating factor 1 receptor (CSF1R). Using this approach, we verified nearly complete microglia depletion in optic nerves and retinae. Surprisingly, preliminary results in cell culture indicate that microglia depletion neither influenced the survival of RGCs nor their capability to regenerate injured axons. The effect of microglia depletion on RGC survival and optic nerve regeneration in vivo is currently under investigation and will be discussed.

Cross-talk between mitochondrial permeability transition and K_{ATP} ion channels in mediating neuroprotection

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Mitochondria play a key role in apoptotic and necrotic cell death. In our study, we have investigated whether the mitochondrial ATP sensitive potassium (mtK_{ATP}) channel blocker 5-hydroxydecanoate (5-HD) and calcium (Ca²⁺) affects permeability transition pore (PTP) alterations in isolated brain mitochondria treated with melatonin (Mel) and cyclosporin A (CsA). Mitochondrial swelling, mitochondrial membrane potential, ROS measurement and mitochondrial respiration were evaluated in isolated brain mitochondria. In our results, mitochondrial swelling stimulated by exposing Ca²⁺ ions and 5-HD associated by mPTP opening as depicted by modulation of CsA and Mel. In addition, Ca²⁺ and 5-HD decreased $\Delta\psi_m$, depleted intracellular ROS, and inhibition of mitochondrial respiration (state 3 and state 4) in isolated brain mitochondria. Addition of Mel and CsA has shown significant restoration in mitochondrial swelling, intracellular ROS measurement and mitochondrial respiration in isolated brain mitochondria. Therefore, we speculate the modulatory effect of Mel and CsA in mitochondria treated with 5-HD and Ca²⁺ hinders the mPTP-mediated mitochondrial dysfunction and cellular oxidative stress. We conclude that inhibition of mPTP is one likely mechanism of CsA's and its neuroprotective actions. Development of neuroprotective agents including Mel targeting the mPTP therefore bears hope for future treatment of severe neurodegenerative diseases.

Spinal versus brain microglial and macrophage activation traits determine the differential neuroinflammatory responses and analgesic effect of minocycline in chronic neuropathic pain

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Substantial evidence indicates involvement of microglia/macrophages in chronic neuropathic pain. However, the temporal-spatial features of microglial/macrophage activation and their pain-bound roles remain elusive. Here, we evaluated microglia/macrophages and the subtypes in the lumbar spinal cord (SC) and prefrontal cortex (PFC), and analgesic-anxiolytic effect of minocycline at different stages following spared nerve injury (SNI) in rats. While SNI enhanced the number of spinal microglia/macrophages since post-operative day (POD)3, pro-inflammatory MHCII⁺ spinal microglia/macrophages were unexpectedly less abundant in SNI rats than shams on POD21. By contrast, less abundant anti-inflammatory CD172a (SIRP⁺) microglia/macrophages were found in the PFC of SNI rats. Interestingly in naïve rats, microglial/macrophage expression of CD11b/c, MHCII and MHCII⁺/CD172a⁺ ratio were higher in the SC than the cortex. Consistently, multiple immune genes involved in anti-inflammation, phagocytosis, complement activation and M2 microglial/macrophage polarization were upregulated in the spinal dorsal horn and dorsal root ganglia but downregulated in the PFC of SNI rats. Furthermore, daily intrathecal minocycline treatment starting from POD0 for two weeks alleviated mechanical allodynia most robustly before POD3 and attenuated anxiety on POD9. Although minocycline dampened spinal MHCII⁺ microglia/macrophages until POD13, it failed to do so on cortical microglia/macrophages, indicating that dampening only spinal inflammation may not be enough to alleviate centralized pain at the chronic stage. Taken together, our data provide the first evidence that basal microglial/macrophage traits underlie differential region-specific responses to SNI and minocycline treatment, and suggest that drug treatment efficiently targeting not only spinal but also brain inflammation may be more effective in treating chronic neuropathic pain.

Effects of *Nymphaea lotus* Linn on Structure of Hippocampal Neurons of Rats in Chronic Stress

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Introduction: Chronic Stress leads to production of pro-oxidant products that may provoke neurodegenerative and neuropsychiatric disorders. In the present study, we investigated the effects of the aqueous flowers extract of *Nymphaea lotus* (*N. lotus*) on hippocampal neuronal death and oxidative stress induced by chronic stress exposure in rats.

Methods: 50 adult wistar male rats were randomly divided into five groups: control, chronic mild stress (CMS), CMS+ Yohimbine (2 mg/kg p.o) and CMS+ *N. lotus* (75 and 200 mg/kg/days p.o) groups received during the stress period. All animals except control group were exposed to chronic unpredictable mild stress for 2 weeks. At the end of the experiment, animals were sacrificed. Brains were divided and processed for determination of some oxidative stress markers and histological analysis through Toluidine blue.

Results: We found that the brain concentration of malondialdehyde (MDA) was significantly higher in the CMS group than in the control group ($p < 0.01$) whereas the reduced glutathione (GSH) level was significantly reduced ($p < 0.001$). Moreover, *N. lotus* not Yohimbine, dose dependently decreased MDA concentration and normalizes GSH levels in the CMS groups. CMS also resulted in significant cell loss in hippocampal CA1, CA3 and hilus. *N. lotus* markedly inhibited the decreases in number of hippocampal CA1 and CA3 ($p < 0.05$) and hilus ($p < 0.05$) neurons caused by chronic stress.

Conclusion: chronic stress induces oxidative stress in brain and specifically damages hippocampal CA1, CA3 and hilus neurons, and *N. lotus* protects hippocampus from these damages induced by chronic stress.

Engineered hNCSs for Targeting Spinal Cord Gliomas: A Neurobiology-based Therapeutic Approach

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There are currently no experimental models showing autonomic dysfunction for intramedullary spinal cord gliomas (ISCG), a lethal disease with no effective treatment. We recently developed a rat model of ISCG and investigated whether genetically engineered human neural stem cells (hNSC) could be developed into potent therapies for ISCG. ISCG rats received injection of hNSC.CD-TK, hNSC.CD or hNSC.CD-TK debris adjacent to the tumor epicenter 7 days after glioma cell implantation, followed with administrations of prodrugs (i.e., 5-FC and GCV; i.p. till the end of the study). Post-tumor survival was determined by time lasted before loss of body weight-bearing stepping in the hindlimb. Also evaluated were autonomic functions and tumor growth rate in vivo. ISCG rats with hNSC.CD-TK treatment showed significantly improved survival than controls that received hNSC.CD or hNSC.CD-TK debris ($P < 0.05$, median rank test), with better maintained autonomic function and reduced tumor growth rate. hNSC.CD-TK cells migrated diffusively into ISCG clusters to mediate oncolytic effect in manners that enabled sparing of spinal cord projection pathways. Through impeding glioma growth and preserving spinal cord neurobiology, dual gene-engineered hNSC regimen significantly prolonged survival in a rat model that emulated sensorimotor and autonomic dysfunctions of human cervical ISCG. Our findings may provide a stem cell-based multimodal approach to treating ISCG and help formulate a recovery neurobiology-based therapeutic strategy for gliomas.

ErbB2 inhibition as a novel treatment option for Traumatic Brain Injury

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Traumatic Brain Injury (TBI) is a major cause for morbidity, mortality and disability, and imposes therefore a relevant health issue worldwide. Ethanol intoxication in TBI patients results, according to clinical studies, in a decrease of mortality and serves therefore as a possible neuroprotective agent. Our aim is to determine the consequences and the mechanisms of ethanol impact on TBI and in doing so, identify new molecular players and potential drug targets.

We performed the TBI by using a blunt weight (333 gram) with a height of 2 cm on the closed skull of the mouse. We treated mice with ethanol (5 mg/kg) pre-TBI, by oral gavage. We screened signaling cascades involved in TBI and ethanol/TBI interaction with a RTK- antibody phosphor-array on cortical whole-tissue lysates sampled three hours after TBI. Mouse motor performance was assessed at 2, 4, 5 and 7 days post TBI using standard Neurological Severity Score (NSS) (exit circle, beam walk, grip test and startle response) as well as open field assay, novel object recognition and elevated beam walk tests.

TBI affected negatively the motor performance, as expected; however ethanol intoxication caused a significant improvement in performance detected already at the first time point tested. TBI caused the up-phosphorylation of multiple RTKs (including ErbB2, FGFR, HGFR, FLT3, VEGFR1 and Ephrin Family) and ethanol pre-treatment resulted in the suppression of the up-phosphorylation of a subset of them (notably ErbB2, PDGFR, FGFR and Ephrin family). ErbB2 was further investigated because of its translational potential.

Immunostaining revealed that Erbb2 phosphorylation was upregulated in neurons, and it was suppressed by ethanol pretreatment, confirming the array data. By phosphor-antibody array, we studied the impact of Erbb2 inhibition and ethanol administration on downstream neuroprotective and metabolic pathways involving Akt, mTor and Erk signaling, showing a considerable suppression of TBI-induced Akt and mTor activation. By immunostaining, we verified that mTor activation, as revealed by phosphorylated S6, is uniquely restricted to neurons.

Finally, we verified the impact of ErbB2 inhibition on motor performance, along with three additional classes of RTK inhibitors, namely FGFR, PDGFR, Ephrin family inhibitors. ErbB2 inhibitor (Tyrphostin AG825, Tocris) pretreatment resulted in a significant improvement in motor performance at 2 days, with sustained benefit over the testing period; the extent of improvement was comparable to the effect of ethanol, whereas PDGFR inhibitor was ineffective. Surprisingly, Ephrin family inhibitor was also effective in improving anxiety performance and deserves further investigations.

Immunofluorescence staining demonstrated ErbB2 over-expression specifically in neurons after TBI and a downregulation by ethanol. Injection of an ErbB2 inhibitor pre-TBI showed significant improvements in the beam walk, exit circle and open field assay.

Our data strongly suggest that ethanol has a positive effect on TBI outcome, associated with the inhibition of multiple RTK signaling. ErbB2 inhibition recapitulates ethanol effect, suggesting that it may be a prime target of ethanol-induced neuroprotection. ErbB2 inhibitors are already approved for clinical use, and they may be repurposed for the acute treatment of TBI.

Erythropoietin dampens injury-induced microglial activity

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Traumatic brain injury causes progressive brain atrophy and cognitive decline. An early treatment with recombinant human erythropoietin (rhEPO), a growth factor with a wide range of neuroprotective properties, prevents both brain atrophy and cognitive deficits observed in lesioned mice. Microglia, the resident macrophages of the brain parenchyma, swiftly respond to injury-released factors, such as ATP, creating a milieu that can under certain conditions drive pathologies. Here we show by advanced imaging and innovative analytical tools that rhEPO, a clinically established and neuroprotective growth factor, dampens microglial activity, as visualized also in vivo by strongly attenuated injury-induced cellular motility. Furthermore, by using a pH-sensitive dye attached to apoptotic cells, we show that the EPO system is involved in phagocytosis of microglia in vitro.

EXPRESSION OF CD73, CD39 AND CD39L1 IN THE LUMBAR SPINAL CORD DURING THE COURSE OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Ecto-5'-nucleotidase (eN) and ectonucleoside triphosphate diphosphohydrolases 1-2 (NTPDases1-2) are purine nucleotide metabolizing enzymes expressed in all mammalian cell types. The enzymes catalyze the hydrolysis of extracellular ATP released from the cells in response to any type of cell insult, including metabolic insult, hypoxia and inflammation. NTPDases1-2 hydrolyze ATP and ADP to AMP, while eN hydrolyzes AMP to adenosine. In the central nervous system, ATP and adenosine have opposite roles on glial cells physiology - while ATP promotes activation of astrocytes and microglia, adenosine acts on them as an immunosuppressive and anti-inflammatory agent. Therefore, eN and NTPDases have interrelated roles in the neuroinflammation as well. Moreover, eN (CD73) and NTPDase1 (CD39) belongs to the cluster of differentiation family, implicated in cell adhesion. There is strong evidence that eN/CD73 and NTPDase1/CD39 expressed at lymphocytes and endothelial cell have the role in the induction of experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. However, the role of the enzymes expressed at glial cells during EAE is less clear. We have previously shown that eN/CD73 and NTPDase1/CD39 activities were markedly upregulated in the lumbar spinal cord during EAE. The present study is aimed to explore the molecular pattern of eN/CD73 and NTPDase1-2 expression over the course of EAE and cell type(s) accountable for the CD73 and CD39 induction.

EAE was induced in Dark Agouti rats by immunization with the spinal cord tissue homogenate and adjuvant. Animals were sacrificed 8, 15, and 28 days after immunization (D8, D15, and D28), i.e., at the time points corresponding to the presymptomatic, symptomatic, and post-symptomatic phases of the illness. Significant increase in eN/CD73 and NTPDase1/CD39 activities were observed at D15, together with marked upregulation of both enzymes at the gene and the protein levels. Highly reactive astrocytes and microglia, abundantly present in the lumbar spinal cord parenchyma were identified as the principal cell types with elevated eN/CD73 and NTPDase1/CD39 expression, respectively. On the other hand, although overall expression of NTPDase2/CD39L1 decreased, both at the gene and protein level, the enzyme was abundantly present at reactive astrocytes exclusively in the spinal cord white matter. The possible (patho)physiological meaning of the altered expression of eN/CD73, NTPDase1/CD39 and NTPDase2/CD39L1 during EAE will be further discussed.

Expression of NKG2D ligands in glioma stem cells in situ and *in vitro*

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Objectives: Glioblastoma multiforme (GBM) is a highly malignant brain tumor. Tumor stem cells have a major influence on tumor malignancy and progression, and immunological escape mechanisms, involving the Natural Killer Group 2, member D (NKG2D) receptor-ligand system, are key elements in controlling tumor progression. Cell-bound NKG2D-ligands (NKG2DL) such as MHC class I related molecule A and B (MICA and MICB), and the UL-16 binding protein family (ULBP1-6) are recognized by the NKG2D-receptor (NKG2D) and trigger cytotoxic effector activity in NK- and T-cells. By releasing soluble NKG2DL, tumor cells inhibit the killing potential of effector cells. Numerous studies documented the importance of the NKG2D-system in *in vitro* GBM-model systems, but the role for glioma stem cells has not been described yet.

Materials: We analyzed the expression profile and localization of NKG2DL (MICA, MICB, ULBP1, 2,3) and embryonic and neural stem cell markers (Klf-4, Oct-4, Sox-2, Nanog, Musashi-1) in solid human GBM and stem-cell like cells isolated from glioma cell lines using quantitative RT-PCR and two colour immuno-staining. We also evaluated the effect of temozolomide (TMZ), a chemotherapeutic agent for GBM standard treatment, on NKG2DL and stem cell marker expression in stem-like cells derived from glioma cell lines by qRT-PCR.

Results: Whereas Musashi-1 and Oct-4 were rarely costained with NKG2DL, Sox-2 and Nanog showed partial costaining. For Klf-4, we observed a complete costaining with MICA, MICB, ULBP1 and ULBP2. NKG2DL were found in a distinct tumor cell subpopulation and were broadly costained with each other, though single positive cells were found as well.

qRT-PCR of stem-like cells derived from glioma cell lines T98G and U251MG in comparison to differentiated cells revealed that T98G stem-like cells were predominantly positive for MICB and Klf-4, whereas MICA, ULBP2, Sox-2, Nanog and Musashi-1 were more pronounced in U251MG stem-like cells. Upon differentiation, T98G displayed significantly less NKG2DL, whereas in U251MG expression of most stem cell markers decreased. Stimulation with TMZ led to a significant upregulation of Klf-4 and Oct-4 in U251MG. Effects on stem cell markers in T98G were not significant. Whereas MICA, MICB and ULBP1 were upregulated in T98G, MICB and ULBP2 were higher expressed in U251MG.

Conclusion: The role of the NKG2D system concerning glioma stem cells is complex: in solid glioblastomas NKG2DL are found in a subset of tumor cells that coexpress some but not all investigated embryonic and neural stem cell markers. Stem-cell like cells derived from glioma cell lines show a heterogeneous picture concerning NKG2DL and stem cell marker expression, with some modulation in response to stimulation with TMZ. As stem-cell like cells from GBM cell lines *in vitro* show a higher expression of NKG2DL than more differentiated tumor cells, the NKG2D/NKG2DL system might play an important role in regulation of tumor stem cell survival.

Ferritin in Microglia

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Iron-dependent pathways guide a variety of inter- and intracellular responses to oxidative stress. In the Fenton reaction, oxidation of ferrous iron in the presence of hydrogen peroxide generates highly reactive hydroxyl radicals. Therefore, iron sequestration is pivotal to the maintenance of cell and tissue integrity since it will prevent excessive, oxidative stress based damage of healthy tissue. Cellular iron homeostasis is regulated by iron import and release and intracellular iron transfer and storage. Most of the iron-binding proteins involved in cellular iron metabolism (e.g., transferrin, divalent metal transporter DMT-1, ferroportin) bind only a few iron ions. In contrast, the iron storage protein ferritin may hold up to 4500 iron atoms, which renders this protein an ideal, high capacity iron buffer and intracellular antioxidant limiting iron availability for the above-mentioned generation of reactive oxygen species. Importantly, ferritin has also been identified in the blood plasma, the levels being increased under certain pathological conditions such as inflammation or malignancy. Moreover, small amounts of ferritin are also present in the cerebrospinal fluid (CSF), however, little is known about the physiological role of serum or CSF-ferritin. Recently, it has been proposed that ferritin serves as iron transporter across the blood-brain-barrier and inside the brain. In particular, the increased iron needs of oligodendrocytes are considered to be covered by ferritin uptake. Ferritin is a 24 multimer of heavy (H) and light (L) chains, the H:L ratio defining different tissue specific isoforms. The H-chain bears a ferroxidase centre which oxidizes Fe^{2+} to Fe^{3+} and together with the L-chain which has nucleation sites for iron mineralization is essential to an efficient iron sequestration. Brain tissue is generally considered to be rich in H-chain isoforms, however, little is known on the ferritin composition of different cell types. Here we present an investigation on the composition of ferritin in microglial cells, resembling macrophage-like cells in the nervous system, which may play a critical role in the neuroinflammatory processes. By immunoblot analysis employing polyclonal antibodies, we identified three bands at about 70, 21 and 14 kDa in cell lysates from BV-2 cells and two bands at 21 kDa, and 14 kDa in primary microglial cells. While the 21 and 14 kDa band represent monomeric subunits, the 70 kDa band most likely represents an unusual trimer not described so far for primary cell types. Western-blot experiments using isoform-specific monoclonal antibodies revealed an increased expression of the H-chain in BV-2 cells compared to primary microglial cells which could indicate a transformation – related change of the ferritin subunit composition. Immunocytochemical studies revealed that ferritin was not uniformly distributed in the cytoplasm, but was concentrated in numerous spot-like structures.

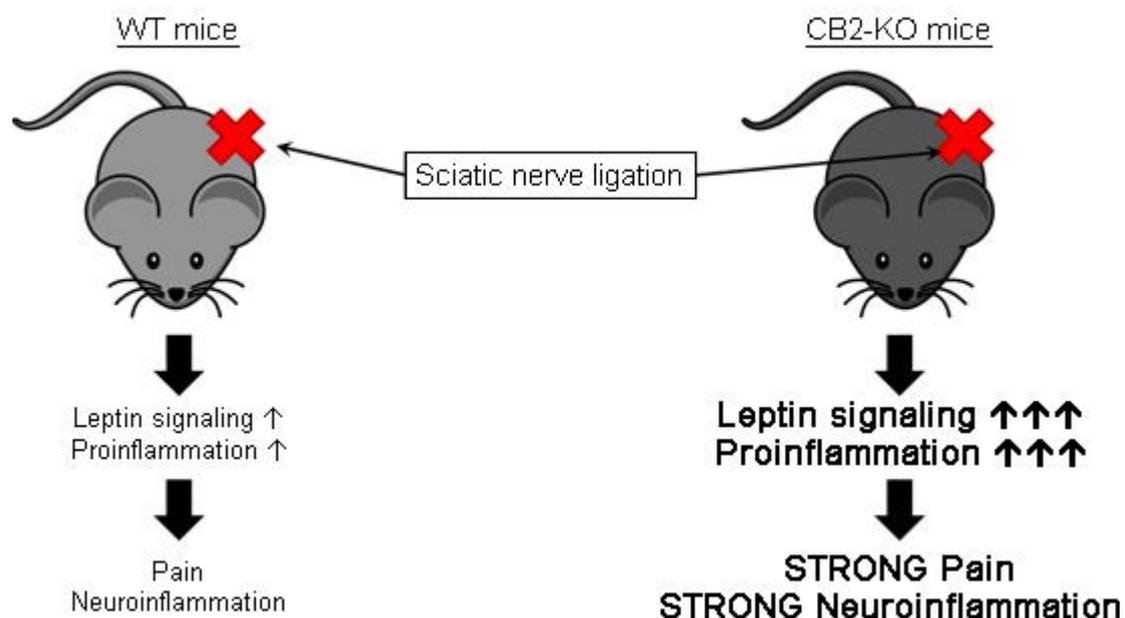
Iron regulation may be important in the long-term modulation of neuroinflammatory processes. As ferritin is not only an intracellular iron storage protein but also an intercellular iron transporter, ferritin could play a crucial role in the coordination of iron-dependent pathways in a variety of neural and immune cells.

Genetic ablation of CB2 receptors enhances neuropathic pain development via boosted leptin signaling in peripheral nerves

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We previously reported that cannabinoid type 2 (CB2) receptor takes an important role for neuropathic pain development. Thus, sciatic nerve injury induced stronger pain and spinal neuroinflammation in CB2 deficient animals. However, detailed mechanisms including why and how CB2 receptors regulate the development of pain and neuroinflammation still remains unclear. In present study, we focused on leptin signaling at sciatic nerves because recent study showed that peripheral leptin activity modulates neuropathic pain development. We found that leptin receptor expression is robustly upregulated on injured sciatic nerve in CB2 deficient mice. We also found that peripheral STAT3 activity is significantly upregulated in nerve ligated CB2 knockouts. Interestingly, upregulation of STAT3 activity in CB2 knockouts has been observed not only on injured nerves but also on non-injured nerve. We also confirmed the infiltration of F4/80 positive macrophages on both injured and non-injured nerves in CB2 knockout mice, whereas it was observed only on injured nerve in WT animals. Furthermore, we discovered that perineurally administered leptin inhibitor could reduce both neuropathic pain and peripheral neuroinflammation, while intrathecal injection of leptin blocker had no effect on the neuroinflammation. These results suggest that CB2 deficiency may augment the nerve injury-induced neuroinflammation and neuropathic pain via enhanced leptin signaling on peripheral nerve. Furthermore, present study reveals the peripheral leptin signaling as the important therapeutic target of neuropathic pain and neuroinflammation.



Immunization with S100 leads to increased complement activation in an experimental autoimmune glaucoma model

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Purpose: Little is known about the mechanisms that lead to the loss of retinal ganglions cells (RGC) in glaucoma, beside an intraocular pressure. In the last years, studies showed a contribution of the immune system within the RGC loss. Here, we investigated the role of the complement system and toll-like-receptor 4 (TLR4) in the retinas and optic nerves in an experimental autoimmune glaucoma model.

Methods: Rats were immunized with S100 protein, while controls (Co) received sodium chloride. In order to evaluate the different cell markers, cryo-sections of the retina and optic nerve were stained against C3, MAC (membrane attack complex), MBL (mannose-binding-lectin), and TLR4 at different points in time after immunization. Groups were compared using Student's t-test. Additionally, retinas were analyzed via quantitative real time PCR (qRT-PCR) and these data were evaluated using REST software.

Results: In regard to the C3 staining in retinas, no changes were observed in the S100 group at 3 and 7 days ($p>0.05$). At 14 days, significantly more C3 depositions were noted ($p=0.04$). Also, an increased C3 expression was seen via qRT-PCR at this point in time. No alterations could be observed in the optic nerves at all points in time ($p>0.05$). The evaluation of MAC revealed no changes between the S100 and Co group neither in the retinas nor in the optic nerves at all points in time ($p>0.05$). MBL, a marker of the lectin pathway, was significantly increased in the retinas at 3 days ($p<0.001$), while no changes were observed later on ($p>0.05$). In the optic nerves, the analyzed of TLR4 showed no alteration between the S100 und Co group at all points in time ($p>0.05$).

Conclusion: Immunization with S100 leads to an early activation of the complement system via the lectin pathway. This activation occurred before RGC loss and optic nerve degeneration. Interestingly, no changes could be noted in the optic nerves. We assume that the activation of the complement system triggers RGC death in the retinas and might play a role in degeneration of the optic nerve at later points in time.

Influence of acid sphingomyelinase deficiency on brain damage after mild focal ischemia in mice

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The hydrolysis of sphingomyelin is one potential pathway for generation of ceramide. It is well known that lack of acid sphingomyelinase (Asm) activity results in the severe lysosomal storage disease Niemann-Pick in humans. In Asm deficient mice, a similar phenotype including impaired growth, neurodegeneration and shortened lifespan due to accumulation of sphingomyelin can be observed. In the present study, male Asm deficient mice in the age of 8 or 13 weeks were subjected to 30 minutes of middle cerebral artery occlusion (MCAO) followed by 24 hours reperfusion. In subsequent analyses, we showed that infarct size, brain edema and disseminated neurological injury were significantly increased in Asm deficient mice compared to wildtype littermates. In addition, no significant effects of Asm deficiency in brain damage could be observed in models of severe stroke induced by 60 or 90 min MCAO. Interestingly, Asm heterozygous mice were protected from ischemic brain injury. Furthermore, we could show an increase in intercellular adhesion molecule 1 (ICAM-1) abundance in Asm deficient non-ischemic and ischemic brain tissue. Besides, wildtype mice treated with the Asm inhibitor amitriptyline showed an ameliorated outcome in the acute phase after MCAO and enhanced vessel density in the sub-acute phase. The precise mechanisms how Asm deficiency leads to exacerbation of ischemic damage after mild focal cerebral ischemia and how these processes differ from more severe ischemia remain to be elucidated.

Microglia activation in the Interferon- α mouse model of depression

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Interferon- treatment is known to cause depression in patients with a chronic state of inflammation/sickness. The administration of this immune-stimulating cytokine can also induce a depressive-like behavior in rodents. To detect an altered behavior in mice, we used the Interferon-alpha mouse model of depression combined with the forced swim test. After daily i.p. injections of Interferon- or PBS for two weeks, we observed a change in the immobility time for the experimental group and could therefore subdivide the group in vulnerable and non-vulnerable mice. This allowed us the identification of an Interferon- -related development of depressive-like symptoms, which provides a more reliable comparison to human studies of Interferon- treated patients. To investigate the pathophysiological mechanisms of these pro-inflammatory processes, we will focus on microglial change in both morphology and expression pattern in depression-associated brain regions.

Molecular pathophysiology of human anti-glutamate receptor 2 autoantibodies on AMPA-receptor mediated synaptic transmission

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Autoantibodies (AB) to alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) were recently described in patients with autoimmune encephalitis. We purified AB from plasma exchange material patients with autoimmune encephalitis to determine the molecular effects of the patient IgG fraction on the synaptic glutamatergic transmission. AMPAR IgG was highly specific to the critical glutamate receptor 2 (GluR2) subunit of AMPAR as shown by using transfected HEK293 cells, brain slices of GluR1 or GluR2 deficient mice, and mass spectrometry. Primary hippocampal neurons preincubated with anti-GluR2-specific IgG showed decreased amplitude and inward rectification of AMPAR mediated excitatory postsynaptic currents (EPSCs) at depolarized membrane potential. We iontophoretically applied glutamate to individual synapses of cultured hippocampal neurons that were identified by intravital FM1-43-labelling and measured single-synapse evoked EPSCs by whole-cell patch-clamp recording. We found reduced AMPAR-mediated EPSC amplitudes and disturbed receptor kinetics after preincubation with anti-GluR2-specific patient IgG. dSTORM super-resolution imaging revealed specific loss of the synaptic expression of the AMPAR GluR2 subunit and an increase of GluR1 expression. In hippocampal slice recordings, non-stationary fluctuation analysis of AMPAR-mediated EPSCs showed a reduction of AMPAR number but an increase of receptor channel conductance. We suggest that anti-GluR2-specific patient IgG leads to a decrease of GluR2-containing receptors in active synapses (e.g. by internalization). Synaptic scaling mechanisms may generate a compensatory overexpression of other than GluR2 subunits resulting in synaptic AMPA-receptor insertion with higher conductivity and increased calcium-permeability. The present data show a possible mechanism of how anti-GluR2 specific IgG can directly affect synaptic transmission in the hippocampus possibly resulting in cognitive deficits and behavioral abnormalities in anti-AMPA mediated autoimmune encephalitis.

NO/cGMP signaling via Guanylyl Cyclase isoform 1 (NO-GC1) affects neuronal networks and blood-brain barrier integrity after traumatic brain injury in somatosensory cortex of mice

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Traumatic Brain Injuries (TBI) can lead to cortical dysfunctions induced by the mechanical impact, and it is a main cause of death in young humans (<45 years). It often results in deafferentation, neuronal loss, disruption of the blood-brain barrier (BBB), edema, neurodegeneration, and in neurological deficits. Among the molecules involved in the pathophysiology of TBI, nitric oxide (NO) is considered to be one key factor. NO activates the soluble guanylyl cyclase (NO-sGC), which exists in 2 isoforms NO-GC1 and NO-GC2. This leads to the formation of the second messenger cGMP, which mediates numerous functions including control of cerebral blood flow, synaptic functions, intracellular signal transmission, and release of neurotransmitters under both physiological and pathophysiological conditions. Here we addressed the role of NO/cGMP signaling in experimental TBI, knockout mice lacking NO-GC1 gene (NO-GC1^{-/-}) were compared with wild-type (NO-GC 1^{+/+}) mice in a model of controlled cortical impact (CCI) in somatosensory cortex or sham surgery as control. Neurological deficits were evaluated by basal excitatory synaptic transmission and intrinsic neuronal properties on pyramidal neurons in layers II/III of somatosensory cortex, brain edema was measured by brain water content, and BBB permeability was assessed by Evans Blue (EB) dye extravasation. Our results showed that TBI decreased the frequency of miniature excitatory postsynaptic currents (mEPSC) as well as action potential firing in pyramidal neurons of layer II/III at 500 μ m distance from the lesion in wild-type mice, however these changes were not visible in the NO-GC1-KO mice. In addition, NO-GC1-KO animals subjected to TBI exhibited less brain edema formation and BBB permeability disruption compared to the wild-type mice. These results suggest that cortical dysfunction observed after TBI is mediated via NO/cGMP signaling. Furthermore, this could make NO-GC1 a promising candidate for future therapeutical strategies to reverse neuronal dysfunctions and deficits after TBI, and to rescue, at least in part, the BBB integrity following traumatic brain injuries.

Occurrence of tau-reactive antibodies in plasma of cognitively normal individuals

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Objectives

Passive vaccination using monoclonal and polyclonal antibodies targeting tau protein is a current trend in the treatment of Alzheimer's disease (AD). However, little is known about the natural pool of anti-tau antibodies already present in the blood of patients with AD not mention in cognitively normal older individuals. Thus, we aimed to characterize natural antibodies reactive with tau protein present in plasma of young and old control individuals and compare their reactivity with antibodies from AD patients. Moreover, we also measured levels of tau antigen in the form of oligomers by anti-tau oligomeric antibody in the blood of AD patients and aged-matched cognitively normal individuals.

Methods

Antibodies from pooled plasma samples either of old cognitively normal individuals or AD patients and IVIG Flebogamma product (representing the pool of purified plasma IgG of young individuals) were isolated against tau 1-441 aa. Reactivity of isolated antibodies was assessed by blotting techniques and ELISA against several forms of tau protein present in brains of AD patients and controls. ELISA assay for measurement of tau oligomers in blood was developed. We enrolled four groups of subjects: cognitively normal controls (n=112), subjects with mild cognitive impairment (n= 15), subjects with mild cognitive impairment due to AD (prodromal AD, n=17), patients with AD (n=68) and subjects with different neurodegeneration than AD (n=21).

Results

The antibodies from control subjects reacted with pathological forms of tau protein found in the brain of AD patients. Moreover, we observed significantly higher levels of tau oligomers in the serum of control subjects when compared to MCI and prodromal AD groups. On top of that the serum tau oligomer levels increased with the age of the subjects.

Conclusions

Altogether these results suggest that the occurrence of tau oligomers in the sera of healthy individuals could be a clearance pathway of tau protein toxic forms from the brain and the humoral immune system could play an important role in this process. The latest studies showed physiological clearance of extracellular tau from the brain along paravascular pathways. The chronic impairment of this pathway may cause an accumulation and aggregation of tau in the brain and as a consequence cause the neurodegeneration. This study was supported by the project Nr. LO1611 with the financial support from the MEYS under the NPU I program, GACR 13-26601S, GACR P304/12/G069 and by Alzheimer Endowment Fund (AVASTipendium for the human brain 2014).

Progranulin protects against exaggerated secondary consequences of experimental traumatic brain injury in mice

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Traumatic brain injury (TBI) is the leading cause of death and disability in adults under 45 years. Treatment options are restricted to surgical intervention and supportive therapies but do not address secondary TBI sequels (McConeghy et al., 2012). Secondary processes in TBI include the activation of CNS-resident microglia and astrocytes and the release of pro- and anti-inflammatory cytokines and chemokines which are major modulators of the immune response following TBI (Ziebell and Morganti-Kossmann, 2010). Also, TBI causes delayed neuronal cell death and axonal injury (Maxwell, 2015). Therefore, factors with anti-inflammatory and neuroprotective properties may alleviate secondary consequences of TBI.

Here, we investigated the role of the neurotrophic and anti-inflammatory glycoprotein Progranulin (PGRN) after experimental TBI in mice using the controlled cortical impact model. We performed (immuno-) histology, gene and protein expression analyses in wild-type and PGRN-deficient mice 5 days after TBI. Behavioral assessment using a neurological severity score was performed at 1, 3, and 5 days after TBI. These analyses revealed stronger neurological impairment and increased pro-inflammatory cytokine gene expression as well as exaggerated astrogliosis and axonal injury in PGRN-deficient mice compared to wild-type mice. In order to verify that the lack of PGRN is responsible for these effects, we administered recombinant PGRN immediately before trauma induction by intracerebroventricular (ICV) injection to PGRN-deficient mice. Indeed, ICV injection of recombinant PGRN reduced brain damage and neurological deficits as determined by lesion volumetry and behavioral assessment. Furthermore, recombinant PGRN attenuated the TBI-induced up-regulation of pro-inflammatory cytokine genes *TNF α* , *IL-1 β* and *IL-6*. Moreover, recombinant PGRN restored normal levels of axonal injury and astrogliosis 5 days after TBI, as demonstrated by immunohistochemistry, immunoblot and gene expression analyses, respectively. Taken together, our results show that endogenous and recombinant PGRN limit secondary consequences of experimental TBI in mice and suggest therapeutic potential of PGRN in TBI.

- Maxwell, W.L. 2015. Development of Concepts in the Pathology of Traumatic Axonal and Traumatic Brain Injury.
- McConeghy, K.W., J. Hatton, L. Hughes, and A.M. Cook. 2012. A review of neuroprotection pharmacology and therapies in patients with acute traumatic brain injury. *CNS Drugs*. 26:613-636.
- Ziebell, J., and M. Morganti-Kossmann. 2010. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics*. 7:22-30.

Atorvastatin mitigates neuroinflammation through downregulating cytokine and NF- κ B activity in PTZ-kindled mice

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Aims: Present study was aimed to investigate the mechanism of action of atorvastatin (ATV) in inflammation through modulation of cytokines viz. IL-1, TNF-, IL-6 and involvement of IL-1Ra and NF- κ B pathway. Previous studies have reported that ATV is a powerful anti-inflammatory and antiepileptic agent. However, there are no reports on ATV treatment and amelioration of IL-1Ra along with NF- κ B activation during PTZ-kindling in mice.

Methods: In Swiss albino mice a subconvulsant dose of PTZ, i.e 25mg/kg.i.p, was administered on every alternate day. ATV (20, 40 and 80mg/kg/p.o) was given daily till the development of kindling. After the last injection animals were assessed for Transfer latency (TL) and step down latency (SDL) cognitive tasks. In the end of the study inflammatory markers were investigated in different brain regions viz. hippocampus and cortex tissues using ELISA kits. Expression of NF- κ B was analyzed using immunohistochemistry.

Results: In TL ATV significantly enhances memory when compared to vehicle+ PTZ group ($p < 0.001$) and in step down latency test it reduces memory dysfunction ($p < 0.05$). ATV suppressed the production of IL-1, IL-1Ra, and TNF- and IL-6 levels when compared to vehicle +PTZ group ($P < 0.001$). However, NF- κ B activation was controlled by ATV administration.

Conclusions: Here we conclude that ATV treatment mitigates the NF- κ B expression thereby attenuating IL-1, IL-1Ra, IL-6 and TNF- level. ATV suppressed neuroinflammation through curbing the over expression of these cytokine levels. Our finding suggests ATV as a potent anticonvulsant candidate during experimental epileptogenesis.

Proteome profile of IL-17 and IL-18 in blood serum, cerebrospinal fluid and conditioned media of BM-MSC culture of ALS patients A

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Cytokines such as interleukin/IL/-17 and 18 are well known proinflammatory factors taking part in the inflammatory response of many neurodegenerative disorders, for instance amyotrophic lateral sclerosis (ALS). Therefore, the purpose of this study was to investigate the proteome profile of IL-17 and IL-18 in blood serum, cerebrospinal fluid (CSF) and conditioned media collected from bone marrow-derived mesenchymal stem cell (BM-MSC) culture of ALS patients.

Blood and CSF samples as well as bone marrow were drawn from 4 patients suffered from amyotrophic lateral sclerosis. Cytokine proteome profile has been checked in blood serum, CSF and conditioned media of BM-MSC culture (after first passage). To measure IL-17 and IL-18 level in the above-mentioned fluids, the Human XL Cytokine Array Kit (R&D Systems, Minneapolis, MN, USA) has been used. The pixel density was determined using Image software (NIH, Bethesda, MA, USA). Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by the Bonferroni test and statistical significance was defined as a p-values below 0.05.

The level of IL-17 in the conditioned media of BM-MSC culture was greater ($P < 0.05$) than in the blood serum and CSF. In turn, the highest ($P < 0.001$) amount of IL-18 was found in the blood serum, compared to CSF and conditioned media. Our preliminary report shows that increased level of IL-17 in conditioned media of BM-MSC culture may indicate on immunomodulatory properties of this cytokine. Moreover, increased level of IL-18 in blood serum of ALS patients suggests the importance of this factor in pathogenesis of ALS. However, further studies were undertaken in this direction.

REGULATION OF NOD-LIKE RECEPTORS AND INFLAMMASOME ACTIVATION IN CEREBRAL ENDOTHELIAL CELLS

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Cerebral endothelial cells forming the BBB (blood-brain barrier) are at the interface of the immune and the central nervous systems and thus may play an important role in the functional integration of the two systems. Here we investigated how brain endothelial cells recognize and respond to pathogen- and damage-associated molecular patterns in order to regulate the functions of the neurovascular unit. First we detected the expression of several NLRs – including NOD1, NOD2, NLRC4, NLRC5, NLRP1, NLRP3, NLRP5, NLRP9, NLRP10, NLRP12, NLRA and NLRX – in brain endothelial cells. Inflammatory cytokines, such as IFN-gamma, TNF-alpha, and IL-1beta had stimulatory effect on the transcription of many of these receptors. Expression of key inflammasome components (NOD2, NLRP3 and caspase-1) along with inflammasome-activated IL-1beta could be induced by priming with lipopolysaccharide (LPS) and activation with muramyl dipeptide (MDP). In addition, combined treatment with LPS and MDP resulted in IL-1beta secretion in a caspase- and ERK1/2 kinase-dependent manner. Our findings demonstrate that NLRs and inflammasomes can be activated in cerebral endothelial cells, which may confer a yet unexplored role to the BBB in neuroimmune and neuroinflammatory processes. Supported by NKFIH-OTKA K-116158.

Removed perineuronal nets and damaged, but persisting GABAergic neurons in the ischaemia-affected nucleus reticularis thalami of wildtype and 3xTg mice

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Currently, the treatment of ischaemic stroke is limited to intravenous thrombolysis and mechanical thrombectomy as recanalizing approaches. The urgently requested translation of additional treatment options requires clinically relevant stroke models which should consider co-morbidities, age-dependency of neuropathological alterations and the concept of the neurovascular unit (NVU).

Main components of the NVU are neurons, vessels, glial cells and the extracellular matrix (ECM) with perineuronal nets (PNs). Such chemically complex, highly anionic and chondroitin sulphate proteoglycan-rich PNs ensheath frequently fast-firing GABAergic neurons. While they were characterized by numerous studies in the naive brain, the role of PNs during ischaemia and as potential target for stroke treatment is hardly understood. The present study is therefore focused on stroke-induced alterations of PNs and GABAergic neurons with emphasis on the nucleus reticularis thalami (NRT).

Focal cerebral ischaemia in mice was induced by unilateral occlusion of the middle cerebral artery, resulting in clinically relevant, predominantly striatal lesions. For these experiments we used 3- and 12-month-old wild-type mice and co-morbid triple-transgenic (3xTg) mice displaying age-dependent Alzheimer-like alterations. All animals were perfused 1 day after ischaemia onset, and frozen coronal sections comprising the NRT were applied to double fluorescence labelling of biotinylated *Wisteria floribunda* agglutinin (WFA) as established marker for PNs and the calcium-binding protein parvalbumin in fast-firing GABAergic neurons. Subsequent semiquantitative analyses comprised 28 animals and revealed staining intensities, staining areas as well as cell counts for WFA and parvalbumin.

A drastic decline of WFA-staining was found in the ischaemia-affected NRT when compared with the contralateral, non-affected side. Concomitantly, ischaemia-affected parvalbumin-immunoreactivity decreased to a much lesser degree. Further immunostaining demonstrated the ischaemia-induced loss of aggrecan and neurocan in PNs and allocated neuropil. Additional multiple immunofluorescence labelling detected apparently damaged GABAergic neurons in the ischaemic NRT, persistently expressing the calcium-binding proteins parvalbumin and calbindin, the potassium channel subunit Kv3.1b and the glutamate decarboxylase isoforms GAD65 and GAD67 as their marker enzymes.

Taken together, our data show PNs as highly vulnerable constituents of the ECM under ischaemic conditions. In conclusion, components of the extracellular matrix appear as promising targets of future neuroprotective strategies in acute ischaemic stroke. Such approaches might also include ECM-degrading enzymes such as metalloproteinases, chondroitinases and aggrecanases.

Role of dopamine agonists in mitochondrial dysfunction mediated focal cerebral ischemia in rodents

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Ischemic stroke results in extensive brain injury and is a substantial medical burden because of its ensuing morbidity and mortality worldwide. Alterations in mitochondrial permeability transition and organelle damage are key players in the development of the cerebral ischemic tissue injury due to associated modifications in ATP turnover and cellular apoptosis/necrosis. Early restoration of blood flow and improvement of mitochondrial function might reverse the situation and help in recovery following stroke onset. Mitochondria and related bioenergetics can be effectively used as pharmacological targets. Dopamine-D2-agonists have been found neuroprotective in various models of neurological diseases through number of mechanisms. This large data prompts us to investigate the possible role of these agonists in mitochondrial mediated neuroprotective mechanism in ischemic stroke of rat. In this study, we have shown the positive effect of these agonists administration behavioral deficits and mitochondrial health in ischemic/stroke injury model of tMCAO. Findings showed a significant improvement in dopamine agonists treated animals in comparison to the only tMCAO group. 2, 3, 5- triphenyltetrazolium chloride staining of isolated brain slices from dopamine agonists treated rats showed a reduction in the infarct area in comparison to vehicle group indicating presence of more viable mitochondria. The treatment was also able to attenuate the mitochondrial ROS as well as modulated the mitochondrial permeability transition pore (mPTP) in the tMCAO injury. We conclude that, dopamine agonists might act as a potential therapeutic agent for designing pharmacological target in ischemic stroke.

Role of glial NF- κ B signalling in IL-1 β mediated central effects

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Infections and inflammatory disorders lead to autonomic (e.g. fever) and behavioural (e.g. anorexia and immobility) responses. Inflammatory cytokines, such as IL-1, IL-6 and TNF are produced and activate the proinflammatory transcription factor nuclear factor kappa B (NF- κ B).

However, it is not known which cell types in the central nervous system are responsible for fever and sickness behaviour as well as how the communication between the peripheral and central compartments is organized.

To investigate the relevance of glial NF- κ B signalling in IL-1 induced CNS responses, we generated glial knockouts for the NF- κ B essential modulator (Nemo, Nemo^{gliaKO}). The effects of IL-1 on food intake, body temperature, brown adipose tissue (BAT) thermogenesis and body weight were tested in wild-type and Nemo^{gliaKO} mice.

IL-1 administration activated the NF- κ B pathway mostly in tanycytes, a glial subpopulation in the ventricular wall of the mediobasal hypothalamus, as shown by an upregulation of NF- κ B target genes in tanycytes. In parallel, IL-1 transiently increased the body temperature and the temperature of the BAT, suggesting an increase in thermogenesis. In Nemo^{gliaKO} mice, the effects of IL-1 were abolished. IL-1 stimulation in Nemo^{gliaKO} mice did not increase body temperature and the activation of the BAT was normalised.

We conclude that IL-1 activates the NF- κ B pathway in tanycytes, which might contribute to the function of tanycytes in the fever and sickness response during inflammatory diseases.

Role of the anti-inflammatory cytokine IL-37 in the brain

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The anti-inflammatory cytokine Interleukin-37 (IL-37) is a member of the Interleukin-1 (IL-1) family. It is structurally homologous to the IL-18 protein, an important modulator of immune responses, and is able to bind to its receptor. Of interest, IL-37 is the only member of the IL-1 family that is lacking an identified murine homolog. However, a transgenic mouse model has been described that expresses human IL-37 (hIL-37tg) and allows to study this cytokine *in vivo*, since the human protein is functional in the mouse. Studying hIL-37tg animals in combination with a variety of mouse models of innate immune activation revealed that IL-37 in splenocytes decreases during an immune reaction the expression of pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α , while the secretion of anti-inflammatory cytokines is increased. These results suggest an immune modulatory role of IL-37, by which this cytokine is able to reduce inflammation and may act as an important suppressor of innate immunity. Recently, it has been shown, that there are beneficial effects of hIL-37 after spinal cord injury. It could be demonstrated that hIL-37tg mice show reduced numbers of activated microglia and macrophages. Furthermore, functional deficits (locomotor skills) and tissue damage (neuronal sparing) were decreased in these mice. These results open the question for the role of IL-37 in the central nervous system (CNS).

In this study we investigated the potential functions of IL-37 in the brain. Using the hIL-37tg mouse strain, we explored the effects of IL-37 on cytokine production, neuronal architecture and behavior. Robust expression of hIL-37 in the CNS of transgenic animals was induced by intraperitoneal (i.p.) injections of LPS in transgenic animals. The morphology of individual Dil labeled dendrites of the hippocampus was analyzed in 400 μ m brain slices isolated from the naïve and the neuroinflamed CNS of hIL-37tg animals and non-transgenic littermates. Our results show no differences in spine density of hippocampal neurons between wildtype mice compared to hIL-37tg mice in the naïve brain. However, by stimulating the immune system with i.p. applications of LPS, wildtype mice showed a reduced spine density, whereas in contrast hIL-37tg mice exhibited no spine loss. Currently, we are extending these studies on the structural aspects of neuronal plasticity by measurements of long-term potentiation (LTP), which will allow us to analyze functional changes of synaptic plasticity in the naïve and the neuroinflamed brain of IL-37tg animals. In addition, Morris Water Maze tests are used to determine potential effects of transgenic IL-37 expression on spatial learning and memory. Finally, we are assessing the impact of recombinant IL37 expression in the rodent brain on microglial cells by flow-cytometric measurements of cell surface markers and by the ability of this cell type to express and secrete cytokines which are associated with an ongoing neuroinflammatory processes.

The comparison of glucose, lipid and nitric oxide metabolism parameters between schizophrenic patients with metabolic syndrome and internal medicine patients with metabolic syndrome

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Objective. Incidence rates of metabolic syndrome (MetS) are significantly higher in patients with schizophrenic compared to the general population. The differences in MetS features representing an increased risk of developing cardiovascular diseases in schizophrenic patients in comparison to not psychiatric patients has not been systematically investigated. The objective of this study is to evaluate the differences in cardiovascular biomarkers including glucose, lipid and nitric oxide metabolism parameters in MetS among a group of schizophrenic patients and a group of internal medicine patients.

Methods. We enrolled 70 schizophrenic patients with MetS who were taking antipsychotic drugs from the Mental Health Research Institute of Tomsk National Research Medical Center of the Russian Academy of Sciences (Tomsk, Russia) and 155 internal medicine patients with MetS treated in the Kaliningrad Regional Clinical Hospital (Kaliningrad, Russia). In all patients the presence of MetS was established according to International Diabetes Federation (IDF) criteria (2005). Anthropometric (body mass index (BMI), waist and hip circumference, waist to hip ratio) and biochemical parameters (fasting blood glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride and nitrite levels) were evaluated in both samples.

Results. Internal medicine patients with MetS showed significantly higher BMI and waist circumference as compared with schizophrenic patients with MetS (Table 1). Fasting glucose levels were also higher in internal medicine patients with MetS than in schizophrenic patients with MetS. In contrast, lipid metabolism parameters in schizophrenic patients with MetS (higher total and low-density lipoprotein cholesterol levels, lower high-density lipoprotein cholesterol levels) differed from those in internal medicine patients with MetS. Serum nitrite levels were significantly higher in schizophrenic patients with MetS as compared with internal medicine patients with MetS as well.

Conclusion. Lipid metabolic alterations involved in MetS development were more highly expressed in schizophrenic patients than in internal medicine patients. Antipsychotic therapy probably contributes to observed lipid abnormalities and increased serum nitrite levels in schizophrenic patients with MetS.

Acknowledgments. This study was supported by grant 17-04-00735 A from the Russian Fund of Fundamental Research.

Table 1. Comparison of anthropometric and biochemical characteristics in studied samples

Variables	Schizophrenic patients with MetS (n = 70)	Internal medicine patients with MetS (n = 155)	p level
Age (years)	46 (36; 58)	44 (38; 50)	0.1398
Male (n, %)/Female (n, %)	19 (27.14%)/51 (72.86%)	60 (38.71%)/ 95 (61.29%)	0.0924
Body mass index(kg/m ²)	31.99 (30.86; 36.72)	40.10 (34.80; 45.20)	<0.0001
Waist circumference (cm)	108 (92; 112)	113 (105; 122)	0.0376
Hip circumference (cm)	108.5 (89; 119)	120 (112; 133)	0.0591
Waist-to-hip ratio	1.00 (0.86; 1.11)	0.92 (0.85; 1.00)	0.4608
Fasting glucose (mmol/l)	5.60 (5.30; 5.80)	6.20 (5.70; 6.70)	0.0105
Total cholesterol (mmol/l)	5.60 (4.80; 5.90)	4.41 (3.78; 5.00)	0.0004
High-density lipoprotein cholesterol (mmol/l)	0.90 (0.88; 1.00)	1.15 (0.95; 1.30)	0.0367
Low-density lipoprotein cholesterol (mmol/l)	3.45 (3.10; 4.50)	2.64 (2.14; 3.17)	0.0001
Triglycerides (mmol/l)	1.50 (1.06; 1.80)	1.26 (0.84; 1.85)	0.3491
Nitrites (mcmmol/l)	30.73 (4.52; 45.78)	3.86 (3.20; 4.93)	0.0008

Values of quantitative traits are median (quartile 1, quartile 3).

The orphan cytokine receptor CRLF3 is involved in erythropoietin induced neuroprotection in *Tribolium castaneum*

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The cytokine erythropoietin (Epo) is well known for its role in erythropoiesis in vertebrates, but it also mediates potent neuroprotective functions in both vertebrates and insects. However, different Epo receptors seem to mediate erythropoiesis and neuroprotection, opening the possibility to stimulate both functions independently with ligands that differentially activate one or the other receptors. While erythropoiesis is promoted by the “classical”, homodimeric Epo receptor (EpoR), neuroprotection relies on tissue-specific Epo receptors, which may either be heteromeric complexes including units of classical EpoR or completely different receptor proteins. Since some alternative Epo receptors, e.g. EpoR/beta common chain receptor complexes or ephrine B4 receptor, have been shown to transduce protective effects in some cell types but not in others, expression of tissue-protective Epo receptors might be specific to cell type, developmental status and type of physiological challenge.

We studied whether the human orphan cytokine receptor-like factor 3 (CRLF3) plays a role in Epo-mediated neuroprotection. Like classical EpoR, CRLF3 is a member of the cytokine type I receptor family that mediates its cellular functions through activation of JAK/STAT signaling pathways.

Previous studies demonstrated that Epo-mediated neuroprotection in mammals and in insects shares similar transduction mechanisms. Hence, tissue protection may be an ancient beneficial function of Epo-like signaling which evolved in a common ancestor of vertebrates and insects and later became adapted for erythropoiesis in vertebrates. We found a single orthologue of the human orphan cytokine receptor CRLF3 in the beetle *Tribolium castaneum* (*TcCRLF3*) that shares major predicted functional domains and ~ 28% amino acid sequence similarity with its human and mouse orthologues. In order to investigate its contribution to neuroprotection, we established protocols for primary brain cell cultures from *T. castaneum* late pupal stages and *in vitro* RNA interference with double stranded RNA (dsRNA) constructs applied to the culture medium (“soaking RNAi”) to knock down protein expression in cultured neurons. We first demonstrated that recombinant human Epo (rhEpo) and the neuroprotective but non-erythropoietic human Epo splice variant EV-3 significantly increased the survival of serum-deprived *T. castaneum* brain cells. Moreover, rhEpo (0.8 ng/ml) completely prevented neuronal apoptosis induced by 36 h exposure to hypoxia. Pre-treatment with dsRNA targeting the expression of the *TcCRLF3* orthologue completely abolished the neuroprotective effect of rhEpo. Potential off-target effects by RNAi-mediated suppression of additional proteins besides *TcCRLF3* were excluded by conducting two series of experiments with different dsRNA construct targeting different non-overlapping fragments of the *TcCRLF3* transcript. Our experiments show that the orthologue of the human orphan cytokine receptor CRLF3 is crucial for Epo-induced neuroprotection in *T. castaneum*. Whether mammalian CRLF3 also serves as a neuroprotective Epo receptor, which may be selectively targeted by Epo variants without erythropoietic activity, remains to be studied.

Poster Topic

T13: Cognitive, Emotional, Behavioral State Disorders and Addiction

- [T13-1A](#) Anxiety-related behavior in Cre-mediated inducible Tph2 knockout (icko) mice
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- [T13-4A](#) Bipolar disorder: Neurobiological mechanisms in a virus-induced animal model
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- [T13-6A](#) *Cacna1c* haploinsufficiency leads to a developmental delay in the emission of isolation-induced ultrasonic vocalizations in rat pups
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- [T13-7A](#) Effect of Lithium in the Glutamine synthetase (GS)- reporter mouse
Charlotte Mezö, Dominik K.E. Beyer, Andreas Fallgatter, Michael Schwarz, Nadja Freund
- [T13-8A](#) Emotional regulation and social behavior: effects of oxytocin
Olga Lopatina, Yulia K. Komleva, Yana V. Gorina, Anna A. Shabalova, Alla B. Salmina
- [T13-9A](#) EFFECTS OF SHORT-TERM NEONATAL HYPERTHERMIA IN KRUSHINSKY-MOLODKINA AUDIOGENIC SEIZURE PRONE RAT STRAIN.
Irina Fedotova, Natalya Surina, Georgy Nikolaev, Zoya Kostina, Inga Poletaeva
- [T13-1B](#) Effects of CB1 receptors in the ventral tegmental area on the potentiation of morphine rewarding propertiesA
Leila Zarepour

- [T13-2B](#) Effects of selective deletion of the gamma 2 subunit of GABAA receptor on the neuronal activity of dopaminergic cells
Aleksandra Trenk, Magdalena Walczak, David Engblom, Tomasz Blasiak
- T13-3B moved to [T13-9A](#)
- [T13-4B](#) Functional Network Differences between the ADHD and Normal Groups
Reza Khanbabaie, Masood Nemati Andavari, Ali Asgharnia, Mina Asadifar, Amirhossein Ghaderi, Mohamadali Nazari
- [T13-5B](#) Haploinsufficient *Cacna1c* rats display increased anxiety-related behavior, impaired sensorimotor gating, and alterations in inflammatory markers
Moria Dening Braun, Theresa M. Kisko, Clara Raithel, Tobias M. Redecker, Christine Hohmeyer, Marcella Rietschel, Stephanie Witt, Rainer K. W. Schwarting, Holger Garn, Markus Wöhr
- [T13-6B](#) Hippocampal disruption of NOS-I PDZ-interaction: Effects on learning and memory
Florian Freudenberg, Esin Candemir, Aet O`Leary, Lena Grünewald, Miriam Schneider, Andreas Reif
- [T13-7B](#) Knockdown of the ADHD Candidate Gene *Diras2* in murine neuronal primary cells
Lena Grünewald, Florian Freudenberg, Christoph Schartner, Heike Weber, Claus-Jürgen Scholz, Andreas Reif
- [T13-8B](#) MORC1, a gene associated with early life-stress and depression - A study in the rodent brain
Annakarina Mundorf, Nadja Freund
- [T13-1C](#) NEUROPEPTIDE S RECEPTOR-DEFICIENT MICE ARE MORE PRONE TO DEVELOP PTSD-LIKE FEAR MEMORY AFTER CORTICOSTERONE INJECTIONS.
Malgorzata Helena Kolodziejczyk, Markus Fendt
- [T13-2C](#) Periaqueductal gray/ dorsal raphe dopamine neurons control associative learning of fear
Florian Grössl, Thomas Munsch, Susanne Meis, Johannes Griessner, Pinelopi Pliota, Dominic Kargl, Sylvia Badurek, Klaus Kraitsy, Arash Rassoulpour, Volkmar Lessmann, Wulf Haubensak
- [T13-3C](#) Post-weaning social isolation results in ultrasonic communication deficits, cognitive impairments and alterations in microRNA-dependent *Ube3a1* function on neuronal plasticity in rodents: Implications for autism
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- T13-4C Retracted
- T13-5C Retracted
- [T13-6C](#) Self-regulatory behavior of rats being on different hierarchical level in chronic psychogenic stress model
Tamar Domianidze, Tamar Matitaishvili, George Burdjanadze, Mikheil Khananashvili

- [T13-7C](#) Serotonin Transporter Dependent Activation of the Amygdala after Negative Stimuli: A fMRI Study in 5-HTT Knockout Mice
Jann Frederik Kolter, Markus F. Hildenbrand, Stephan Nauroth, Julian Bankmann, Klaus-Peter Lesch, Peter M. Jakob, Angelika G. Schmitt_Böhrer
- [T13-8C](#) Sex-dependent Effects of Cacna1c Haploinsufficiency on Juvenile Social Play Behavior and 50-kHz Ultrasonic Vocalizations in Rats
Theresa Marie Kisko, Moria D Braun, M Bartz, A Pützer, C Hohmeyer, M Rietschel, SH Witt, Rainer KW Schwarting, Markus Wöhr
- [T13-1D](#) Social impairments, olfactory dysfunction, and inattention in neuronal nitric oxide synthase (Nos1) knockdown mice
Aet O`Leary, Florian Freudenberg, Esin Candemir, Lena Grünewald, Andreas Reif
- [T13-2D](#) Stress-induced aggression in mice and evidence for preventive effects of drugs with pro-neurogenetic activity
Nataliia Bazhenova, Jonas Waider, Dolores Bonopartes, Ekaterina Veniaminova, Nataliia Markova, João Costa-Nunes, Evgeniy Zubkov, Anna Gorlova, Dmitii Pavlov, Anna Morozova, Klaus-Peter Lesch, Tatyana Strelkova
- [T13-3D](#) Sustained effect of ketamine is mediated by homeostatic regulation of synaptic function and reconfiguration of gene expression
Debarpan Guhathakurta, Santosh Pothula, Anna Fejtova
- [T13-4D](#) The effect of arsenic exposure on learning and memory in rats of various age groups
Tamar Bikashvili, Tamar Lordkipanidze, Nana Gogichaishvili, Nino Pochkhidze
- [T13-5D](#) The regulatory role of trace amine-associated receptor 1 in acute and chronic effects of nicotine
Mariia Dorofeikova, Antonina Dolgorukova, Artem Dorotenko, Raul R. Gainetdinov, Ilya Sukhanov
- [T13-6D](#) Time-dependent modulation of visual motion prediction in humans.
Motoharu Takao
- [T13-7D](#) Trace Amine-Associated Receptor 1 agonist attenuates adjunctive water drinking in rat model of compulsive behavior
Artem Dorotenko, Antonina Dolgorukova, Raul R. Gainetdinov, Ilya Sukhanov
- [T13-8D](#) First report of interesting awake craniotomy of a famous musician in history; The suprasellar tumor surgery of Pianist Clara Haskil in 1942 without general anaesthesia
Elena Romana Gasenzer, Ayhan Kanat, Edmund A. M. Neugebauer

Anxiety-related behavior in Cre-mediated inducible Tph2 knockout (icko) mice

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Background: Dysregulation in serotonergic neurotransmission has been implicated in mood-related disorders in humans and laboratory animals. However, the molecular mechanisms of serotonin (5-Hydroxytryptamine; 5HT) function as well as specific time point for the development of such neuropsychiatric conditions are yet to be fully explored. Tryptophan hydroxylase 2 (Tph2) is the rate limiting enzyme in the 5HT biosynthetic pathway and plays an important role in the regulation of 5HT function. Inducible Cre-mediated deletion of Tph2 genes selectively in 5HT neurons of all raphe nuclei in transgenic mice provides the instrument for molecular dissection of Tph2 gene function during early and late periods of life.

Aim: Characterization of a mouse model for acute brain serotonin depletion on anxiety.

Method: Mice (10-12weeks old) expressing Cre-recombinase under the serotonergic cell specific Tph2 promoter and with the Tph2 gene flanked by lox P sites were injected with tamoxifen (2mg, daily for 5 consecutive days). The level of 5HT in the brain was measured by High performance Liquid Chromatography (HPLC) after injection. The mice were tested for anxiety-related behaviour in the light-dark-box and open-field arena.

Results and Discussions: HPLC analysis of different brain regions, indicated complete gene recombination. However, immunofluorescent staining showed up to 83% reduction in 5-HT immunoreactive neurons in the raphe nuclei. This resulted in significantly increased exploratory and reduced anxiety-like behavior in Open-Field test but no significant differences in the Light-Dark-Box.

Conclusion: Cre-mediated inducible Tph2 deletion may be able to model the impact of central 5HT depletion on anxiety-like behavior in a time-specific manner.

Behavior of dominant and submissive rats in the chronic informational stress and depression model

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Modern society relations and problems, such as a variety of socio-political, economic and other environmental factors under which the people permanently live and work leads to serious mental disorder problems. For prevention and effective treatment of human like mental disorders, it is important to use animal models in order to study and correct physiological, biochemical and behavioral changes of rats at different stages of the developed mental disorders. According to the numerous experimental studies, chronic psychogenic stress which develops in response to long-term, moderate stress is the initiating agent causing psycho-neural diseases including depression. We used “informational” stress model for the purpose of modeling chronic psychogenic stress and depression. The aim of the research was to study behavior of rats on various hierarchical level at different stages of “informational” stress.

Model of “informational” stress is a modified version of active avoidance reaction. Initially, we instigated in rats active avoidance reaction towards conditional signal metronome and then towards tone. After development of active avoidance reactions separately towards metronome and tone, testing of 2 active avoidance reactions was implemented during one experimental session. Under high defensive motivation background, limited time and a lack of pragmatic information the animal (rat) was unable to fulfill adequate behavior, which led to the development of “informational” stress. The rats were subject to day-to-day stressing procedure within 45 days. In order to identify dominant and submissive animals in small groups of rats we used two methods enabling the stronger animal to gain a victory during food and water obtaining process. In order to study anxiety, depression and aggressive behavior of rats we used “forced swim”, “elevated cross maze”, “open-field” and “muricidal” tests. We determined the concentration of serotonin in hypothalamus, corticosterone and testosterone in plasma of rats. The obtained results showed that the condition of “informational” stress led to the increased anxiety and aggressive behavior in rats. Chronic “informational” stress developed depression both in dominant and submissive rats.

Research work is supported by Shota Rustaveli National Science Foundation grant FR/260/7-270/13

Behavioural, molecular and metabolic consequences of cholesterol-enriched diet and ameliorating effect of dicholine succinate

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The “Western diet”, a diet enriched with fat and cholesterol, is associated with obesity and development of hypercholesterolemia, insulin resistance, metabolic syndrome, type II diabetes and numerous other medical conditions including affective disorders. In our study we used the model of non-alcoholic fatty liver disease (NAFLD) that can be induced in female mice by feeding with cholesterol-enriched diet during three weeks. Our aim was to characterize behavioral profile, inflammatory state and metabolic changes of mice housed on high cholesterol diet. It was found that in NAFLD model a cholesterol-enriched diet evokes numerous behavioral changes: anxiety- and depressive-like behaviours, impulsivity, abnormal social interactions during group housing and food competition, deficient hippocampus-dependent performance in the contextual fear conditioning and food pellet displacement paradigms, and reduced object exploration. Dietary-challenged mice demonstrated elevated levels of blood cholesterol and leptin, impaired glucose tolerance, hepatic dystrophy and triglyceride accumulation, increase in gene expression of inflammatory marker, Toll-like receptor 4 (TLR4), and decrease in expression of mitochondrial activity marker, the peroxisome proliferator-activated-receptor-gamma-coactivator-1-beta (PPARGC1b), in brain and liver and decrease in peroxisome proliferator-activated-receptor-gamma-coactivator-1-alpha (PPARGC1a) expression in brain. In the present study we also investigated whether dicholine succinate (DS), a mitochondrial complex II substrate that enhances the effects of insulin on its receptor and therefore is regarded as its sensitizer, can interfere with the effects of cholesterol-enriched diet. Dosing with DS for the three-week period of dietary intervention normalized depression- and anxiety-related behaviours and gene expression of TLR4 and PPARGC1b and prevented NAFLD-associated decrease of glucose tolerance. In summary, our study demonstrates that exposure of mice to high amounts of dietary cholesterol can result in depression- and anxiety-like changes, impulsivity, social avoidance, deficits in hippocampal plasticity, brain and liver inflammation and metabolic disturbances. Based on the data of dicholine succinate effects in our model, it can be suggested that enhancement of insulin receptor mediated signalling is a promising approach in therapy of affective disturbances and metabolic consequences of NAFLD.

Bipolar disorder: Neurobiological mechanisms in a virus-induced animal model

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Bipolar disorder is characterized by a switch between manic and depressive episodes. Recently, Freund and colleagues¹ developed a lentiviral-induced animal model that is able to model both phases in one animal. By manipulating the dopamine D1 receptor (D1R) in rats' medial prefrontal cortex (mPFC) they were able to induce either mania- or depressive-like behavior one after the other. Lentiviral overexpression of the D1R in rats' PFC induced mania-like behavior whereas termination of this D1R overexpression resulted in depressive-like behavior.

For a better understanding of the neurobiology connected to the observed behavior we now analyze the changes in the brain accompanied by induced mania- or depressive-like behavior. We quantified the gene expression of D1R in rats' mPFC via quantitative real-time PCR in our animal model with the D1R overexpression and after the termination of the overexpression compared to controls. First experiments indicated that D1R expression following termination after D1R overexpression decreases to baseline level with no differences between animals after D1R overexpression and controls. Therewith the depressive-like behavior cannot be explained by reduced D1R gene-expression after the termination of the overexpression. Further investigation of gene expression patterns in several depression associated brain regions, like nucleus accumbens, dorsal raphe nucleus, hippocampus and ventral tegmental area will give evidence if downstream effects contributed to the observed behavioral changes.

Mass spectrometry revealed that the overexpression of D1R and its termination induced several protein level changes within the mPFC. A number of proteins could exclusively be detected in all tissue samples of one specific group (animals with D1R overexpression; after the overexpression and respective controls). Within the nearly 2000 proteins that were detected in all samples 49 proteins significantly differed in their expression levels depending on group and if virus was expressing or if expression was stopped. Identification of these proteins and their common pathways will help to improve our understanding of the changes in the brain, which cause the behavioral changes resembling the dramatic alternations in mood observed in patients with bipolar disorder. Our big goal is to improve and fully establish this animal model for bipolar disorder to advance our understanding of cause, environmental influences and neurobiology of the human condition.

¹ Freund, N., Thompson, B. S., Sonntag, K., Meda, S. and Andersen, S. L. (2016). "When the party is over: depressive-like states in rats following termination of cortical D1 receptor overexpression". *Psychopharmacology* 233: 1191 - 1201

Both, early phase and later phase of life affect neuronal morphology in serotonin transporter deficient mice

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Stress is a risk factor for developing psychiatric disorders, e.g. anxiety disorders and depression. Several hypotheses postulate that the interaction between early and later life stress is crucial for the development of psychopathology. Two currently discussed hypotheses deal with that topic: the allostatic load, and the mismatch hypothesis. The former considers the accumulation of environmental adversity over the lifetime as the major risk factor. The latter postulates highest vulnerability to diseases when there is a mismatch between the individual's experience during early and later phases of life. Moreover, variants of serotonergic system genes interact with life events moderating the susceptibility and/or resilience for psychiatric disorders. 5-HTT knockout (KO) mice display increased anxiety-like behavior compared to wildtype (WT) mice, per se.

Bodden and coworkers (2015) showed effects of four different life histories (with matching and mismatching situations) on the behavior of mice varying in 5-HTT genotype.

Using Golgi-stained sections of these mice we analyzed the morphology of pyramidal-like neurons in the lateral amygdala and of the infralimbic cortex with the NeuroLucida system (MicroBrightField).

In the lateral nucleus of the amygdala we revealed statistically significant early phase of life by late phase of life by 5-HTT gene interactions on spine density of apical/basal dendrites with significant genotype differences exclusively in the group with adversity throughout whole life. In this group 5-HTT HET mice exhibit significantly lower spine densities compared to WT and to KO mice. In general, early adversity seems to promote the generation of more spines resulting in higher spine densities in basal dendrites. Surprisingly, late adversity seems to have the opposite effect with lower spine densities.

Early life experience matters - Late life experience matters. Adversity throughout life (cumulative stress) provokes different neuroadaptions to stress in the brain of mice with different 5-HTT genotypes.

***Cacna1c* haploinsufficiency leads to a developmental delay in the emission of isolation-induced ultrasonic vocalizations in rat pups**

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CACNA1C, especially the rs1006737 allele, is considered one of the best replicated vulnerability genes for affective disorders, including bipolar disorder, but also schizophrenia. Mutations in *CACNA1C* have further been identified to cause Timothy syndrome, whose features include autism spectrum disorder along with severe cardiac arrhythmia and developmental abnormalities. The gene encodes an alpha-1 subunit of the voltage-dependent L-type gate calcium channel Cav1.2, mediating depolarization-dependent calcium influx. Studies on the effects of *Cacna1c* deletions in genetically modified rodents on behavioral readouts with relevance to neuropsychiatric disorders are yet sparse. In particular, little is known about the effects of *Cacna1c* deletions on early developmental measures. Therefore, the aim of the present study was to describe the behavioral phenotype of *Cacna1c* haploinsufficient rat pups. To this aim, we used the newly generated *Cacna1c* rat model and compared behavioral phenotypes displayed by *Cacna1c* heterozygous (+/-) rat pups to wildtype (+/+) littermate controls with specific emphasis on ultrasonic vocalizations (USV). Rats emit distinct types of USV, with call emission strongly depending on developmental stage. Isolation-induced USV emitted by pups are mainly driven by separation from mother and littermates and it is believed that they serve important communicative functions in regulating mother-infant interaction, e.g., through eliciting maternal search and retrieval behavior. Isolation-induced USV often increase during the first week of life and decrease thereafter, giving rise to an inverted U-shaped pattern of call emission. In rodent models for neurodevelopmental disorders, such as autism spectrum disorder, this inverted U-shaped developmental call emission pattern is commonly delayed and/or distorted. Therefore, in the present study, isolation-induced USV were measured at postnatal days 5, 7, 9, and 11. The behavioral phenotyping approach further included the homing test as well as the assessment of early developmental milestones and somatosensory reflexes. In addition, a pup discrimination task was applied in order to test whether mothers display a genotype-dependent pup preference. Our results show that *Cacna1c*^{+/-} rats are viable, with the expected 50/50 genotype and sex ratios being evident in *Cacna1c*^{+/+} x *Cacna1c*^{+/-} breedings. At the behavioral level, the most prominent genotype effect was seen in the emission of isolation-induced USV. While *Cacna1c*^{+/+} littermate controls displayed the expected inverted U-shaped developmental call emission pattern, with call rate peaking at postnatal day 9, call emission was severely delayed in *Cacna1c*^{+/-} rat pups. Genotype effects on the acoustic features of isolation-induced USV as well as early developmental milestones and somatosensory reflexes were mild or absent. Moreover, in the homing test, both *Cacna1c*^{+/+} and *Cacna1c*^{+/-} rat pups displayed a clear preference towards the environment containing home cage bedding over fresh bedding material, reflecting intact social olfactory abilities and high levels of social motivation. Finally, mothers did not display a clear genotype-dependent pup preference. Together, our findings indicate that *Cacna1c* is involved in the developmental regulation of pup ultrasonic calling. The observed delay is consistent with a variety of rodent models for neurodevelopmental disorders. Funding: Deutsche Forschungsgemeinschaft (DFG; FOR 2107, WO 1732/4-1).

Effect of Lithium in the Glutamine synthetase (GS)- reporter mouse

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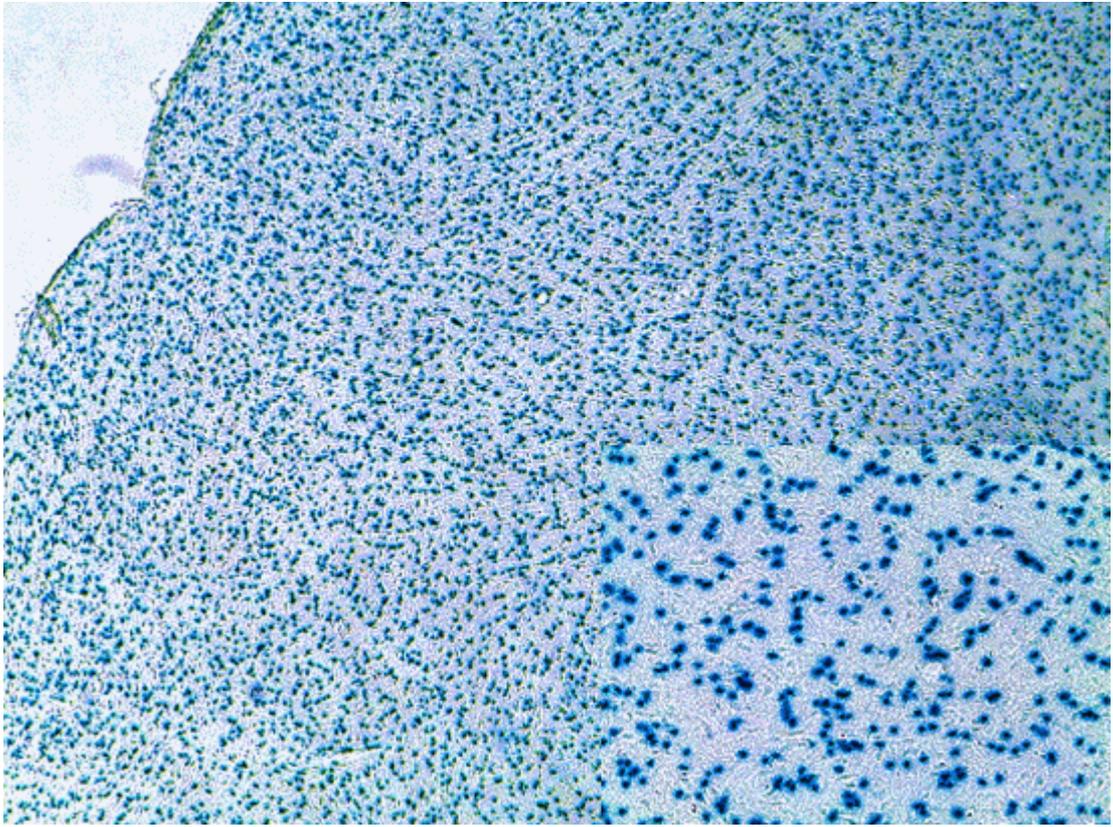
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About 1 to 5 percent of the people all over the world suffer from bipolar disorder (BD). The rate of patients, who commit suicide is dramatically high, it amounts about 10 to 15 percent. Therefore, it is important to find reliable and effective treatments. Lithium carbonate is one of the most successful treatment options for BD. In addition, several studies found that long-term treatment of BD patients with lithium carbonate reduces suicidal risk. Despite clear evidence of therapeutic value, the specific mode of action of lithium is still not completely understood. Potentially, lithium may act via modulation of β -catenin downstream signaling through inhibition of glycogen synthase kinase 3. A downstream target of this signaling pathway is glutamine synthetase (GS). To acquire a deeper understanding of lithium's neurobiological mechanisms, we investigated the effect of the drug on a GS enhancer/promoter-driven reporter gene in a respective transgenic mouse line. As reporter, lacZ, encoding β -galactosidase, was utilized.

In total, 57 three-month old male and female GS-reporter mice were equally and randomly assigned to one of three treatment groups. Group 1 was given a daily injection of 25mg/kg lithium carbonate, group 2 was given a daily saline injection and group 3 mice were untreated and served as no stress control. Weight of all animals was monitored every other day. After one week of treatment, all mice were transcardially perfused and their brains were postfixed, cryoprotected and sliced. To detect β -galactosidase positive cells, the indication of GS expression in the brain, serial slices were stained with 5-bromo-4-chloro-3-indolyl- β -D-galacto-pyranoside (X-Gal). Brain sections were imaged and the blue intensity was analyzed.

Our results showed a slight, but significant reduction in weight of lithium-treated animals, not seen in controls. Staining with X-Gal resulted in blue staining throughout the entire brain. Preliminary analysis of female brains showed no obvious differences in overall GS-reporter expression between groups. Additional analyses in males and comparisons of staining between specific brain regions as well as investigation of cell density and reporter activity in specific neuronal cell types are under way. We hope that the GS-reporter mouse model may help to improve our understanding of the neurobiological mechanisms of lithium, which may help to establish more effective medications against BD in the future.



Emotional regulation and social behavior: effects of oxytocin

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Regulation of emotional and social behavior in the appropriate physiological and social conditions require strict control secretion of neuropeptides. The hormone oxytocin is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and transported to the posterior pituitary which accumulates and is excreted into the blood and into the central nervous system. Oxytocin is involved in implementing various types of social behavior of animals and human, including sexual behavior, social interactions between individuals, maternal and paternal behaviors, the interaction of the mother with a newborns.

Molecular mechanisms of oxytocin-dependent behavioral responses may be associated with CD38/ADP-ribose cyclase - bifunctional receptor/enzyme found in many body tissues which catalyzes formation of cADP-ribose and NAADP. cADP-ribose and NAADP are endogenous mobilizers of intracellular Ca²⁺ and oxytocin release. Mice (due to their social and socialization, a high degree of homology to the human genome) is the most appropriate model to investigate brain plasticity and repair at the molecular-cellular level responsible for the development of brain adaptation in different environment experience. Studying the role of specific environmental factors in the pathology of the brain plays an important role in determining the molecular and cellular mechanisms of action of environmental stimuli.

Our research is aimed to study the changes in cognitive function, emotional state and social behavior in CD-1 mice kept for three weeks under different conditions: enriched environment, normal group housing or single isolation. We evaluated behavioral changes and plasma oxytocin levels dynamically one time per week during the experiment. At the end of different environment stimulations the full battery of behavior tests was performed. Brain samples (hypothalamus, pituitary) were collected to assess the level of oxytocin and ADP-ribose cyclase activity, oxytocin release from isolated nerve ending of hypothalamus and pituitary. We found environment-induced changes in oxytocin levels associated with development of behavioral patterns specific for all the conditions tested. And oxytocin treatment modulates behavioral responses in fear conditioning test.

EFFECTS OF SHORT-TERM NEONATAL HYPERTHERMIA IN KRUSHINSKY-MOLODKINA AUDIOGENIC SEIZURE PRONE RAT STRAIN.

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Audiogenic epilepsy (AE) is the development of epileptiform seizures in response to a loud sound (which include wild run stages as well as stages of clonic and tonic convulsions). The physiological mechanisms of AE proneness were investigated with considerable details, while the biological basis of this pathology is less well understood. The selection of Russian audiogenic prone rat strain KM (Krushinsky-Molodkina) started at the end of 1940 and the strain was extensively used as the model of epilepsy and catatonic muscle state. Practically 100% of KM strain animals develop the AE seizure of highest intensity with very short latency.

The remote effects of short (5 min) single hyperthermia (HPT) exposure (41-42 °) of 14 days old KM rat pups will be presented. This data were obtained in 4 series of experiments (AE seizures, being tested at the age of 1 and 4 months). The heating of animals was performed using either the infrared lamp (series 1, 2 and 4), or (series 3) by placing animals in the thermostat (41 °). The data were obtained for 85 rats in HPT group (48 males and 37 females) and for 96 control animals (42 males and 54 females). No significant differences between male and female groups in maximal AE seizure intensities were found at the ages of 1 and 4 months in both experimental and control groups and the respective data were pooled. At the age of 1 month part of animals was tested in the elevated plus maze (EPM) and their pain sensitivity thresholds (test tail flick) were determined. At the age of 1 month the animals from the HPT group developed significantly ($p < 0.01$) less intense AE seizures than control rats, while the proportion of animals with most intense seizures was significantly lower in HPT group, and the proportion of rats with lack of seizures in response to sound was higher in treated animals (at the age of 1 month not all KM rats develop maximal AE fit). The postictal catalepsy, which is characteristic for KM rats, was significantly larger in HPT group.

The pain sensitivity and elevated plus maze behaviour, tested at 1 month age, revealed significant differences from control group as well, which indicated the increased sensitization to pain in HPT animals together with increase in EPM test indices, which could be regarded as the plausible evidence of changes in anxiety. At the age of 4 months no significant differences between HPT and controls in AE proneness were found. The hyperthermia effects on seizures were previously analysed by other authors groups using status epilepticus model (subthreshold serial kainite or corazol injections), while the HPT effect on AE seizures after the single short term HPT was demonstrated in our study for the first time. The increase of body temperature as the stressor is accompanied by the increase of heat shock protein genes expression, which in turn could induce rather long-term physiological changes in CNS. The HPT used in this study could presumably induce changes in heat shock proteins expression, which (according to literature data) could be the cause of AE epileptiform seizures decrease.

Supported by RFBR (grant 15-04-01732) and the project NAAA-A16-116021660055-1.

Effects of CB1 receptors in the ventral tegmental area on the potentiation of morphine rewarding propertiesA

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Introduction: The ventral tegmental area (VTA) is a critical part of the brain reward system and has been engaged in mediating rewarding actions. CB1 receptors mediate the effects of cannabinoid and endocannabinoid in the central nervous system. Our aim is to determine the potentiating effects of CB1 receptors within the VTA during the acquisition of morphine-induced conditioned place preference (CPP).

Materials & Methods: Stereotaxic surgery was performed bilaterally on each rat to administrate WIN55,212-2 (1, 2 and 4 mmol/0.3µl DMSO) as CB1 receptor agonist and AM251 (15, 45 and 90 mmol/0.3µl DMSO) as CB1 receptor antagonist. In all of groups the CPP paradigm was done and CPP score was determined for each rat.

Result: The results showed that two doses of Win55,212-2 (2 and 4 mmol) potentiates the rewarding properties of ineffective dose of morphine (2 mg/kg). We did not see any significant difference between any other doses of Win55,212-2 and vehicle in the group which received the effective dose of morphine (5 mg/kg). Additionally, conditioning scores decreased significantly with the highest administrated dose of AM251 (90 mmol) compared to the vehicle group.

Discussion: Administration of cannabinoid agonist and ineffective dose of morphine concurrently induced morphine-CPP. It seems that the cannabinoid and opioid systems are in interaction with each other and also the pre-synaptic CB1 receptors existing in GABAergic neurons affect dopaminergic and/or non-dopaminergic neurons in the VTA. Additionally, blockade of CB1 receptors may increase GABA release and result in a decrease of acquisition of morphine-CPP in the rats.

Effects of selective deletion of the gamma 2 subunit of GABAA receptor on the neuronal activity of dopaminergic cells

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The dopaminergic system is involved in rewarding processes and many studies revealed that its dysfunction might lead to many disabilities, including drug addiction, depression and other psychiatric diseases. Activity of dopaminergic neurons is regulated by glutamate and GABA. The inhibitory input from GABAergic interneurons is mediated through GABA receptors located postsynaptically on DA neurons. Previously, it was shown that many drugs of abuse can exert indirect effects on dopaminergic system by inhibition of GABAergic cells. The aim of our study was to determine how disruption of GABAA receptors will influence the firing of dopaminergic neurons.

In our study we have used mice that in the adulthood underwent deletion of gamma 2 subunit of the GABAA receptor, selectively in the dopaminergic neurons. Extracellular in vivo recordings of DA-like cells' activity in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) were conducted in urethane anaesthetised mice. Iontophoretic application of GABAA receptor antagonist (bicuculline) and/or agonist (muscimol) was used to test the efficacy of GABAA mediated inhibition of DA-like neurons in control and mutant animals.

Our results show that DA-like neurons of mutant mice are characterised by higher total and extrabursts firing rates, comparing to control animals. Although DA-like neurons in mutant animals were still responsive to muscimol and bicuculline, their reaction to the drugs was significantly weaker comparing to control mice. Changes after drug application involved total firing in case of muscimol and total and extraburst firing in case of bicuculline. Bicuculline enhanced bursting activity in mutant and control animals, but bursts analysis shows that the genotype is not a differentiating factor.

It seems that Gabrg2 may be involved in many pathogenesis as lack of this subunit leads to changes in DA neurons activity. However, the deficiency of the gamma 2 subunit of GABAA receptor leads only to partial dysfunction of the receptor, because some response to administered drugs still remains. Thus it is plausible that Gabrg2 function in GABAA receptors might be substituted by other subunits.

Functional Network Differences between the ADHD and Normal Groups

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In this work we analyze the differences between the functional networks of two groups, Attention-Deficit Hyperactivity Disorder (ADHD) group and normal group. In recent years brain has been analyzed using complex systems and graph theories. One of these methods utilizes weighted graph of connectivity matrix which in this case, one of the methods of investigation is applying threshold to obtain conventional measures of graph theory. We use a new method by deriving minimum spanning tree (MST) graphs from connectivity matrices. This method has several advantages compared to previous methods including better accuracy (removing non-beneficial connections) and less errors (without human intervention for removing non-beneficial connections) [1]. Using MST a sub-graph of connectivity graph is calculated and then the graph measures for these MSTs will be determined. After recording data from individuals, we calculate the weighted connectivity matrix for multiple frequency bands using coherency. The graph measures determine the brain network structure in terms of integration and segregation [2]. There are two extreme shapes for MST. The first type is line-like and the second type is star-like. Four measures are important for the type of the tree: diameter, eccentricity, betweenness centrality (BC) and Leaf number. If an MST has longer diameter, higher eccentricity, lower leaf number and lower BC, it is more line-like configuration.

The resting-state EEG data was recorded for our normal group (14 people) and also for our ADHD group (13 people). The recording time for each individual was 5 minutes and 90 seconds of these data without artifact was selected and analyzed using EEGLAB. At the end the output of this analysis was statistically analyzed by SPSS software.

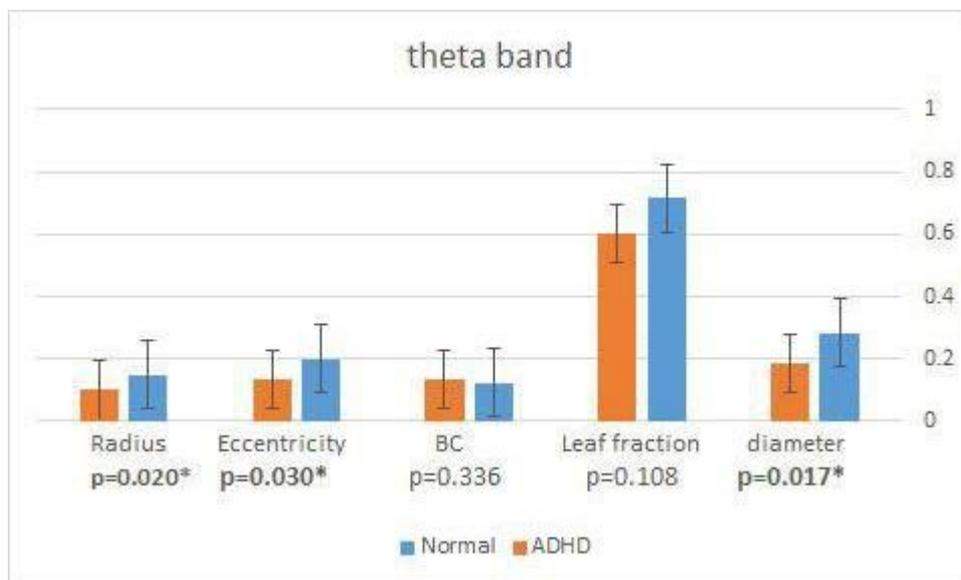
As a result we found some differences in theta band. These differences are significant statistically for three measures: diameter, eccentricity and radius. In this frequency band all measures were higher for normal group compared to ADHD group except for BC. In alpha band we see that the diameter, eccentricity and radius for ADHD group is more than normal, but the leaf number and BC for normal group is more than ADHD group.

[1] Stam CJ. Modern network science of neurological disorders. *Nat Rev Neurosci* 2014;15(10):683–95

[2] Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci* 2009;10(3):186–98

	ADHD	Normal	P-value
<i>δ band</i>			
Diameter	0.3429	0.3560	0.856
Leaf fraction	0.7287	0.7166	0.697
BC	0.1014	0.1010	0.956
Eccentricity	0.2608	0.2587	0.971
Radius	0.1781	0.1874	0.859
<i>θ band</i>			
diameter	0.1865	0.2844	0.017
Leaf fraction	0.6032	0.7166	0.108
BC	0.1335	0.1240	0.336
Eccentricity	0.1326	0.1995	0.030
Radius	0.0992	0.1489	0.020
<i>α band</i>			
diameter	0.3274	0.1966	0.090
Leaf fraction	0.5951	0.6015	0.844
BC	0.1355	0.1364	0.927
Eccentricity	0.2447	0.1429	0.094
Radius	0.1745	0.1069	0.099
<i>β band</i>			
diameter	0.1881	0.3258	0.610
Leaf fraction	0.6154	0.6278	0.667
BC	0.1324	0.1064	0.426
Eccentricity	0.1382	0.1660	0.636
Radius	0.1030	0.1691	0.629
<i>High β band</i>			
diameter	0.3236	0.3258	0.240
Leaf fraction	0.7166	0.6654	0.340
BC	0.1064	0.1169	0.185
Eccentricity	0.2574	0.2483	0.223
Radius	0.1691	0.1718	0.264

Table 1 Values of measures for all frequency bands. The bold numbers show that the differences are significant ($p < 0.005$).



Haploinsufficient *Cacna1c* rats display increased anxiety-related behavior, impaired sensorimotor gating, and alterations in inflammatory markers

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The neurobiological mechanisms ultimately resulting in the outbreak of affective disorders, i.e. major depressive disorder and bipolar disorder, are not fully elucidated yet. Genetic and environmental risk factors contribute critically to their etiology to varying degrees, but the exact pathophysiological pathways how these risk factors influence brain structure and function remain to be uncovered. Important genetic factors include the novel, yet well-established risk gene *CACNA1C*, while maltreatment and beneficial environment are among the most relevant environmental conditions that specifically act in windows of opportunity during early development. Here, we used the newly generated *Cacna1c* rat model to study its role in affective disorders. Firstly, behavioral phenotypes displayed by adult *Cacna1c* heterozygous (+/-) rats were compared to wildtype (+/+) littermate controls in a sex-dependent manner. The behavioral phenotyping approach included, among others, open field, elevated plus-maze, and pre-pulse inhibition, but also the assessment of olfactory abilities, repetitive behavior and sucrose preference. Secondly, *Cacna1c* +/- rats and *Cacna1c* +/+ littermate controls were exposed to post-weaning social isolation as a model for maltreatment or social and physical enrichment to study the effects of beneficial environments on inflammatory markers. Our results show that *Cacna1c* +/- rats are viable, with the expected 50/50 percent genotype and sex ratios being evident in *Cacna1c* +/+ x *Cacna1c* +/- breedings. At the behavioral level, open field exploration was found to be mildly reduced in both male and female *Cacna1c* +/- rats as compared to *Cacna1c* +/+ littermates. Reduced exploratory behavior was seen on both consecutive test days and reflected by a shorter distance travelled and reductions in rearing behavior. Moreover, anxiety-related behavior displayed on the elevated plus-maze was enhanced in *Cacna1c* +/- rats, with heightened anxiety levels being particularly evident in *Cacna1c* +/- females. In males, the genotype effect on anxiety-related behavior was less prominent, possibly due to higher levels of anxiety-related behavior in males than females. Regarding startle responses to auditory stimuli, intact pre-pulse inhibition was seen in *Cacna1c* +/- rats under baseline conditions, yet impaired pre-pulse inhibition was observed in *Cacna1c* +/- females under apomorphine challenge. In males, the apomorphine effect was detected irrespective of genotype. No clear evidence for genotype effects on social and non-social olfactory abilities, repetitive self-grooming behavior, and sucrose preference was obtained. Finally, the measurement of cytokine levels revealed that post-weaning social isolation mostly increased proinflammatory markers, while social plus physical enrichment mainly led to opposite effects. Such changes were most prominently seen in wildtype littermate controls, with relatively minor environmental effects being evident in *Cacna1c* +/- rats. Together, our findings indicate that *Cacna1c* is involved in the regulation of behavioral phenotypes with relevance to neuropsychiatric disorders and that the responsivity to environmental changes at the level of inflammatory markers is reduced in *Cacna1c* +/- rats. Funding by the Deutsche Forschungsgemeinschaft (DFG; FOR 2107): GA 545/5-1, SCHW 559/14-1, and WO 1732/4-1.

Hippocampal disruption of NOS-I PDZ-interaction: Effects on learning and memory

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Background: Neuronal nitric oxide (NO) synthase (NOS-I), the main source for NO in the brain, is linked to the glutamatergic post-synapse through PDZ-interaction with PSD-93 and -95. NOS-I adaptor protein (NOS1AP) directly competes with PSD-93/-95 for interaction with the PDZ-domain of NOS-I. Importantly, both NOS-I and NOS1AP have been associated with several psychiatric disorders including schizophrenia and mood disorders, possibly through disruption of NOS-I PDZ interactions (Freudenberg et al., 2015). Here we aimed to investigate, whether disturbed NOS-I PDZ-interaction results in learning and memory deficits comparable to those observed in these disorders.

Methods: We designed different protein constructs interfering with NOS-I PDZ interaction as previously described (Candemir et al., 2016): (1) full length murine NOS1AP (2) amino acids 396-503 of NOS1AP (NOS1AP₃₉₆₋₅₀₃) encoding the NOS-I interaction motifs, (3) the amino acids 1-133 of NOS-I (NOS-I₁₋₁₃₃) containing PDZ domain, (4) a control construct expressing mCherry. Recombinant adeno-associated virus vectors expressing these proteins were stereotaxically delivered to the dorsal hippocampus of adult C57Bl/6J mice (N=9/group). Behavioural testing started four weeks after virus delivery.

Results: Mice were tested for spatial reference memory on a Y-maze. In this task, mice overexpressing NOS-I₁₋₁₃₃ mice displayed a significantly reduced memory performance ($P=0.014$), while mice overexpressing NOS1AP or NOS1AP₃₉₆₋₅₀₃ were comparable to mCherry controls ($P=0.645$ and 0.235 respectively). When tested for social memory in a social recognition paradigm, all treatment groups showed reduced interaction with a novel social partner, though this was only significant for mice overexpressing NOS1AP₃₉₆₋₅₀₃ ($P=0.011$), but not for those overexpressing NOS1AP ($P=0.372$) or NOS-I₁₋₁₃₃ ($P=0.145$). However, disruption of NOS-I PDZ-interaction does not seem to result in a generalized memory deficit, as contextual and cued fear memory were comparable between all groups ($P>0.3$).

Conclusions: In the present study we were able to replicate some of the learning and memory deficits observed in schizophrenia and mood disorders. These findings, together with our accompanying study (see abstract by Candemir et al.) provide further evidence for an important involvement of NOS-I PDZ interaction in phenotypes relevant for these disorders. Our results will eventually result in a better understanding about NOS-I dependent psychopathogenesis and may lead to optimized treatment options.

References:

Candemir, E., Kollert, L., Weißflog, L., Geis, M., Müller, A., Post, A.M., O Leary, A., Harro, J., Reif, A., & Freudenberg, F. (2016) Interaction of NOS1AP with the NOS-I PDZ domain: Implications for schizophrenia-related alterations in dendritic morphology. *Eur. Neuropsychopharmacol.*, 26, 741–755.

Freudenberg, F., Althoff, A., & Reif, A. (2015) Neuronal nitric oxide synthase (NOS1) and its adaptor, NOS1AP , as a genetic risk factors for psychiatric disorders. *Genes, Brain Behav.*, 14, 46–63.

Knockdown of the ADHD Candidate Gene *Diras2* in murine neuronal primary cells

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Attention-deficit / hyperactivity disorder (ADHD) is the most prevalent childhood behavioral neurodevelopmental disorder which is highly persistent into adulthood and shows an heritability up to 80%. In a previous study, we could show an association of DIRAS2 with adult as well as childhood ADHD. The function of the gene product is still unknown but might include the regulation of cell morphogenesis.

To get more knowledge about the role of *Diras2* in the brain, an adenoassociated virus was used for a knockdown of *Diras2* gene expression in murine hippocampal primary cells. To identify possible signalling cascades in which *Diras2* might play a role, GeneChip Mouse Gene 2.0 ST Arrays (Affymetrix) were used for expression profiling. The results, were verified using Roche RealTime ready Custom panels for quantitative real time PCR (qPCR).

Expression analyses in primary cells with a reduced *Diras2* expression revealed more than 1600 genes whose expression has changed significantly in comparison to the negative control samples. Several candidate genes for ADHD and other psychiatric disorders as *Nos1*, *Dgkh* and *Cdh13* were among the top hits. A considerable amount of differentially expressed genes is coding for synaptic proteins as *Snapin* and *Synaptotagmin XIII* and *XVII* and for ion channels. 29 out of 88 selected genes showed significant expressional changes in a qPCR replication.

The fact that a knockdown of *Diras2* expression in hippocampal primary cells leads to an altered expression of more than 1600 genes suggests an important role of *Di-Ras2* in brain development, synaptic transmission and a possible involvement in the pathomechanisms of ADHD.

MORC1, a gene associated with early life-stress and depression - A study in the rodent brain

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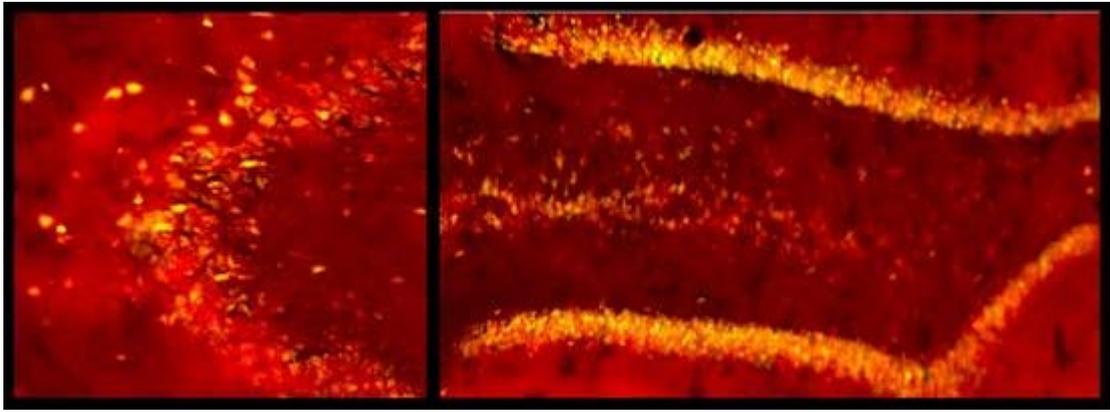
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The human brain exhibits lifelong plasticity. Thereby, in so called sensitive periods the brain is especially accessible for new information but it is also particularly vulnerable. When we are confronted with negative stressors during sensitive periods, e.g. by experiencing psychosocial stress in early childhood, major changes to our brain can be induced. These changes that include altered gene expression increase the risk to develop into a serious psychiatric disorder later in life. Early life stress (ELS), like sexual or physical abuse, loss of a caregiver or a natural disaster, often results in the onset of major depressive disorder (MDD) during early adulthood.

Recently, MORC family CW-type zinc finger 1 (MORC1) was identified as a potential candidate gene that could be responsible for the ELS induced neurobiological changes setting the brain at risk to develop MDD. MORC1 is a zinc finger protein which is linked to signaling-dependent chromatin remodeling and epigenetic regulation. We are now interested in the role of MORC1 in the brain and how it acts to build the link between ELS and MDD. For further investigation we established a staining protocol for immunoperoxidase as well as immunofluorescence staining against the MORC1 protein in rodents.

Analysis of immunohistochemically stained rat and mouse brains revealed new information about the protein's localization in the cell and in the brain. Immunofluorescence double staining with DAPI allowed to locate MORC1 in the cell nucleus and additional staining with the neuronal marker NeuN revealed that MORC1 can predominantly be found in neurons (Figure 1: Double stained rat hippocampus, MORC1 protein is stained in red and NeuN protein in yellow. If MORC1 is in the same cell as NeuN, the color seems orange). Neuroanatomical evaluation of MORC1 staining in the whole brain enabled a clear link between MORC1 and MDD impaired brain structures. Beside the protein's occurrence in cortex areas of motoric, somatosensory, auditory and visual processing, it was also present in structures of the mesolimbic dopaminergic pathway, like the amygdala, lateral hypothalamus and nucleus accumbens. These structures are part of the reward circuitry.

Moreover, MORC1 was found in structures of the limbic system and mood regulation areas, like amygdala, ventral pallidum, parts of the thalamus and hippocampus. The MORC1 stained hippocampus suggests also an influence of MORC1 on cognition, memory processing and emotional behavior. All these structures are impaired in MDD. Given its location in the brain, altered expression of MORC1 due to ELS may make the brain vulnerable to develop MDD. Future rodent studies to prove if MORC1 expression is influenced by ELS and to further investigate the neurobiological consequences of these changes in expression patterns will allow us to better understand the connection between MORC1, ELS and MDD. This better understanding could then lead to the development of prevention and better treatment options for MDD.



NEUROPEPTIDE S RECEPTOR-DEFICIENT MICE ARE MORE PRONE TO DEVELOP PTSD-LIKE FEAR MEMORY AFTER CORTICOSTERONE INJECTIONS.

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Dysfunctions within the mechanisms underlying fear can lead to anxiety disorders which include panic disorder (PD), generalized anxiety disorder (GAD), phobias, acute stress disorder and posttraumatic stress disorder (PTSD). These disorders are characterized by inadequate anxiety states as well as increased and generalized fear learning. Currently, the available treatments consist of the combination of psychotherapy, where the gold standard is cognitive-behavioral therapy, and pharmacotherapy. However, the established pharmacological anxiolytic treatments are not very optimal due to limited efficiency and negative side-effects. Moreover, the neurobiological underpinnings of most anxiety disorders are as yet not completely understood.

Endogenous neuropeptide S (NPS), that has been shown to exert strong anxiolytic effects upon intracerebral injection in rodents, seems to be a promising target for anxiety disorders. Several clinical studies identified a polymorphism in the NPS receptor (NPSR) gene that is associated with an increased incidence of anxiety disorders. Consequently, we hypothesize that NPSR-deficient mice will be more prone to develop a PTSD-like fear memory, i.e. generalized fear.

Data from a previous study of our group suggest that there is an interaction between NPSR genotype, plasma corticosterone levels as well as incubation time after a traumatic event, leading to a generalized, PTSD-like fear memory. In the present study, we further investigated this potential interaction: Female and male NPSR-deficient mice were fear-conditioned (two intense electric stimuli) with or without systemic injections of different doses of corticosterone (2.5 mg/kg or 5 mg/kg). Animals were tested for their fear memory after 1-2 days and one month later. During the fear memory tests, animals were exposed to a novel context, a very similar context to the conditioning context, and the conditioning context in the order to assess the specificity/generalization of the contextual fear memory. During different stages of the experiments, blood samples from the tail were collected to determine corticosterone levels. Additionally, the brains of the animals were analysed for neuroinflammatory changes (microglia activation), which were found in PTSD patients.

Our data show that corticosterone injections after fear conditioning result in a PTSD-like fear memory, i.e. a fear generalization, 4 weeks after fear conditioning. This effect appears to be more pronounced in NPSR-deficient mice. Furthermore, high corticosterone levels during fear memory tests seemed to be associated with impaired context discrimination. Notably, high inactivity before fear conditioning appears to predict the development of PTSD-like fear memory. The data analysis and further data collection are ongoing and will show the influence of other factors like e.g. gender.

This research was funded by the Deutsche Forschungsgemeinschaft (DFG, FE 483/7-1).

Periaqueductal gray/ dorsal raphe dopamine neurons control associative learning of fear

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Functional neuroanatomy of Pavlovian fear has identified neuronal circuits and synapses associating conditioned stimuli with aversive events. However, Hebbian plasticity at these sites may require additional reinforcement for writing particularly salient experiences into long-term memory. Here, we have identified a dopaminergic circuit module, interconnecting the ventral periaqueductal grey/dorsal raphe with the central amygdala, that gates fear learning. Ventral periaqueductal grey/dorsal raphe dopamine neurons release glutamate to increase attention and dopamine to reinforce long-term potentiation of a fear synapse in the central amygdala. Negative feedback limits reinforcement to events that have not been predicted and directs long-term memory to the most informative contingencies. These findings establish a circuit context for dopamine in negative reinforcement learning and dissociate functions of co-released neurotransmitters. Hyperactivation of this circuitry may drive the excessive associations related to post-traumatic stress disorder.

Post-weaning social isolation results in ultrasonic communication deficits, cognitive impairments and alterations in microRNA-dependent Ube3a1 function on neuronal plasticity in rodents: Implications for autism

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Rats are highly social animals and rough-and-tumble play during adolescence has an important role for social development. Post-weaning social isolation, i.e. separation from conspecifics during this phase, is known to induce behavioral phenotypes and changes in neural development relevant to neuropsychiatric disorders like autism. Ultrasonic vocalizations (USVs) are an important component of the rat's social behavioral repertoire and serve as situation-dependent affective signals with important communicative functions. High-frequency 50-kHz USVs are produced in appetitive situations such as rough-and-tumble play and induce social approach behavior, indicating that they serve as social contact calls. Here, we tested by means of our highly standardized 50-kHz USV radial maze playback paradigm if social isolation impairs approach behavior in response to pro-social USVs. Male rats were housed in one of the following conditions: group housing, short-term isolation (24 hours), or long-term isolation (28 days). While group-housed and short-term isolated rats displayed approach behavior in response to pro-social 50-kHz USVs, post-weaning long-term isolation led to pronounced deficits, with rats rather displaying avoidance behavior. Importantly, such deficits could be reversed by one additional week of peer-rearing and were not observed after post-adolescence long-term isolation, indicating a critical period for social development during adolescence. At the neurobiological level, post-weaning isolation, also resulting in poor novel object recognition as expected, led to an increase in an alternative E3 ubiquitin ligase Ube3a transcript, Ube3a1, in the hippocampus; a key regulator of activity-dependent synapse development and plasticity. The increase in Ube3a1 RNA expression following post-weaning isolation was paralleled by elevated levels of microRNA 134, with Ube3a1 knockdown increasing dendritic complexity in the hippocampus in wild-type controls. Ube3a1 RNA knockdown, however, failed to induce dendritic complexity when the miRNA cluster 379-410, including miR-134, was missing, demonstrating that the Ube3a1 function is microRNA-dependent. Taken together, post-weaning social isolation led to ultrasonic communication deficits, cognitive impairments and alterations in microRNA-dependent Ube3a1 function on neuronal plasticity. The finding that environmental factors affecting social behavior and cognition alter Ube3a has important implications, particularly since loss of UBE3A is the leading cause for the neurodevelopmental disorder Angelman syndrome and UBE3A duplications are among the most frequent copy number variations associated with autism.

Self-regulatory behavior of rats being on different hierarchical level in chronic psychogenic stress model

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The aim of research was to study biologically positive self-regulatory, compensatory behavioral manifestations in the rats being on different hierarchical level under psychogenic stress conditions. As a psychogenic experimental model served informational stress model created by us, in which the animal (rat) is under conditions of unfavorable combination of informational triad – high motivation, chronic deficit of time and information. It appeared that against background of strong defence motivation, under conditions of deficit in the time allotted for processing the given information, rat fails to perform adequate behavior that leads to the disturbances specific for depression. Originality of this model consists in that it enables to study not only the occurring pathological changes from the initial stage of stressogenic influence to development of depression, but also the selfregulation mechanisms protecting the organism. It is remarkable that character of development and outcomes of information stress depend on individual peculiarities of the nervous system. Therefore, in our experiments we studied, both in dominant and submissive rats, biologically positive, selfregulatory behavioral manifestations from the initial stage of stressogenic impact to development of a pathogenic condition (depression).

Experiments were conducted on male Wistar laboratory rats. Preliminarily we created small groups of rats composed of 4 male and 1 female rats. One month later we distinguished dominant and submissive animals, against high alimentary motivation, which considers in the groups victory of a stronger one in food procure. Stressing occurred in a modified shuttle-box – “stress-box”. The “stress- box” is composed of three equal sized compartments – central and two side ones with 10cm partitions. Active avoidance reaction is elaborated first in response to the sound of metronome (2Hz) followed by learning to spring over the barrier from the central compartment into the left section. After acquisition of this avoidance reaction we proceed to elaboration of another active avoidance – in response to 500 Hz tone to spring from the central compartment into the right one. Having elaborated and consolidated the two active avoidance reactions separately, we start their combined application in a random way in one experimental session. The rats were stressed every day for 45 days. Animals’ behavior was studied in a “stress-box”, also using “forced-swimming” and “open-field” tests. For stress level assessment corticosterone level in plasma was measured at different stages of stressing.

At the initial stage of information stress in the course of experiment in the “stress-box” one could observe an increase in animals’ motor activity, intersignal movements, rearing and grooming activity, being more clear-cut in dominant rats. In our opinion this kind of behavior is a manifestation of selfregulation behavior.

At prolonged stressing (45 days) the increased motor activity gradually decreases during experiments (rearing and intersignal movements disappear altogether). Dominant rats react to a conditioned signal (CS), in spite of this percentage of correct responses to a signal does not exceed 25-35%. In contrast to dominants, submissive animals no longer react to CS and remain in a central compartment and spring into side sections only in response to painful electroshock.

Thus, in our experiments after 45-day stressing both dominant and submissive rats develop depressive state, but animals’ behavior before development of a pathology is directed toward elevation of organism’s stability in response to a stressogenic influence and it is viewed by us as a manifestation of selfregulation,

defensive mechanism.

Research work is supported by Shota Rustaveli National Science Foundation grant FR/260/7-270/13.

Serotonin Transporter Dependent Activation of the Amygdala after Negative Stimuli: A fMRI Study in 5-HTT Knockout Mice

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The short (s) variant of the serotonin transporter-linked polymorphic region (5-HTTLPR), which results in reduced serotonin transporter (5-HTT) mRNA, 5-HTT protein and 5-HT re-uptake, is associated with several different psychiatric disorders including affective disorders and anxiety disorders. 5-HTT blocking by selective serotonin reuptake inhibitors (SSRIs) is a major target for the treatment of depression. 5-HTTLPR s-allele driven amygdala hyperactivity in response to negative stimuli is confirmed in humans with no psychiatric disorders. Therefore, this genotype effect in the amygdala, a brain region involved in fear processing, has to be examined in 5-HTT knockout (KO) mice, which are the predominant model organism for the investigation of affective and anxiety disorders and have been shown to be significantly more anxious in behavioural tests compared to their wildtype (WT) and heterozygous (HET) littermates. In this study, long term cerebral perfusion changes are measured by ultra-high field functional magnetic resonance imaging (fMRI) in a 17.6 Tesla Bruker Avance 750 WB system with continuous arterial spin labelling (CASL) and serve as indicator for neuronal activation. In several studies predator odours like rat soiled bedding have been used to evoke fear and are also applied in this experiment via a ventilation system. Amygdalar resting state (RS), stimulation state (SS) and post-resting state (PRS) of female and male 5-HTT WT, HET and KO are measured.

Genotype effects seem to be contrary to previous studies in humans, suggesting a heightened amygdala response in 5-HTT WT mice due to an increased neuronal excitability. However further experiments are required, some of which are ongoing.

Sex-dependent Effects of *Cacna1c* Haploinsufficiency on Juvenile Social Play Behavior and 50-kHz Ultrasonic Vocalizations in Rats

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In juvenile rats, the rough-and-tumble play period is an important time for the development of social behavior and communication abilities. During social play, rats emit high numbers of 50-kHz ultrasonic vocalizations (USV). Studies using playback as well as devocalization approaches have found that 50-kHz USV are an important component of the rat's social behavior repertoire, serving various communicative functions in regulating social interactions, for instance as social contact calls. Such 50-kHz USV presumably also reflect a positive affective state ("rat laughter"). If rats are unable to communicate properly during play, the rate of play significantly decreases. Without sufficient play during the critical juvenile period, the risk for developing severe social impairments increases. Therefore, rough-and-tumble play and the concomitant emission of 50-kHz USV appear to be ideal readouts for assessing behavioral deficits in social behavior and communication with relevance to affective disorders, such as major depression and bipolar disorder. A recently implicated gene in affective disorders is the novel yet well-established risk gene *CACNA1C*, especially the rs1006737 allele. The gene encodes an alpha-1 subunit of the voltage-dependent L-type gate calcium channel Cav1.2, mediating depolarization-dependent calcium influx into the cell. Using a newly developed genetic rat model, we investigated rough-and-tumble play behavior and concomitant 50-kHz USV emission in juvenile male and female wildtype (+/+) and heterozygous (+/-) *Cacna1c* rats in the present study. In addition to 50-kHz USV emission in the sender, we assessed behavioral responses displayed by the recipient exposed to 50-kHz USV playback. Typically, rats display social approach behavior in response to playback of 50-kHz USV. Our results indicate that in males there are no significant genotype differences in play behavior. However, in females, evidence for a highly significant difference between *Cacna1c*+/+ and *Cacna1c*+/- rats was obtained, with female *Cacna1c*+/- rats spending more time playing and specifically pinning more than female *Cacna1c*+/+ littermate controls. Based on this finding, it is hypothesized that female *Cacna1c*+/- rats also show a significantly increased number of 50-kHz USV, especially during the pinning sequences of play. In the recipient, however, no evidence for genotype-dependent differences was obtained. Irrespective of sex, *Cacna1c*+/+ and *Cacna1c*+/- rats displayed strong social approach behavior in response to playback of 50-kHz USV, further supporting their pro-social function as social contact calls. Together, the present findings might suggest that *Cacna1c* is implicated in behavioral phenotypes and changes in social development with relevance to affective disorders in the sender but not the recipient. Funding by the Deutsche Forschungsgemeinschaft (DFG; FOR 2107): GA 545/5-1, SCHW 559/14-1, and WO 1732/4-1.

Social impairments, olfactory dysfunction, and inattention in neuronal nitric oxide synthase (*Nos1*) knockdown mice

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Background: The gaseous neurotransmitter nitric oxide, synthesized in the brain by the neuronal isoform of the nitric oxide synthase (NOS-I), has been implicated in various psychiatric disorders, including attention deficit/hyperactivity disorder (ADHD) and schizophrenia [1]. Previous studies have shown that mice lacking the gene coding for NOS-I, *Nos1*, display locomotor hyperactivity, decreased anxiety-like and abnormal social behaviour, cognitive impairments, increased aggression and impulsivity, and proposed these animals as an animal model of ADHD [2, 3]. Here we used male *Nos1*-knockdown (kd) and wild-type (wt) C57Bl/6J mice to extend the previous research by investigating how *Nos1* deficiency modulates various aspects of social functioning, territorial aggression, and impulsivity and attention.

Methods: Male *Nos1*-kd and wt mice (N = 8/group) were tested in the 5-trial social memory assay and sociability/social novelty preference test, olfactory habituation/dishabituation test, resident-intruder paradigm, touchscreen 5-choice serial reaction time task (5-CSRTT), and the nestlet shredding and nest building tests.

Results: Male *Nos1*-kd mice displayed reduced social investigation time, and lack of detection of social novelty in the 5-trial social memory assay ($p < 0.01$ vs wt); whereas discrimination between a familiar and novel social partner was absent in the sociability/social novelty preference test ($p < 0.05$ vs. wt). Detection and discrimination of social but not non-social odours (almond, banana) was significantly impaired in the *Nos1*-kd ($p < 0.001$ vs. wt). *Nos1*-kd were less likely to attack an intruder in the resident-intruder task. In the 5-CSRTT, *Nos1*-kd showed intact acquisition of the task, reduced premature responding, and increased perseverative responding suggestive of cognitive rigidity rather than compulsive behavior ($p < 0.05$ vs. wt). Shortening the stimulus duration tended to increase the rate of omissions only in the *Nos1*-kd ($p = 0.06$ vs. wt). Finally, nestlet shredding ($p < 0.01$ vs. wt) and nest quality ($p < 0.05$) were significantly decreased in the *Nos1*-kd, indicative of compromised self-care.

Conclusions: We found that *Nos1*-deficiency in mice results in marked social impairments, reduced self-care, mild inattention, and cognitive rigidity which particularly resemble the negative symptomatology in schizophrenia, rather than ADHD-like phenotype. Normal social functioning critically relies on the ability to discriminate between social scents, therefore the social abnormalities and overall reduced aggression in the *Nos1*-kd mice can potentially be attributed to their olfactory dysfunction.

[1] Freudenberg, F., Althoff, A., Reif, A., 2015. Neuronal nitric oxide synthase (NOS1) and its adaptor, NOS1AP, as a genetic risk factors for psychiatric disorders. *Genes, Brain and Behav* 14(1), 46–63.

[2] Tanda, K., Nishi, A., Matsuo, N., Nakanishi, K., Yamasaki, N., Sugimoto, T., Toyama, K., Takao, K., Miyakawa, T., 2009. Abnormal social behavior, hyperactivity, impaired remote spatial memory, and increased D1-mediated dopaminergic signaling in neuronal nitric oxide synthase knockout mice. *Mol Brain* 18, 2-19.

[3] Nelson, R.J., Demas, G.E., Huang, P.L., Fishman, M.C., Dawson, V.L., Dawson, T.M., Snyder, S.H., 1995. Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. *Nature* 378(6555), 383-386.

Stress-induced aggression in mice and evidence for preventive effects of drugs with pro-neurogenetic activity

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Several lines of evidence suggest that stress may increase aggression. In the present work we tested two different paradigms of stress and their effect on aggressive behavior. Tryptophanhydroxylase-2 (TPH2) null mutant mice ($Tph2^{-/-}$), which are characterized by increased aggressive behavior. Initial studies revealed that under normal conditions heterozygous mice ($Tph2^{+/-}$) lack significant behavioral changes, while under stress conditions these animals display aberrant behavior similar to $Tph2^{-/-}$ mice. In the first study, $Tph2^{+/-}$ male mice were subjected to 5 days of predator stress and afterwards tested in the Resident-Intruder test. While non stressed $Tph2^{+/-}$ mice showed no changes in parameters of aggressive, dominant-like and social behaviors, in comparison to their wild type littermates, stressed $Tph2^{+/-}$ mice displayed elevated measures of aggressive behavior, such as a latency of the first attack, duration of fighting behavior. In contrast to $Tph2^{+/-}$ mice, wild type mice subjected to predator stress showed a significant reduction in the measures of aggressive behavior, such as an increased latency of the first attack, reduced number and duration of attacks. These data suggest that predator stress evokes opposing effects on aggressive behavior dependently on a genetic deficit of Tph2. HPLC measurements of monoamines revealed a strong positive correlation between aggressiveness and 5HT turnover rate in striatum in stressed wild type animals ($Tph2^{+/+}$), while in naive $Tph2^{+/-}$ mice there was a correlation between aggressiveness and 5HT turnover rate in prefrontal cortex. Therefore, it can be assumed that heterozygous animals use 5-HT to process the situation of RIT through different brain areas.

In another study 3 m.o. Balb/c mice were subjected to a 3-week ultrasound radiation in 20-45 kHz frequencies ranges. It is known that unpredictably altering frequencies of ultrasound between ranges 20-45 kHz correspond with a negative emotional state for small rodents in nature and can induce behavioral aggressive-like changes in this mice strain. We have also studied a potential anti-aggressive effect of substances DF302 and thiamine at doses of 40 mg/kg/day and 200 mg/kg/day respectively. Both DF302 and thiamine are powerful pro-neurogenetic drugs with different mechanisms of action. Aggressive behavior was evaluated in Resident-Intruder test. It was found that mice subjected to a three-week ultrasound exposure, had a significantly decreased latency of attacks, and significantly increased number and duration of attacks, in comparison to the control group, while in mice treated with DF302 and thiamine parameters of aggressiveness didn't differ from the control group. To sum up we can say that

stress is a major factor causing the aggression, but there are substances that increase neurogenesis and able to prevent the negative effect of the stress.

Sustained effect of ketamine is mediated by homeostatic regulation of synaptic function and reconfiguration of gene expression

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Major depressive disorder (MDD) is the most common psychiatric disorder with lifetime prevalence of 16%. Conventional antidepressants require prolonged treatment and have only limited efficacy. Development of faster acting and more effective antidepressants is desirable. Ketamine applied in single sub-anesthetic dose has rapid antidepressant effect that is sustained for several days. Ketamine is rapidly metabolized and becomes undetectable within several hours after application. Similarly, the activation of protein synthesis machinery that was suggested to be key for antidepressant effect of ketamine is only very transient and drops to baseline before manifestation of systemic antidepressant effect. Recent evidences revealed that sustained ketamine application might be associated with synaptogenesis, changes in synaptic transmission and neuronal plasticity, but the underlying molecular and cellular mechanisms are not completely understood. Here, we investigated the effect of ketamine on synaptic function, intracellular signaling and gene expression in dissociated rat cortical cultures. Synaptotagmin1 Ab uptake assay, electrophysiological recordings and quantitative immunostainings were used to determine the changes in pre and postsynaptic function and molecular composition. Genome-wide microarray-based expression analysis was performed to screen for changes in gene expression at several time points after ketamine treatment. Results of the study revealed rapid changes in synaptic function and concomitant molecular remodeling and reconfiguration of cellular gene expression. Interestingly, our analyses revealed biphasic changes and opposite regulation at early (30 mins after treatment) and at delayed (24 hrs after treatment) time points. While acute effects were common to both excitatory and inhibitory synapses, delayed effect was specific to excitatory synapses, suggesting that acute interference with NMDA-mediated transmission by ketamine induces adaptive functional and structural changes that seem to rely on homeostatic neuronal plasticity mechanisms, and lead to a shift in the excitation-inhibition balance. In line with the involvement of homeostatic mechanisms, the regulation of key genes relevant to synaptic transmission, neuronal plasticity, calcium and MAPK signaling pathways, and antidepressant mechanisms was observed. Our results also suggest that changes in the expression of activity-dependent genes upon ketamine treatment might be partially dependent on transcriptional corepressor C-terminal binding protein 1 (CtBP1). The results of this study provide better understanding of cellular and molecular mechanisms involved in rapid antidepressant actions of ketamine and may identify potential targets for exploring the novel therapeutic approaches for MDD.

The effect of arsenic exposure on learning and memory in rats of various age groups

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Chronic exposure to arsenic compounds is one of the serious public-health problems in many developing and some developed countries. Long-term exposure to arsenic from drinking-water and food can cause cancer and skin lesions. It has also been associated with developmental effects, cardiovascular disease, neurotoxicity and diabetes. The aim of this study was to investigate the effects of chronic exposure to arsenic on learning and memory processes.

The effect of arsenic exposure on rat behavior was studied in two series of experiments: a) exposure of arsenic in two different age groups (young and adult); the rats from the experimental groups drank water with final arsenic concentration of 68 mg/L for three months; b) exposure of arsenic in offspring of rats given this dose of arsenic before pregnancy, during pregnancy, and for three weeks after parturition. Animals in control groups were given regular water.

Multi-branched maze was used to estimate the learning and memory process.

The body weight gain abnormalities were observed in all experimental groups. After three months of arsenic exposure the average body weight in both groups and 21-day-old pup's (offspring of arsenic exposure parents) lags behind 20%, 6% and 18%, respectively, in comparison with control animals. Object novelty discrimination index in young and adult control groups, also habituation to the environment (habituation score) in young group was higher in comparison to experimental groups (differences being statistically significant $p < 0.05$). Compared with controls, learning and memory ability declined in rats that were exposed to arsenic. Rats from the experimental groups demonstrated impaired cognitive learning: they made more errors and need more time compared to control animals. However, the difficulty in learning ability was statistically significant ($p < 0.05$) in pups, offspring of rats exposed to arsenic. More significant difference was observed between control and experimental groups in memory test. Statistically significant impairment of memory was seen as in offspring, so in young and adult rat groups.

Neurons also are susceptible to arsenic toxicity, arsenic exposure in rats resulted in individual shrunken cells with condensed cytoplasm and nucleus was seen in the cerebral cortex and hippocampus; both which are critical regions in the brain for learning and memory processes.

The neurotoxic effects of arsenic appear to be most severe in the developing brain. It was revealed as in deficits in learning and memory behavioral tests so in reduced number of neurons found in cerebral cortex of offspring that parents drank water with final arsenic concentration of 68 mg/L for three months.

Acknowledgment. This work was supported by grant from Shota Rustaveli National Science Foundation.

The regulatory role of trace amine-associated receptor 1 in acute and chronic effects of nicotine

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The trace amine-associated receptor 1 (TAAR1) has emerged as a promising target for addiction treatment because of its ability to affect dopamine transmission. Behavioral effects of nicotine are primarily mediated by its stimulating effects on nicotinic receptors-mediated dopamine release within the mesocorticolimbic system. For now, the impact of TAAR1 to effects of nicotine has not been investigated.

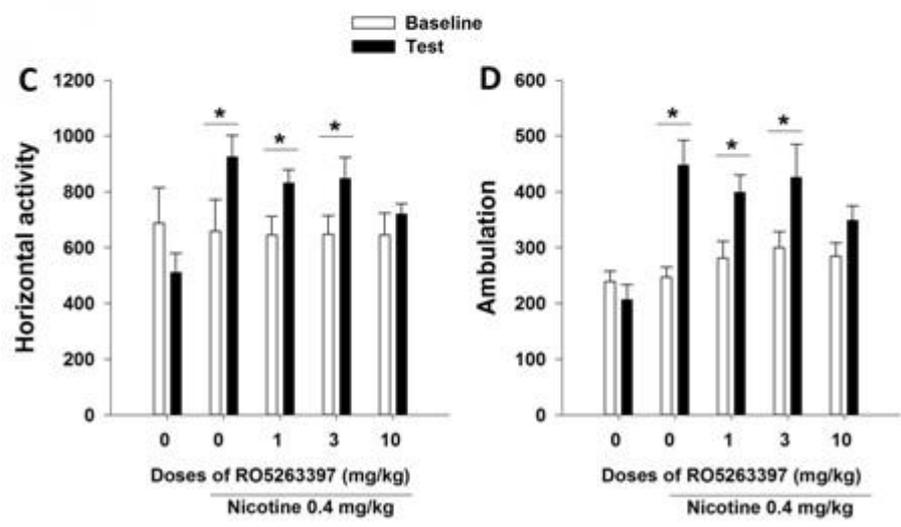
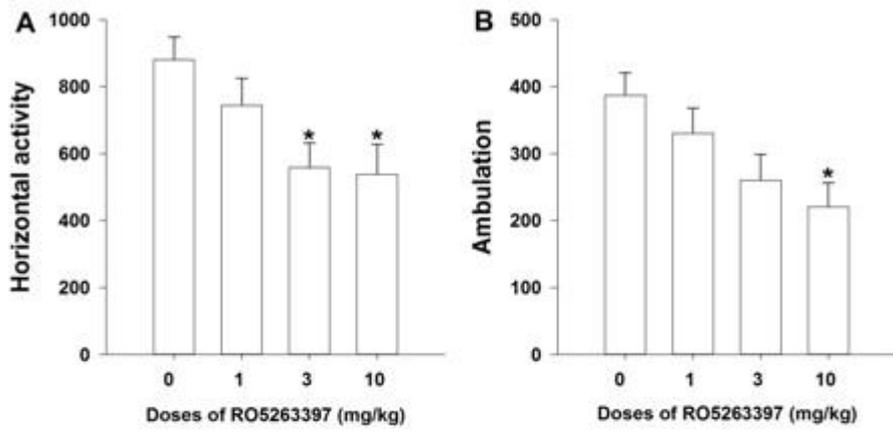
This study was carried out to investigate the effects of TAAR1 on nicotine-induced behavioral activation. We tested the efficacy of RO5263397, TAAR1 agonist, to nicotine-induced hyperlocomotion and nicotine sensitization in rats. Additionally we evaluated locomotor effects of nicotine in TAAR1 KO mice.

Rat locomotor activity was assessed in two sound-attenuating ventilated cubicles each containing five identical photocell-based activity chambers. The acute effects of the RO5263397 in combination with 0.4 mg/kg nicotine on locomotor activity were tested in 8 Wistar rats. After three weeks habituation to the activity monitors, drug tests were started. Dose test order was based on a within-subjects Latin Square design. Animal activity was recorded for 60-min post-injection session. The drug tests were divided by at least 72 h. Pretreatment with RO5263397 dose-dependently decreased the nicotine-induced hyperlocomotion (pic. A, B).

Before acquisition of nicotine sensitization, according to baseline locomotor activity animals were divided into 5 groups: vehicle-saline (n=9), vehicle-nicotine (n=11), 1 mg/kg RO5263397-nicotine (n=10), 3 mg/kg RO5263397-nicotine (n=10), and 10 mg/kg RO5263397-nicotine (n=10). The acquisition of nicotine sensitization included 8 sessions the activity chambers. Before every acquisition session, the rats were pretreated with either RO5263397 or vehicle intraperitoneally and then injected with either nicotine 0.4 mg/kg or saline subcutaneously. 48 h after final acquisition session, the rats were tested for nicotine sensitization. All animals were given an nicotine injection immediately before being exposed to the activity cages for 60 min. During the test the nicotine sensitized rats demonstrated locomotor activity elevated more than twofold compared to the saline treated subjects. Pretreatment with 10.0 mg/kg RO5263397 prevented the development of nicotine sensitization (pic. C, D).

Locomotor effects of nicotine in TAAR1 KO mice were tested in automated Omnitech Digiscan apparatus. Before drug administration, mice were habituated to the activity monitor for 30 min. After nicotine injection (0.3 - 0.8 mg/kg), the locomotor activity of the animals was recorded for additional 60 min. The lack of TAAR1 did not affect locomotor effects of nicotine in mice.

Thus, the results of the present study suggest that the activation of TAAR1 can attenuate behavioral effects of nicotine. These results further support previously proposed view that TAAR1 is promising target for prevention and treatment of drug addiction.



Time-dependent modulation of visual motion prediction in humans.

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Predicting the future position of moving objects is an essential cognitive function used for many daily activities. In spite of a wealth of studies on visual motion prediction by psychophysicists, it has not been investigated with reference to circadian modulation so far. This study examined the diurnal time-of-day modulation of visual motion prediction in a task to predict the position of moving objects at 9:00, 12:00 and 18:00.

In the experiments, the inflating and the deflating computer images were displayed as moving objects to prevent the contaminating effects of eye pursuit. The results demonstrated that participants showed a marked diurnal time-of-day modulation in predicting times related to the inflating images in a light-adapted environment ($p < 0.004$). This motion prediction was more accurate in the afternoon than in the morning. Such diurnal time-of-day modulation was, however, not found in predicting times related to the inflating images.

Our experiments were done in light-adapted environments. Thus, the results can reflect the functions of cone photoreceptors. Cone photoreceptors also have circadian dependency. These cells were shown to contain the clock genes in mouse retina. Moreover, those gene expressions fluctuate throughout the day. In physiological experiments, the b-component of light adapted electroretinogram shows a marked circadian rhythm which peaks at 20:00 hours in human subjects (Danilenko et al., 2011). Therefore, it is plausible that visual motion prediction is circadian-dependent in light-adapted environments. In fact, we have already found that such modulation cannot be shown in dark-adapted environments. The discrepant results may be explained by the gradient distribution of cone photoreceptors in the human retina.

Trace Amine-Associated Receptor 1 agonist attenuates adjunctive water drinking in rat model of compulsive behavior

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Introduction

Compulsive behavior is performing an act persistently and repetitively without necessarily leading it to an actual reward or pleasure. Compulsive behavior is an important characteristic of obsessive-compulsive disorder and drug addiction. Schedule-induced polydipsia (SIP), classified as a form of adjunctive behavior, is one of the most popular animal models of compulsive behavior. In this model scheduled food delivery elicits abnormal and exaggerated drinking behavior (that is not linked to thirst) in rats. Numerous studies indicate that dopaminergic system plays a conspicuous role in regulating of SIP. It has been reported that the Trace Amine-Associated Receptor 1 (TAAR1), one of the most exciting new pharmacological target, is expressed in several brain region including dorsal raphe and the ventral tegmental area and takes part in dopaminergic activity modulation. The present study aimed to evaluate effects of full and partial TAAR1 agonists in SIP.

Materials and methods

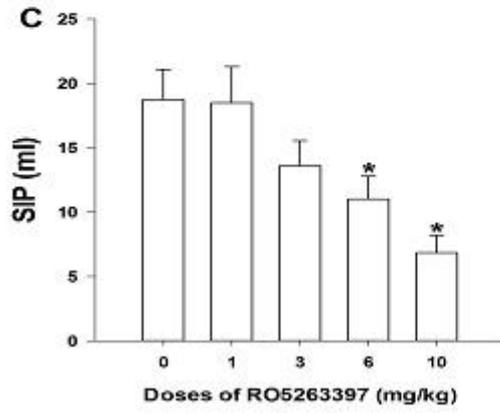
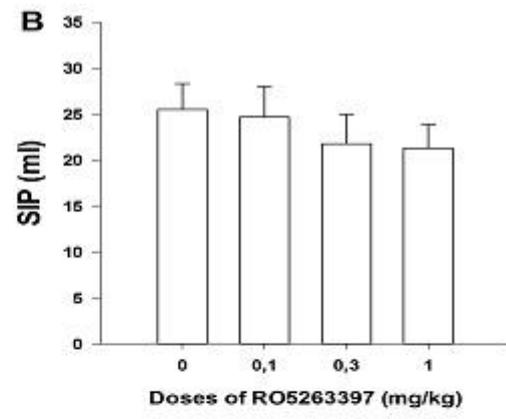
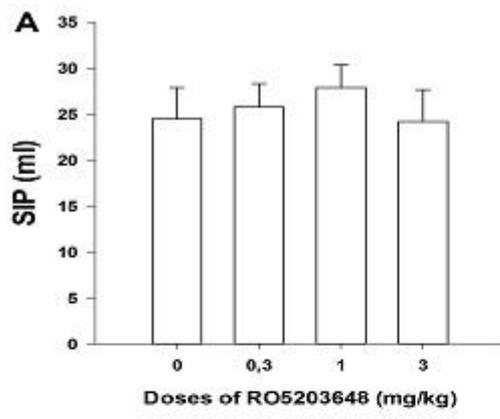
The experiment was conducted in four standard operant conditioning chambers for rats (MED Associates, Inc., East Fairfield, VT). Each chamber was equipped with a white house light, a food dispenser which delivered food pellets and a water bottle. During the 1-h sessions, food pellets were automatically delivered every 60 sec in a response-noncontingent manner (fixed time (FT) 60 sec schedule). While in the home cage, animals had ad libitum access to water but their food consumption was limited. Once the polydipsia was established, rats were given the tests with TAAR1 agonists provided by F. Hoffmann-La Roche (Switzerland), RO5203648 (0.3 - 3 mg/kg) and RO5263397 (0.01 - 10 mg/kg). Dose test orders were based on a within-subjects Latin Square design.

Results

Pretreatment with full TAAR1 agonist, RO5203648, did not affect polydipsia induced by FT 60 sec schedule. However, partial TAAR1 agonist RO5263397 significantly decreased volume of consumed water in a dose dependent manner (fig. 1).

Conclusions

The results of the present study suggest that activation of TAAR1 receptors may decrease schedule-induced excessive drinking. These results may indicate that TAAR1-selective drugs could be effective in the treatment of disorders related to compulsive behaviors such as OCD and drug addiction.



First report of interesting awake craniotomy of a famous musician in history; The suprasellar tumor surgery of Pianist Clara Haskil in 1942 without general anaesthesia

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Case report: Clara Haskil (7 January 1895 - 7 December 1960) was one of the most famous female pianists of the 20th century. In her life and work, she set new standards in piano playing, however, her career beset by poor health and the adversities of the world wars. In her life, Clara Haskil had suffered from three major disorders: Juvenile scoliosis requiring treatment in her adolescence, a tumor of the sellar region requiring surgery at an age of 47 years, and a traumatic brain injury causing her death at the age of 65. Her medical history illustrates the development of surgical methods and rehabilitation in medicine before and after World War II. In 1942, at the age of 47 years, she displayed the first symptoms of a suprasellar brain tumor: headache and hemianopsia. The famous surgeon Marcel David performed surgery on her without general anaesthesia, during which Clara Haskil mentally played Mozart's piano concerto E flat Major KV 271 as a neuronal representation to control her memory and mental abilities. Three months after that operation Clara Haskil played the piano concerto in D Minor, KV 466, by Mozart; which was the start of her career. Her awake craniotomy was remarkable and highlighted new methods in the field. In December 1960, she travelled to Brussels for a concert. In the main station, she stumbled on the stairs and hit her head on one of the steps. Skull fracture and intracranial haematoma were diagnosed. Doctors tried to operate on her but she lost consciousness and died on December 7, 1960. Clara Haskil created new styles in piano playing, and her medical history gives indications of new concepts in neurosurgery.

Methods & Material:

General biographical information, historical investigation, general research.

Aims and results:

There is a vast amount of scientific evidence to demonstrate how listening to music can have a positive effect on a large number of medical conditions. Music has been long known to reduce stress, anxiety and tension, as well as relieve pain in both every day and clinical settings. One of the distinctive features of Mozart's music is the frequent repetition of the melodic line; this determines the virtual lack of "surprise" elements that may distract the listener's attention from rational listening. Many studies related to music and its ability to attenuate discomfort and enhance relaxation in patients, have been reported in medical specialties. Awake cranial surgery can be stressful and anxiety-producing for some patients. In this case report of Clara Haskil, our primary aim to better understand the overall effect of performing Mozart piano music. Mentally played on her awake craniotomy, helped to ameliorate her surgically related stress.

However, no a study has reported the effect of playing Mozart piano music on awake craniotomy patient.

Conclusion:

The case of Clara Haskil also documents the potential of mental training after neurological illness or surgery. Today the use of preferred music helps to recover and reduces agitation after traumatic brain injury or in patients with stroke. Also an auditory stimulation with music has positive effects on intracranial pressure. The case of Clara Haskil presented a new time and new techniques in the field of neurological surgery and gives important suggestions for neurological treatment, intensive care, and rehabilitation as well as music therapy.

Poster Topic

T14: Vision: Invertebrates

- [T14-1A](#) Age-related and light-induced synaptic plasticity in the mushroom-body calyx of the buff-tailed bumblebee *Bombus terrestris*
Nadine Kraft, Johannes Spaethe, Wolfgang Rössler, Claudia Groh
- [T14-2A](#) Colour opponent parallel pathways originate in *Drosophila* photoreceptor terminals
Christopher Schnaitmann, Väinö Haikala, Eva Abraham, Vitus Oberhauser, Thomas Thestrup, Oliver Griesbeck, Dierk F Reiff
- [T14-3A](#) GABAergic signaling shapes motion detecting circuits in *Drosophila*
Teresa Magdalena Lueffe, Luis Ramos Traslósheros, Yvette Fisher, Marion Silies
- [T14-1B](#) Integration of polarized and chromatic sky-compass cues in the central complex of the desert locust
Uta Pegel, Keram Pfeiffer, Uwe Homberg
- [T14-2B](#) Light intensity can override wavelength as an orientation cue during honeybee waggle dances
Niklas Kühn, Keram Pfeiffer
- [T14-3B](#) Receptive field organization of neurons required for motion detection in the *Drosophila* visual system
Luis Giordano Ramos Traslósheros López, Sebastian Mauricio Molinda Obando, Marion Silies
- [T14-4B](#) Receptive fields of polarization-sensitive neurons of the central complex in the desert locust
Frederick Zittrell, Keram Pfeiffer, Uwe Homberg
- [T14-1C](#) Response properties of first-order interneurons in the fly visual system
Katja Sporar, Teresa Magdalena Lueffe, Burak Gür, Marion Silies
- [T14-2C](#) Rhodopsin 7 (Rh7) is crucial for fine-tuning light sensitivity in *Drosophila melanogaster*
Pingkalai R Senthilan, Rudi Grebler, Christa Kistenpfennig, Matthias Schlichting, Christiane Hermann-Luibl, Joachim Bentreop, Stephan Schneuwly, Charlotte Helfrich-Förster
- [T14-3C](#) Saccadic strategy in walking *Drosophila melanogaster*
Kristina Corthals, Martin C. Göpfert, Bart R.H. Geurten
- [T14-4C](#) Studying the Heterochrony of Central Complex Development
Max Stephen Farnworth, Marita Buescher, Nikolaus Dieter Bernhard Koniszewski, Gregor Bucher

- [T14-1D](#) Systematic identification of ocellar ganglion interneurons and their projections in the brain of *Drosophila melanogaster*
Jens Goldammer, Gerald M. Rubin, Kei Ito
- [T14-2D](#) Temporal dynamics of *E*-vector responses of CL1 neurons of the desert locust *Schistocerca gregaria*
Ronja Hensgen, Keram Pfeiffer, Uwe Homberg
- [T14-3D](#) The distance code in honeybee waggle dance
Randolf Menzel

Age-related and light-induced synaptic plasticity in the mushroom-body calyx of the buff-tailed bumblebee *Bombus terrestris*

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In social insects both intrinsic factors such as age and extrinsic factors such as the exposure to light are associated with structural changes within the brain. Bumblebees present an ideal model organism for investigating structural neuronal plasticity in the brain in relation to age as the worker caste does not exhibit an age-dependent division of labor, suggesting that possible structural changes might be primarily correlated with neuroanatomical maturation rather than a change in task performance. Furthermore, bumblebee workers exhibit a distinct size polymorphism, which allows for the investigation of a possible correlation between body size and volume plasticity as well as their structural organization of brain centers such as the mushroom bodies (MBs). The MBs are prominent neuropils in the insect brain involved in higher-order processing, sensory integration and learning and memory processes. Their input regions, the MB calyces, are organized in modular synaptic complexes (microglomeruli, MG). Each MG consists of a single bouton of olfactory or visual projection neurons surrounded by several postsynaptic profiles, which are mainly formed by dendritic spines of MB intrinsic Kenyon cells. Synaptic organization in the MB calyces is highly plastic, providing the substrate for possible adaptations to changing intrinsic or extrinsic factors. In the present study, we investigate how the factors age, body size and light exposure affect the volume of the olfactory MB lip and visual collar region as well as the density of MG in these subregions in the worker brain of the buff-tailed bumblebee *Bombus terrestris*. Colonies were reared either in constant darkness (DD 24 h, 50 % rH) to investigate how the factor age acts on neuronal structural plasticity or under a light-dark regime (LD 12:12 h, 50 % rH) to examine possible light-induced changes in the respective structures. In both cases, freshly emerged workers were age-marked every day. At defined ages, whole-mount brains of workers were dissected for synapsin immunolabelling. Using confocal laser scanning microscopy and the 3D reconstruction software AMIRA, we measured the volumes of the olfactory lip and visual collar and quantified synaptic complexes in both subregions. In age-controlled bumblebees reared under DD we found that age had a significant effect on synaptic density, leading to a pruning of MG already within the first two days of adult life. The number of MG and MB volume positively correlated with body size, while MG density remained constant, supporting the idea that size differences between bumblebee workers are in fact isometric, even at the synaptic level. Light exposure did not affect volume or synaptic organization in the calyx subcompartments. We conclude that in this species neuroanatomical maturation seems to proceed much faster than in other social insect species that have been studied so far, suggesting that these structural changes might be correlated with behavior.

Supported by the DFG, SFB 1047 "Insect timing" (projects B3, B5 and B6) to JS, CG, and WR.

Colour opponent parallel pathways originate in *Drosophila* photoreceptor terminals

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Colour vision requires comparing the signals of photoreceptors with different wavelength sensitivities in colour-opponent neurons. In *Drosophila*, four types of spectrally distinct inner photoreceptors R7y/R8y and R7p/R8p provide the major input to the colour vision system and are harboured in 'yellow' and 'pale' types of ommatidia, respectively. Due to the lack of any physiological recordings of the colour vision circuit is not clear which colour-opponent pathways exist and whether colour opponent processing is generated at the photoreceptor level or in postsynaptic neurons in the medulla. Using two-photon calcium imaging we report that colour-opponency emerges already in inner photoreceptor terminals. Genetic perturbation of photoreceptor function disclosed reciprocal inhibition between R7 and R8 of the same type of ommatidia. This processing gives rise to distinct 'pale' (UV_{short}/blue) and 'yellow' (UV_{long}/green) colour-opponent pathways that are each generated with ON/OFF and OFF/ON contrast in R7 and R8 terminals, respectively. Our study reveals common spectral processing mechanisms in vertebrates and insects and establishes *Drosophila* as a powerful model for investigating the cellular physiology of colour vision.

GABAergic signaling shapes motion detecting circuits in *Drosophila*

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The visual system plays an important role in the perception and interpretation of the environment in many species, ranging from humans to invertebrates such as the fruit fly *Drosophila melanogaster*. Visual motion cues are used to for example navigate through the environment, find prey or escape predators. Motion computation requires a comparison of contrast changes over space and time, leading to direction-selective signals, a hallmark of motion detection. Two distinct computational models describe how direction-selective signals can be extracted: First, the *Hassenstein-Reichardt-Correlator (HRC)* relies on a non-linear amplification of preferred direction (PD) inputs. The HRC has long been the prominent model to describe responses to motion cues in insects. Second, the *Barlow-Levick model* describes how direction-selective signals can be extracted through the suppression of signals that move in the non-preferred, or null direction (ND). This model has been favored in the vertebrate retina. Recent data show that direction-selectivity in the visual system of *Drosophila* arises through a combination of these two mechanisms (Fisher et al. 2015, Leong et al. 2016), but the circuits that implement null-direction suppression are not understood.

In the *Drosophila* visual system, visual information travels from the retina through the lamina, the medulla and the lobula complex. While several inputs to motion-detecting circuits in the lamina and medulla have been identified, these inputs respond to light regardless of the direction of motion. The first direction-selective neurons are the T4 and T5 neurons of the lobula complex (Maisak et al. 2013), which already display directional tuning in their dendrites (Fisher et al. 2015). These cells split up into four different subtypes, preferring objects moving into the four cardinal directions (front-to-back, back-to-front, upwards and downwards) in a layer-specific manner in the lobula plate, allowing to record direction selective signals in *in vivo* calcium imaging experiments. Such experiments revealed that T4 and T5 neurons also display orientation tuning orthogonal to the respective axis of motion preference. This orientation tuning is thought to more tightly tune the direction-selectivity of the T4 and T5 neurons (Fisher et al. 2015). A pharmacological approach revealed that both direction-selectivity and orientation tuning require the contribution of GABAergic circuitries. Upon application of the GABA-A receptor antagonist Picrotoxin, both orientation and directional tuning are lost. Therefore we are interested in the GABAergic circuitries in the fly visual system that contribute to orientation tuning and direction selectivity in T4 and T5 cells. To reveal more details on these circuitries we combine genetic silencing and behavioral analysis with an immunohistochemical approach to identify GABAergic neurons that are required for motion-detection. Given the broad expression of many GABAergic signaling components, we are also aiming to develop stochastic approaches that allow to visualize pre- or postsynaptic components of GABAergic signaling in a cell type specific way. Together, we hope to identify the inhibitory circuitry that shapes direction-selectivity and orientation tuning in the fly visual system. Given the emerging similarities between motion computation in *Drosophila* and vertebrate system, we hope to identify general mechanisms that shape behaviorally critical neural computations.

Integration of polarized and chromatic sky-compass cues in the central complex of the desert locust

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Like other migratory insects, the desert locust likely uses a sky compass mechanism for spatial orientation. In the sky, several cues including direct sunlight, the polarization pattern of the sky and the chromatic and intensity gradient can be exploited for compass orientation. Previous work showed that the central complex in the locust brain holds a polarotopic internal representation of celestial *E*-vectors and may therefore act as an internal sky compass (Heinze and Homberg 2007, *Science* 315:995). To explore whether other celestial cues contribute to this internal compass, we examined whether polarization-sensitive (POL) neurons of the central complex receive additional input from the chromatic gradient of the sky. The intensity gradient of long wavelengths (green light) and the uniform distribution of short wavelengths (UV light) across the sky lead to a chromatic gradient with highest intensity difference between long and short wavelengths near the sun and smallest difference in the antisolar hemisphere (Coemans et al. 1994, *Vision Res* 34:1461). We tested the responses of central-complex neurons to zenithal polarized light and a green- and UV light spot rotating at an elevation of 45° around the head of the animal. Sensitivity to the unpolarized light spots was found in various types of neuron in the central-complex network. In many neurons the tunings to the azimuth of the stimulus was virtually independent of wavelength. It might therefore be possible, that the light spot represents the sun, independent of its wavelength. The preferred position of the unpolarized light spots and the innervated slice of the protocerebral bridge (PB) were linearly correlated in certain columnar cell types, suggesting a topographic organization of solar azimuth positions in the PB.

Light intensity can override wavelength as an orientation cue during honeybee waggle dances

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During foraging trips, honeybees use a sky compass as well as olfactory cues and landmarks to maintain oriented. After returning to the hive the position of a lucrative food source is communicated to the nest mates via a distinct series of movements called waggle dance. The dance consists of two circular motions with a straight waggle phase in between, which signals the direction of the food source. Under natural conditions on a vertically oriented honeycomb, gravity is used as a reference direction. When the honeycomb is turned into a horizontal position, thus eliminating gravity as a reference, bees are able to orient their dances with respect to the sun or artificial light sources. In such a setting, bees orient their dances to a green stimulus as if it were the sun, while an ultraviolet (UV) stimulus is interpreted as lying somewhere in the antisolar hemisphere. This is thought to reflect the distribution of wavelength in scattered skylight, where the ratio between long wavelengths (green) and short wavelength (UV) is larger at the solar azimuth than on the opposite side. Beyond wavelength, the distribution of different light intensities is a distinctive sky-compass cue. The intensity of the sun is substantially higher than the intensity of scattered light from the sky.

In this study we therefore focused on the effect of light intensity on dance orientation. Bees dancing on a horizontal comb were either presented with a green or a UV LED at an elevation of 45°. Either LED could be presented at two different intensities (10^{12} or 10^{14} photons/cm²*s). We found that light intensity had several effects on the waggle dances. 1. Dances under high light intensities were generally oriented more precisely than under low intensities. 2. Dance directions obtained in the presence of high UV-light intensities were similar to those under green light conditions, suggesting that a high intensity light spot is generally interpreted as the sun. 3. At low UV intensity, the dance direction was shifted between 90° and 180° compared to dances under high UV-light intensities. These changes were significantly larger under clear weather conditions than under cloudy conditions, suggesting that the chromatic information was deemed more important than intensity information if the animals had just experienced the natural chromatic gradient of the sky prior to testing. Under cloudy conditions, this gradient is virtually gone and the bees rely more on the intensity cue.

Taken together our results show that both chromatic and intensity cues are used to orient waggle dances and that intensity can override color.

Receptive field organization of neurons required for motion detection in the *Drosophila* visual system

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Many animals use visual motion cues to navigate through the environment. Classical models of motion computation rely on temporal correlations of incoming light signals from two different spatially restricted sensors. These models can be applied to explain motion responses in species as diverse as humans and insects. Thus, mapping the underlying neural circuits has been a long-standing interest to understand how paradigmatic neural computations are implemented in neuronal networks. The fruit fly *Drosophila* has been shown to display extensive behavioral responses to motion cues. Given its advantages as a genetic model organism with a comparably small brain, significant progress has been made towards mapping motion detection circuits using a combination of connectomics, physiology, genetics, and behavior. Two distinct motion pathways connect photoreceptors to direction-selective neurons in the lobula and the lobula plate through interneurons in the lamina and the medulla. These two pathways are specialized to detect moving light increments (ON) and moving light decrements (OFF). Core circuits have been identified which are believed to represent the neural substrate of the two-point correlation motion computation models. Nonetheless, the functional architecture of the circuits remains incompletely understood. In particular, a neuron type with wide-field responses (under some stimulus conditions) is behaviorally required in the OFF pathway for motion computation. This finding challenges the purely local two-point correlation assumption. Furthermore, recent physiological studies support a model that implements three-point correlations. Therefore, it is important to understand the circuit at the level of single cell visual response properties to be able to map the algorithmic steps leading to motion detection. Here we investigate the receptive field properties of behaviorally critical neurons for motion detection using in vivo two-photon calcium imaging with the genetically encoded GCaMP6f sensor using a variety of visual stimuli. We are testing how receptive fields are modulated under varying stimulus conditions, and we are probing the function of individual presynaptic circuit elements. We aim to understand how receptive fields are, e.g., shaped by wide-field neurons, or by locally restricted presynaptic inputs. We will ultimately understand how subcomponents of a receptive field can shape neural computations and animal behavior using measurements of downstream direction-selective neurons or optomotor responses.

Receptive fields of polarization-sensitive neurons of the central complex in the desert locust

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The desert locust *Schistocerca gregaria* is able to perceive the polarization pattern of the sky, which is characterized by electric field vectors, *E*-vectors, arranged tangentially along concentric circles around the sun. This pattern depends directly on the sun's position in the sky and may be used to determine geographic directions.

Specialized photoreceptors in the dorsal rim area of the compound eyes are sensitive to the plane of oscillation (polarization) of light. The central complex plays a key role in the integration of this information: Parallel 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 *E*-vector orientation is represented topographically, mapping a range of 360° of *E*-vector orientations across the slices of the bridge. While the *E*-vector tuning of the involved neuron types is relatively well known, their receptive fields regarding the celestial position of polarized light stimuli 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 on the hemisphere above the locust. Neurobiotin tracer injection allowed for identification of neuron types and reconstruction of anatomical relationships. We found that individual columnar neurons have receptive fields directed at different parts of the sky and that their *E*-vector tunings are arranged in concentric circles, matching the sky-polarization pattern of a distinct solar position. This matched-filter property theoretically allows a single cell to unambiguously determine the solar azimuth from the sky-polarization pattern alone. Funded by DFG grant HO 950/24-1.

Response properties of first-order interneurons in the fly visual system

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The interpretation of the visual world by the brain is vital for proper behavioral responses and thus survival. 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 navigation. In the *Drosophila* visual system, the core motion-detecting circuits have recently been characterized. There are two distinct pathways that are specialized to detect moving contrast increments and moving contrast decrements, or ON and OFF motion cues. These two parallel pathways split directly postsynaptic to photoreceptors, in the lamina. Whereas the lamina neuron L1 provides input to the ON pathway, the OFF pathway utilizes several inputs. The two first-order interneurons L2 and L3 are the major inputs to circuitry that detects OFF edge motion. While they are both downstream of the photoreceptors, their physiological properties are very different: calcium signals in response to prolonged light stimuli in L2 axon terminals are transient, whereas calcium responses in L3 axon terminals are sustained. Therefore, the transient L2 provides downstream circuits with information about recent changes in luminance and thus encodes contrast. However, the sustained properties of the L3 pathway might be more consistent with the encoding of overall luminance. Why do we need such fundamentally different parallel OFF pathways and what are the mechanisms that initially shape them? Given that L2 and L3 are just postsynaptic to photoreceptors, response kinetics in L2 and L3 cannot arise from distinct presynaptic circuits. The distinct physiological properties of the lamina neurons could either be shaped by cell autonomous mechanisms or by downstream circuits. We are using a combination of genetic manipulation and in vivo two photon calcium imaging to distinguish these possibilities. Preliminary data suggest that the underlying mechanisms for shaping the sustained calcium signals in L3 are at least partially cell-autonomous. We are currently imaging calcium signals in different subcellular compartments to test if the dendritic signals of L3 are already sustained, or if this is a transformation that is happening within these neuron. In order to identify the molecular mechanisms that shape the L3 physiology, we are in the process of testing the function of a set of molecules that shows differential expression in lamina neurons throughout pupal development, [1]. We are functionally testing candidates, using specific genetic tools to either overexpress them or generate cell type specific mutants.

Given that we and others recently mapped the downstream circuitry and know the behavioral function associated with L2 and L3, we can link any of the above molecular or circuit manipulations back to downstream function and behavior.

[1] Tan L, Zhang KX, Pecot MY, Nagarkar-Jaiswal S, Lee PT, Takemura SY, et al. Ig Superfamily Ligand and Receptor Pairs Expressed in Synaptic Partners in *Drosophila*. *Cell* 2015;163:1756–69. doi:10.1016/j.cell.2015.11.021.

Rhodopsin 7 (Rh7) is crucial for fine-tuning light sensitivity in *Drosophila melanogaster*

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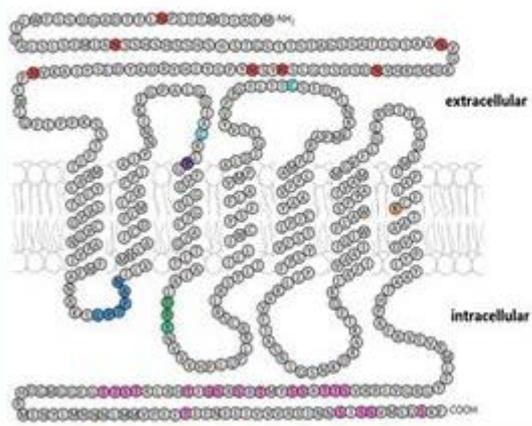
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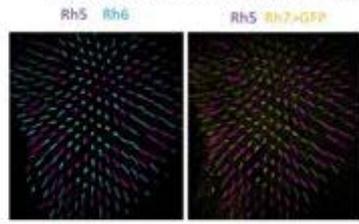
Rhodopsins are the major photopigments in the fruit fly *Drosophila melanogaster*. *Drosophila* express six well-characterized Rhodopsins (Rh1–Rh6) with distinct absorption maxima and expression pattern. Phylogenetic trees based on protein sequences suggest that Rhodopsin 1, 2, and 6 are very similar to vertebrate melanopsins, while Rhodopsin 3, 4, and 5 are more similar to other insect opsins. In 2000, when the *Drosophila* genome was published, a novel Rhodopsin gene was discovered: *Rhodopsin7* (*Rh7*), which is highly conserved among the *Drosophila* genus and is also found in other arthropods. Even though *Rh7* is related to other insect opsins, it also differs from them by its nucleotide and protein properties and forms its own subgroup within the group of insect opsins.

Although *Rh7* was discovered in the genome of *Drosophila melanogaster* 16 years ago, its ability to encode a functional Rhodopsin protein had not been determined yet. Here we show that Rh7 is indeed expressed in receptor cell 8 (R8) of the fruit fly compound eyes. Electrophysiological analyses indicate that Rh7, unlike other Rhodopsins, reduces the amplitude of the electroretinogram. This is true at all wavelengths and especially pronounced after dark-adaptation. Furthermore, Rh7 seems to be important for modulating light signaling in the Rh6-positive R8 cells and seems to prevent dark-adapted flies from over responding to light in the morning. However, Rh7 can neither activate the phototransduction cascade nor substitute for Rh1 in the outer receptor cells (R1-6). Our results indicate that Rh7 is crucial for fine-tuning light sensitivity of the flies' compound eyes by interacting with Rh6 and, therefore, point to a new mechanism of Rhodopsin function.

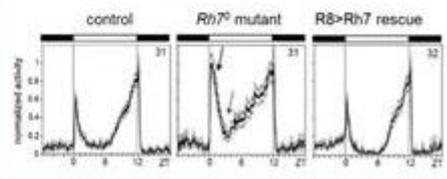
A Rhodopsin 7 (Rh7) protein structure



B Rh7 expression in *Drosophila* retina



C Rh7 influences the activity pattern



Saccadic strategy in walking *Drosophila melanogaster*

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Due to the small stereobasis and interocular overlap, most insects lack stereoscopic vision. Therefore, other cues for distance estimation become prevalent, for example the retinal image shift induced by self-motion (optic flow). During translational movements, close objects will travel faster across the retina than distant ones, whereas during rotational movement all objects move with the same speed. Therefore, only translational movements provide distance information¹. Insects overcome this problem by using a saccadic strategy, which consists of very short and fast rotations, called saccades^{2,3}, that disrupt long translational movements. This strategy has been found in different insects⁴⁻⁶. We found that walking *Drosophila melanogaster* perform body saccades but omit head-saccades. A simple mathematical model revealed that the lack of head saccades might be due to the rather large acceptance angles of the photoreceptors and the resulting coarse spatial resolution⁷.

We further investigated other causes of adaption to the saccadic strategy. Different types of visual impairment and darkness have a severe influence on the saccadic walking strategy. Wildtype flies show an anticorrelation of trust and yaw velocities, whereas slip and yaw movement are strongly correlated. Depriving flies from daylight for several generations attenuates these correlations.

Thus, even though the saccadic strategy is vital to the optimisation of optic flow, it is still a plastic behaviour.

1. Koenderink, J. J. & Doorn, A. J. Facts on optic flow. *Biol. Cybern.* 56, 247–254 (1987).
2. Collett, T. S. & Land, M. F. Visual Spatial Memory in A Hoverfly. *J. Comp. Physiol.* 100, 59–84 (1975).
3. Geiger, G. & Poggio, T. On head and body movements of flying flies. *Biol. Cybern.* 25, 177–180 (1977).
4. Schilstra, C. & van Hateren, J. H. Stabilizing gaze in flying blowflies. *Nature* 395, 654 (1998).
5. Geurten, B. R. H., Kern, R., Braun, E. & Egelhaaf, M. A syntax of hoverfly flight prototypes. *J. Exp. Biol.* 213, 2461–75 (2010).
6. Boeddeker, N. The fine structure of honeybee head and body yaw movements in a homing task. *Proc. R. Soc. B Biol. Sci.* 277, 1899–906 (2010).
7. Geurten, B.R.H., Jähde, P., Corthals, K. & Göpfert M.C. Saccadic body turns in *Drosophila melanogaster*. *Front. Behav. Neurosci.* 8:365 (2014)

Studying the Heterochrony of Central Complex Development

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The central complex is an insect brain neuropil with higher order functions such as locomotor control or spatial orientation. Its structure is highly conserved in adult insects, but astonishingly the developmental timing differs greatly between species. For instance, in *Schistocerca gregaria* the central complex forms during embryogenesis while in *Drosophila* it develops postembryonically. *Tribolium* takes an intermediate position where the central complex forms partially during embryogenesis and is completed postembryonically. The cellular and molecular basis of such a heterochronic shift of brain development and how a conserved adult structure can show vastly different developmental schemata remains unknown.

In this work we want to elucidate the cellular mechanisms and the exact developmental timing of heterochrony by comparing central complex development of *Tribolium castaneum* and *Drosophila melanogaster*. In order to mark comparable cell groups in both species, we are generating antibodies and transgenic tools (using CRISPR/Cas9 techniques) to mark cells that share the expression of the highly conserved transcription factor *retinal homeobox (rx)*. *rx* is expressed in cells contributing to the central complex of both species and after knockdown a central complex phenotype is found in *Drosophila* as well as *Tribolium*. First, homology of a potential sub-group of *rx*-positive cells will be established in the adult brain of *Tribolium* and *Drosophila*. The developmental process will then be followed back from the adult to the embryo where *rx* expression starts. The detected differences will be correlated with neuroblast numbers, types, apoptosis, proliferation and cell division. This will hopefully provide insights into the cellular mechanisms underlying the heterochronic shift as well as its exact timing. We propose that the genetic marking of homologous cells and their comparison between species promises to reveal the cellular and developmental basis of brain evolution.

Systematic identification of ocellar ganglion interneurons and their projections in the brain of *Drosophila melanogaster*

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Many flying insects possess a secondary visual system besides compound eyes - the so-called ocelli. The ocelli are involved in measuring ambient light intensity as well as detecting the horizon during flight (e.g. Taylor and Krapp 2007). The fruit fly *Drosophila melanogaster* has three ocelli located on top of the head, two lateral ocelli in the posterior and one medial ocellus in the anterior. Photoreceptors of each ocellus converge into a tripartite neuropil structure, the ocellar ganglion, which is attached to the dorsal part of the fly brain. As yet, we do not know how neurons in the ocellar ganglion convey visual information to the brain.

We screened several thousand GAL4 expression driver lines of *Drosophila* to identify and describe the morphology of neurons which project from the ocellar ganglion into the brain. By using the split-GAL4 intersectional strategy (Luan et al. 2006, Pfeiffer et al. 2010), we were able to generate stable fly lines whose expression patterns are specific to ocellar ganglion interneurons. We also used the multi-color flip-out technique to provide detailed information about single cell morphology.

We found that there are at least eight distinct types of ocellar interneurons. Terminal projections of these neurons can mainly be found within the posterior neuropils of the brain, e.g. inferior bridge, posterior slope, and posterior lateral protocerebrum. Except for a few most types project ipsilaterally. These ocellar interneuron-specific driver lines can further be tested with optogenetic stimulation or silencing experiments to facilitate better understanding of their functions in visual processing.

Taylor and Krapp (2007) Adv. Insect Physiol. 34:231-316

Luan et al. (2006) Neuron 52:425-436

Pfeiffer et al. (2010) Genetics 186:735-755

Temporal dynamics of *E*-vector responses of CL1 neurons of the desert locust *Schistocerca gregaria*

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Like many other insects the desert locust *Schistocerca gregaria* is able to use sky-compass cues for spatial orientation. One of these cues is the pattern of polarized light in the sky. This pattern is caused by the scattering of sunlight in the atmosphere and consists of electric-field vectors (*E*-vectors) oriented tangentially along concentric circles around the sun.

Specialized photoreceptors, located in the dorsal rim area of the compound eye of the locust, transmit the polarization signals to transmedulla neurons that provide input to the anterior optic tubercle. Information is then passed via the bulbs of the lateral complex to tangential neurons of the lower division of the central body. Columnar neurons (CL1) connect the lower division of the central body to the protocerebral bridge and appear to be crucial elements for the establishment of a topographic representation of *E*-vectors across the slices of the bridge (Heinze and Homberg 2007, *Science* 315:995). Four different types of CL1 neurons have been distinguished by morphological features (CL1a-d, Heinze and Homberg 2009, *J Neurosci* 29:4911). The present study investigates signaling properties of CL1 neurons in order to better understand the physiological mechanisms leading to the compass-like representation of *E*-vectors in the protocerebral bridge.

We performed intracellular recordings from CL1 neurons and investigated their activity changes during presentation of rotating and stationary polarizers above the animal. For morphological identification, Neurobiotin tracer was injected into the recorded neurons. We characterized the signaling properties of CL1 neurons with regards to background activity, excitation and inhibition, as well as time course of adaptation during stimulus presentation. We used confocal laser scanning microscopy to compare morphological features between individual CL1 neurons.

All recordings so far were obtained from CL1a neurons. Physiological and morphological features suggest that at least two types of CL1a neurons exist. Both types of neuron exhibit sinusoidal changes in their activity pattern to dorsally presented, rotating *E*-vectors. Type I showed maximal inhibition during the presentation of a certain *E*-vector orientation (θ_{\min}) and no or minimal inhibition at an *E*-vector orientation 90° different from the θ_{\min} -value. In contrast type II showed polarization opponency, i.e. maximal activity increase at a certain *E*-vector orientation (θ_{\max}) and maximal inhibition during the presentation of an *E*-vector with perpendicular orientation (θ_{\min}). The presentation of stationary *E*-vector orientations elicited phasic-tonic responses in both types of neuron that were characterized by a decrease in response level at θ_{\min} over several seconds. Morphologically, the two types showed distinct differences in the trajectory of their axonal fiber to the gall of the lateral accessory lobe.

Supported by DFG grant HO 950/24-1.

The distance code in honeybee waggle dance

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The distance code in honeybee waggle dance

The human observer can read the distance (and direction) of the outbound flight to a food source from the waggle dance in honeybees. But how does the recruited bee decode this information? We measured the variance of several dance parameters and compared them with the variance of the outbound flights of recruits. Dance parameters were read from video recordings and recordings of the modulated electrostatic field emanating from the dancing bee. The flights were recorded by harmonic radar. The dancing bees were trained to food sources up to 4.5 km from the hive.

We found that the number of waggles per waggle run correlates best with the indicated distance. Each waggle phase (right/left) codes for 60 m in the range of up to 1.5 km. Further distances are encoded by longer ranges per waggle. The precise function for the distance encoding will be described by the Weber-Fechner parameter. Since the variance of the distance code in the waggle dance is larger than the variance of the outbound flights of recruits, we conclude that the recruits integrate (average) dance rounds of one or several dancers.

Since the electrostatic fields of dancers can easily be recorded at high temporal resolution large numbers of dances (> 20 million) haven been monitored so far and allow a precise location of the indicated food sources and their changes over time. These estimations are based on a model that includes the data from the decoding process of recruits.

Poster Topic

T15: Vision: Retina and Subcortical Pathways

- [T15-1A](#) A possible role for ON-bipolar cells in congenital nystagmus
Maj-Britt Hölzel, Maarten Kamermans
- [T15-2A](#) AAV mediated PTPN11 knockdown stimulates TrkB activity in neuronal cells in culture and in rat retina
Nitin Chitranshi, Vivek Gupta, Yogita Dheer, Stuart Graham
- [T15-3A](#) Analysing spatial integration in the mouse retina
Dimokratis Karamanlis, Tim Gollisch
- [T15-4A](#) Combining In-vivo and Ex-vivo Methods for Studying Blood-Brain Barrier Passage of Nanoparticles
Mohamed Tawfik
- [T15-5A](#) Connectivity map of outer retinal neurons in the mouse
Timm Schubert, Christian Behrens, Yue Zhang, Silke Haverkamp, Thomas Euler, Philipp Berens
- [T15-6A](#) Differential localization of CaMKII- and - indicates CaMKII- as a specific element in connexin36-containing gap junctions
Stephan Tetenborg, Bianca Brüggem, Ulrike Janssen-Bienhold, Karin Derek
- [T15-7A](#) Effect of early eye removal on the morphology of a multisensory neuron in the chicken optic tectum
Stefan Weigel, Katharina Lischka, Jiamin Yan, Harald Luksch
- [T15-8A](#) Electrophysiological characteristics and background activity of retinal ganglion cells under rat model of artificial hyperglycemia
Nataliia Martyniuk
- [T15-1B](#) Expression patterns of NF200, Na_v1.6, Ankyrin G and related proteins in a multimodal cell type of the avian optic tectum
Katharina Lischka, Simone Ladel, Harald Luksch, Stefan Weigel
- [T15-2B](#) Functional characterization of the signal processing chain in the mouse early visual system
Miroslav Román Rosón, Philipp Berens, Yannik Bauer, Thomas Euler, Laura Busse
- [T15-3B](#) Functional diversity of mouse retinal ganglion cells in 4096-electrode CMOS array recordings
Fernando Rozenblit, Vidhyasankar Krishnamoorthy, Tim Gollisch

- [T15-4B](#) HDAC6 inhibition by tubastatin A protects retinal cells against oxidative stress and induces autophagic clearance
Janina Leyk, Christiane Richter-Landsberg
- [T15-5B](#) Linear and nonlinear chromatic integration in the mouse retina.
Mohammad Hossein Khani, Tim Gollisch
- [T15-6B](#) Local signal processing in horizontal cells
Camille Anastasia Chapot, Christian Behrens, Philipp Berens, Sinziana Pop, Tom Baden, Thomas Euler, Timm Schubert
- [T15-7B](#) Localization of the excitatory amino acid transporters EAAT2 and EAAT5 in the neuronal network of the mouse retina
Stefan Esser, Anja Mataruga, Frank Müller
- [T15-1C](#) Morphological and functional implications of the retina in multiple system atrophy
Kathrin Kaehler, Hartwig Seitter, Bettina Tschugg, Adolf M. Sandbichler, Edith Sturm, Nadia Stefanova, Alexandra Koschak
- [T15-2C](#) On the role of common age-related beta-synuclein between visual cortex and neuroretina
Karina Hadrian, Katrin Brockhaus, Harutyun Melkonyan, Solon Thanos, Michael R Böhm
- [T15-3C](#) Optimization of electroretinographic recording from the isolated and superfused murine retina.
Toni Schneider, Jan Niklas Lüke, Jürgen Hescheler, Felix Neumaier, Walid Albanna
- [T15-4C](#) Optogenetics in the eye – development of a light-inducible gene therapy for pathological neovascularization
Sidney B. Cambridge, Eric Brandhorst, Hans-Peter Hammes
- [T15-5C](#) Pericentrin, identified at the basal-body complex in mammalian photoreceptor cells, interacts with Nesprin protein Syne-2 in the retina
Andreas Gießl, Nathalie Falk, Kristin Kessler, Marlene Lösl, Johannes Glöckner, Karsten Boldt, Marius Ueffing, Ronald Roepmen, Christian Thiel, Angelika A. Noegel, Johann Helmut Brandstätter
- [T15-6C](#) Response properties in bipolar cells and their impact on ganglion cells in the retina
Helene Marianne Schreyer, Michael Weick, Tim Gollisch
- [T15-7C](#) Retinal ganglion cell activity of Cav1.4 mutant mice
Lucia Zanetti, Hartwig Seitter, Alexandra Koschak
- [T15-1D](#) Rewiring of bipolar cells in a congenital stationary night blindness type 2 mouse model
Irem Kilicarslan, Hartwig Seitter, Enrica Strettoi, Alexandra Koschak
- [T15-2D](#) Spike correlations indicate electrical coupling between heterotypic ganglion cells
Christian Puller, Matthew I. Grivich, Alexander Sher, Greg D. Field, Jeffrey L. Gauthier, Alan M. Litke, E.J. Chichilnisky, Martin Greschner

- [T15-3D](#) Spontaneous emergence of structured responses in a random neural network *in-vitro*
Manuel Schottdorf, Julian Vogel, Hecke Schrobsdorff, Walter Stühmer, Fred Wolf
- [T15-4D](#) Synergy in random motion decoding from a population of direction-selective retinal ganglion cells
Norma Krystyna Kühn, Tim Gollisch
- [T15-5D](#) THE DYNAMICS OF ADAPTATION PROCESS TO DIFFERENT LIGHT LEVELS IN THE MOUSE RETINA STUDIED WITH ELECTRORETINOGRAMS
Anneka Joachimsthaler, Tina Tsai, Jan Kremers
- [T15-6D](#) Two-Photon Calcium Imaging of Dendritic Integration in Mouse Retinal Ganglion Cells
Yanli Ran, Katrin Franke, Tom Baden, Thomas Euler
- [T15-7D](#) Visualization of second messengers in the mouse retina using optogenetic sensors
Safaa Belaidi, Jana Gehlen, Anna Sieben, Frank Müller
- [T15-8D](#) What can a small fish teach us about visual processing?
Ronen Segev

A possible role for ON-bipolar cells in congenital nystagmus

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In the mammalian retina bipolar cells are the connections between the light-sensitive photoreceptors and ganglion cells, which relay the retinal information to higher visual centers in the brain. The cones, the horizontal cells and the bipolar cells contact each other via the ribbon synapse (Wässle, 2004). A defect in this synapse might lead to oscillations in the direction selective ganglion cells and thereby to altered signaling to the AOS and the vestibule-cerebellum. This causes symptoms that seem to be equal to which found in patients with Nightblindness-Associated Transient Tonic Downgaze (NATTD; Simonsz et al., 2009) and show mutations in genes found for congenital stationary nightblindness (CSNB, Bijveld et al., 2013).

To investigate the role of the ON-bipolar cells in NATTD three mice strains with different mutations in proteins of the ON- bipolar cell synapse will be used: nob (NYX; Gregg et al., 2003), nob2 (CACNA1F, Chang et al., 2006), nob3 (GMR6, Maddox et al., 2008). Furthermore, the ganglion cell response of the Cav1.4 IT mice, which present a model for the congenital stationary night blindness type 2 (Knoflach et al., 2013), will be determined. To identify the ON-dsGCs for the single cell recordings, the mice will be crossbred with SPIG1 GFP mice (Yonehara et al., 2008; Yonehara et al., 2009).

This mouse models show, like the human NATTD patients, an altered ERG phenotype reflected in a reduced a-wave and an absent b-wave (Chang et al., 2006; Maddox et al., 2008; Mansergh et al., 2005). The measurement of the optokinetic reflex showed eye movement oscillations at a frequency of 5 Hz, which are also in line with the human patients (Winkelman et al., unpublished). MEA results show oscillations at about 5 Hz in nob mice (Gregg et al., 2007). It is therefore very likely that the oscillations of the ON-dsGCs lead to altered AOS signaling and therefore to the nystagmus mediated by the oculomotor neurons.

The poster will overview the PhD-project "A possible role of ON- bipolar cells in congenital nystagmus". This contains beside the preliminary results, light responses of ON-dsGCs originating from the NATTD mouse models obtained by whole-cell patch clamp recordings.

AAV mediated PTPN11 knockdown stimulates TrkB activity in neuronal cells in culture and in rat retina

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Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (Shp2), encoded by the PTPN11 gene, is a non-receptor phosphotyrosine phosphatase. PTPN11 acts as a regulator of tyrosine kinase receptor signaling, particularly TrkB. Brain Derived Neurotrophic Factor (BDNF) and its high affinity receptor TrkB, play important role in protecting the neuronal cells and retinal ganglion cells (RGCs). Rendering of neurotrophin factors has unsuccessful stories on BDNF/ TrkB activation and gene therapy to modulate BDNF/TrkB has also shown only short-lived protective effects on the neuronal cells, RGCs. Here, we investigated the effects of PTPN11 knockdown on the TrkB activation. PTPN11-shRNA sequences was incorporated into the AAV2 vector under cytomegalovirus chicken actin hybrid promoter linked with green fluorescent protein (GFP). SHSY5Y cells were transduced with each of the PTPN11-shRNA and scramble control viral constructs. PTPN11 knockdown did not exhibit any significant effects on TrkB but TrkB Y515 activity increased. The role of PTPN11 and TrkB in mediating endoplasmic reticulum (ER) stress were evaluated by treating neuronal cells with TrkB antagonist CTX-B, which resulted in upregulation of ER stress proteins. Interestingly, the ER stress response associated with CTX-B treatment was decreased upon subsequent AAV mediated PTPN11 knockdown. AAV scramble transduction of CTX-B treated cells were used as controls. Selective AAV2 mediated PTPN11 knockdown in the neuronal cells pre-treated with neurotoxic soluble A-beta42 (A 42) however, resulted in ablation of this response for ER stress markers proteins as detected using western blotting. Transduction of AAV2 expressing scramble sequence was used as experimental control. The effects of PTPN11 knockdown on the rat retina were evaluated by intravitreal injection of PTPN11 AAV2 construct along with corresponding scramble controls individually into the rat eyes. Briefly, 5µl of AAV2 constructs were injected intravitreally into SD rats (2×10^{10} vg). Anti-GFP staining in retinal sections demonstrated AAV2 transduction in the RGC layer. Anti-NeuN/ anti-PTPN11 staining confirmed the ganglion cell specific knockdown of PTPN11. Rat retinas were assessed for inner retinal functional changes using scotopic threshold response (STR) measurements. Retinal structural changes were assessed using histological analysis. Results indicate that pSTR amplitude which primarily reflects the function of the RGCs was not diminished in the PTPN11 knockdown model. Hematoxylin and eosin staining also revealed no degenerative changes primarily associated with the inner retina. Assessment of optic nerve sections using Bielschowsky's staining further identified no reduced axonal density. On the other hand, AAV mediated PTPN11 knockdown lead to increased Y515 phosphorylation of TrkB compared to the scramble control in rat RGCs. Concluding, our findings strongly indicate that PTPN11 genetic knockdown holds the promise to regulate neuronal cell survival through its effects on TrkB activation. PTPN11 knockdown exerts no detrimental impact on the inner retinal health. Further studies involving rescue of RGCs damage and other retinal disorders phenotype using PTPN11 knockdown in animal models will substantiate these observations and provide mechanistic insights into the roles of neurotrophic factor regulation in retina.

Analysing spatial integration in the mouse retina

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Retinal ganglion cells receive excitatory input in their dendritic trees from bipolar cell terminals. The bipolar cells form the spatial subunits of a ganglion cell's receptive field center. The output of those subunits has to be integrated and then translated into a ganglion cell's spike train. It has long been suggested that this integration can be either linear or nonlinear, and different types of nonlinearities have been identified via closed-loop experiments [1]. Different types of integration might be used by retinal ganglion cells, according to the features of the light scene they are extracting.

We characterize ganglion cells in the mouse retina based on their spatial integration properties via classical open-loop experiments. The retina is stimulated *in vitro*, while the ganglion cell responses are being recorded with a multielectrode array. For each stimulus presentation, a pair of light contrasts is randomly selected and assigned to the tiles of a checkerboard-like pattern. The size of the tiles is such that they can stimulate individual subunits inside a cell's receptive field center. We then identify, for each cell, pairs of contrasts that elicited the same spike counts (iso-response stimuli) offline. This method can indicate the type of integration that precedes the ganglion cell-intrinsic nonlinearity [1]. Based on that information, we classify cells according to the type of integration they show. Furthermore, we compare this classification to others based on specific retinal computations, such as direction selectivity.

References:

1) Bölinger D, Gollisch T. Closed-loop measurements of iso-response stimuli reveal dynamic nonlinear stimulus integration in the retina. *Neuron*. 2012 Jan 26;73(2):333-46.

Acknowledgements

This work is supported by the DFG (GO 1408/2-1 and SFB 889, C1). Dimokratis Karamanlis is supported by a DAAD Scholarship for Graduates of All Disciplines.

Combining In-vivo and Ex-vivo Methods for Studying Blood-Brain Barrier Passage of Nanoparticles

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Objectives

The method of in-vivo Confocal Neuroimaging of the retina (ICON), which was established in our laboratory several years ago, has been proven as a valuable tool for investigating the passage of purpose-designed, fluorescent nanoparticles (NPs) across the blood-brain barrier (BBB) in living rats. However ICON has a number of limitations, including an optical resolution which is not high enough to detect (sub-) cellular structures. To improve the outcome of experiments, in addition to ICON, we utilized an ex-vivo whole mount retina preparation. Imaging of the excised retina will allow us to determine the NPs' distribution within the tissue, i.e. whether NPs remain within the lumen of vessels, accumulate in the vessel walls or cross the BBB and, in the latter case, in which structure of the parenchyma NPs are localized, up to sub-cellular localization.

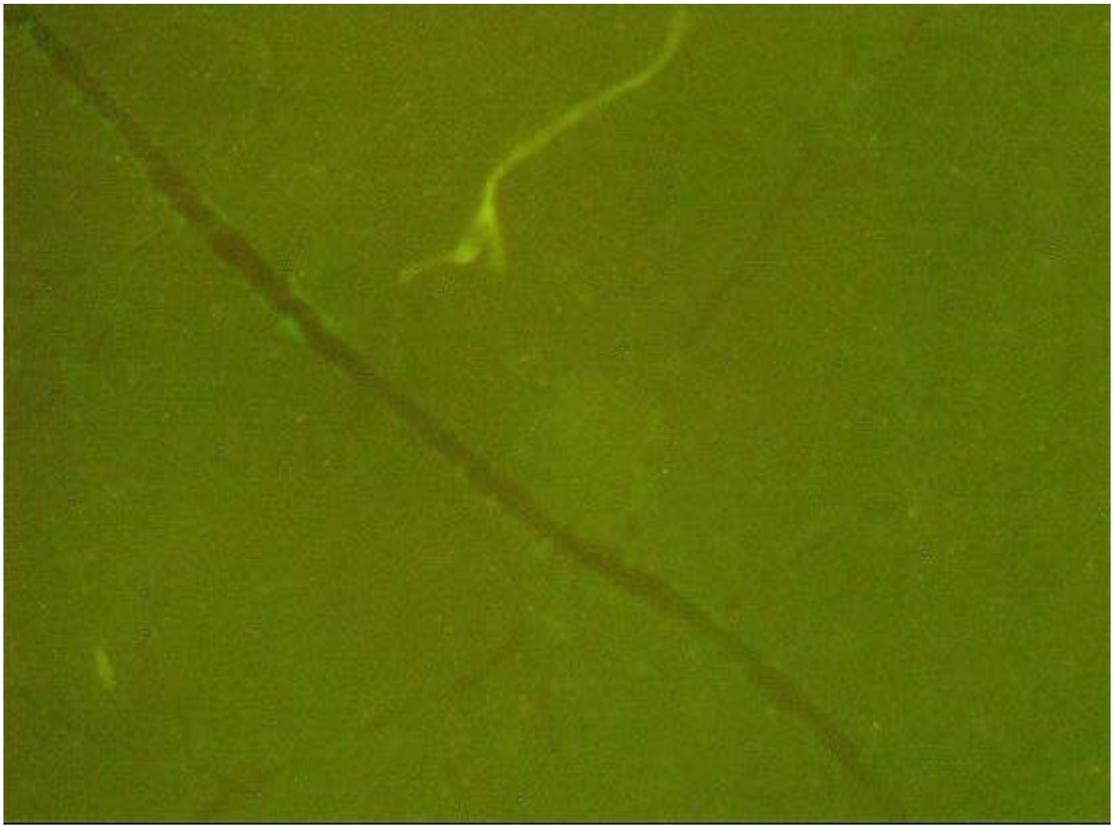
Methods and Results

The fluorescent-labelled polymeric NPs, alone or together with a fluorescent dye (FITC-dextran) were injected into the tail vein of Lister-Hooded rats and ICON experiments were performed. ICON allowed us to confirm the successful application of the dye and NPs by imaging the fluorescence in the retina. With this technique we followed in-vivo the kinetic of the NPs' distribution, and determined further whether the distribution of the fluorescent signal indicated a vascular and/or parenchymal distribution. After injection a first initial flash was detected with ICON when the fluorescent solution applied reached the retinal vessels. When the intensity of the fluorescent signal in the retina stabilized, the rats' eyes were enucleated and whole mount retina was prepared. In our standard procedure the retina was transferred on a 12 mm Millicell insert membrane and kept in Hepes buffered saline on ice. Images were taken from multiple areas of the retina with a laser confocal microscope (image A) or standard fluorescence microscope using video camera (image B)

With this combined in-vivo / in-vitro protocol it was shown that the vessel walls were regularly labeled by FITC-dextran, which suggests that the dye can be up taken by the endothelial cells. Depending on the nature of NPs applied, the fluorescence could be detected also in the retinal tissue. Further experiments using multiple labeling will allow us to determine cell type and structures of NPs accumulation.

Conclusion

The described method combines the advantages of both in vivo and in vitro experiments, as the NPs' administration and distribution takes place in vivo, i.e. with an intact BBB and without the artifacts of cell culturing. The analysis of distribution, however, can be performed with high resolution fluorescent imaging without the disruptive procedures of histological preparation.



Connectivity map of outer retinal neurons in the mouse

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In the mouse retina, two types of cone photoreceptors – short (S-) and medium (M-) wavelength-sensitive – provide input to 13 types of cone bipolar cell (CBC). Type 1 CBCs are thought to exclusively receive input from M-cones, whereas type 9 CBCs selectively contact S-cones (Breuninger et al., 2011). However, it is unknown to which degree the input of the other CBC types is dominated by S- and/or M-cones. In addition, the exact micro-connectivity for most CBC types is unclear, e.g. if a CBC contacts all cones within its dendritic field and how densely individual cones are contacted by the different CBCs. Furthermore, it was proposed that some rod bipolar cells (RBCs) contact cones, and that some OFF CBC types contact rods (Tsukamoto & Omi, 2014). In addition, the photoreceptor output is modulated by reciprocal feedback provided by horizontal cells. However, the complex connectivity pattern of photoreceptors, horizontal and bipolar cells at the level of the photoreceptor axon terminal has not been studied quantitatively.

Here, we exploited the serial block-face scanning electron microscopy data of mouse retina published by Helmstaedter et al. (2013) to systematically analyze the connectivity between photoreceptors, horizontal cells and bipolar cells. Using volume segmentation, we reconstructed horizontal cells and photoreceptor axon terminals, and identified S-cones based on their specific contacts with type 9 CBCs. Using an automated classification approach, we quantitatively analyzed the contacts between photoreceptors, horizontal and bipolar cells.

We confirmed that type 9 ON CBCs (CBC9) sample mostly from S-cones, whereas type 1 OFF CBCs almost exclusively contact M-cones. None of the other CBC types showed any cone type-specific preference, suggesting that CBC9 (blue ON) and CBC1 (green OFF) represent the sole chromatic bipolar cell channels in the (dorsal) mouse retina. In contrast, most other CBC types contacted almost all cones within dendritic reach. Interestingly, CBC8 and XBCs contacted substantially fewer cones than expected from their large dendritic arbors. Surprisingly, XBCs rarely made invaginating contacts but instead had “tip-to-tip” contacts at the basal zone of the cone terminal, rather resembling “classical” OFF than ON bipolar cell synapses. In line with earlier studies, only CBC3a, CBC3b and CBC4 of the OFF bipolar cells contacted rods. CBC3b sampled from substantially more rods than the other two OFF CBCs, suggesting that CBC3b forms the dominant OFF channel for rod-mediated vision. On the other hand, ~80% of the cones provided output to at least one RBC, and ~75% of the RBCs contacted at least one cone, indicating that cones may make a significant contribution to the (primary) rod pathway during photopic conditions.

In summary, we provide a detailed, quantitative “connectivity map” of the outer retina of the mouse. We reveal some unexpected features (e.g. very sparse, atypical cone contacts of XBCs, substantial cone contribution to RBCs) with potentially interesting functional consequences for retinal pathways.

Funding: DFG (EXC 307 to TE; BE 5601/1-1 to PB; SCHU 2243/3-1 to TS), BCCN Tübingen (FKZ 01GQ1002 to PB).

Differential localization of CaMKII- α and - β indicates CaMKII- β as a specific element in connexin36-containing gap junctions

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Gap junctions formed by the neuronal connexin36 (Cx36) couple numerous cell types in the mammalian retina. These connections are fundamental for the processing of visual information and were shown to underly a remarkable plasticity: several signalling pathways regulate the extent of gap junctional coupling in a light-dependent manner. All amacrine cells, key interneurons of the most light-sensitive retinal pathway, are strongly coupled under mesopic conditions but weakly coupled under scotopic and photopic light levels. Previous studies described that phosphorylation of Cx36 at serine 293 by calcium/calmodulin-dependent kinase II (CaMKII) is correlated with increased All amacrine cell coupling (Kothmann et al., 2012). However, it remains unknown which one of the two neuron-specific isoforms, CaMKII- α or - β , mediates these effects. In order to get a detailed view on the isoform involved in gap junction regulation, we analyzed the distribution of both isoforms at Cx36-containing gap junctions in mouse retinas. Although CaMKII- α and - β are able to form heteromers (Lisman et al., 2002), we found a differential localization of both isoforms: CaMKII- β was primarily expressed in starburst amacrine cells, in which electrical coupling is absent. In contrast, CaMKII- α showed almost complete colocalization with Cx36. Confocal and STED microscopy revealed that this colocalization was connexin isoform-specific as most Cx45-containing gap junctions lacked CaMKII- α . Co-immunoprecipitations confirmed these results and showed a selective association between Cx36 and CaMKII- α but not between Cx36 and CaMKII- β . As our data suggest a specific role for CaMKII- α in Cx36-containing gap junctions, we further analyzed the expression of CaMKII- α in Cx36-deficient retinas. Deletion of Cx36 was accompanied by strong down-regulation of CaMKII- α expression, suggesting the correlated expression of both proteins. This was also observed during the postnatal development of the retina because CaMKII- α expression was closely linked to the formation of Cx36-containing electrical synapses. In conclusion, our data indicate that both CaMKII isoforms serve distinct functions in the mammalian retina. Most importantly, CaMKII- α was identified as specific element of Cx36-containing electrical synapses, likely mediating the light-dependent phosphorylation of Cx36 in retinal neurons.

References

Kothmann, W.W., Trexler, E.B., Whitaker, C.M, Li, W., Massey, S.C., and O'Brien, J. (2012).

Nonsynaptic NMDA receptors mediate activity-dependent plasticity of gap junctional coupling in the All amacrine cell network. *J. Neurosci.* 32, 6747–6759.

Lisman, J., Schulmann, H., and Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat Rev Neurosci.* 3, 175-190.

This work was supported by the Deutsche Forschungsgemeinschaft (grant DE1154/5-1 to KD and JA 854/3-1 to UJB; stipend to BB within

Effect of early eye removal on the morphology of a multisensory neuron in the chicken optic tectum

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The vertebrate midbrain is an important subcortical area involved in different functions such as integrating different sensory modalities, movement initiation and bottom-up attention. In chicken, the optic tectum (TeO, homologous to the mammalian superior colliculus) is organized in 15 layers with distinct in- and output regions. Visual input targets the superficial layers, while auditory input terminates in deeper layers. In the precocial chicken, these sensory pathways have to be established in a highly precise manner during development through a complex pattern of molecular guiding cues. Several studies examined the influence of peripheral structures on the developing central nervous system by ablation of a sensory input. In the TeO, Kelly and Cowan (1972) reported that the cellular patterning and layer size change when retinal ganglion cell afferents are absent in the superficial layers. Luksch and Poll (2002) showed that a neuron type, which receives visual input from the retinal ganglion cells, does not change its gross morphology after early enucleation of the eye anlagen. Only the shape of dendritic endings was changed presumably due to missing presynaptic input during synaptic pruning.

We are interested in the effect of deafferentation of one sensory input in multisensory neurons. A candidate for a multisensory processing cell type is the Shepherd's crook neuron (SCN) in the TeO. SCNs have distinct dendritic branches in retinorecipient layers (superficial layers 1 to 5 & 7) and in layers where auditory input terminates. Therefore, this candidate for multimodal integration is ideal to study the effect of deafferentation of one sensory input without affecting the other sensory inputs.

Our hypothesis is that the lack of retinal ganglion cell afferents in the superficial layers only influences the morphology of the apical dendrites in SCNs, while other dendritic structures remain unchanged. Therefore, we removed the eye anlagen unilateral at stage HH 11 (day 2 of embryogenesis). We retrogradely labeled SCNs at different stages later in embryogenesis to visualize the morphology of SCNs in lesioned and non-lesioned embryos.

Our preliminary data show a changed morphology of SCNs after unilateral enucleation. The lack of retinal ganglion cell terminals leads to an altered growth of the apical dendrites of SCNs in the superficial layers, while we did not observe changes in the basal dendrites in the deeper layers.

Literature

Kelly J P and Cowan W M (1972) Studies on the development of the chick optic tectum. III. Effects of early eye removal. *Brain Research*, 42, 263-288.

Luksch H and Poll A (2002) Early enucleation does not alter the gross morphology of identified projection neurons in the chicken optic tectum. *Neuroscience Letters*, 331, 41-44.

Electrophysiological characteristics and background activity of retinal ganglion cells under rat model of artificial hyperglycemia

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It is known that impairment of visual function has been detected in the early stage of diabetes but the underlying neural mechanisms are still unknown. Our work was aimed on the investigation of electrophysiological properties; sodium, potassium, calcium currents and background activity of isolated retinal ganglion cells (RGCs) of two-month rats using the model of streptozotocin (STZ)-induced diabetes mellitus (DM). Experiments were performed using the patch-clamp techniques in the whole cell configuration (voltage-clamp mode). Our result demonstrated that after 1 months of diabetes resting membrane potential of RGCs decreased significantly by 13%, while other characteristics such as input impedance and capacitance of the membrane remained unchanged. At this stage of diabetes, we detected that generation frequency of background excitatory postsynaptic currents (EPSCs) was three times less than in RGCs of control animals. Analysis of time constant (τ) of some EPSCs pointed that deterioration in frequency of spontaneous spiking activity of RGCs in DM rats partly became a result of reduced inhibitory signalling transmission to the cells. Furthermore, we found significant suppression of potassium and sodium currents.

Thus even after one month third of STZ-induced diabetes rats had a significant reduction in the frequency of background activity, moderate deterioration electrophysiological characteristics and currents of RGCs. Results shown that the suppression of frequency of background activity was not caused by changes in its electrophysiological characteristics of RGCs due to its relatively small oppression. Reflect changes were a result of induced pathology. Hence, diabetes on the early stages affects the neurons of retina and synaptic transmission between them.

Expression patterns of NF200, Na_v1.6, Ankyrin G and related proteins in a multimodal cell type of the avian optic tectum

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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 computation of multisensory integration. 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.

Here we present immunohistochemical data of the structural proteins NF200 and Ankyrin G, ion channels Na_v1.6 and K_v1.2 and myelin. The structural protein NF200 is strongly expressed in the entire axon from its origin on. In contrast, the distribution of Na_v1.6 channels on the axon is primarily located at a specific region on the axon. The voltage-gated potassium channel subtype 1.2 is restricted to the cell soma. The structural protein Ankyrin G is mainly expressed from the axon origin and ends at the position where Na_v1.6 expression begins. Combining these expression patterns we can locate the initial segment of the axon. Interestingly, we find variations in the expression patterns which suggest diverse SCN subtypes. The molecular identification of the axon and ion channel distribution in SCN allows delineating the information flow and particularly the integration of sensory modalities in the TeO of the avian midbrain.

Functional characterization of the signal processing chain in the mouse early visual system

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More than 30 types of retinal ganglion cell (RGC) represent parallel channels transmitting different aspects of visual information from the retina to various parts in the brain. Retinal output is most directly conveyed to the cortex via the retino-geniculo-cortical pathway, comprised of RGCs, relay cells in the dorsolateral geniculate nucleus (dLGN) and the primary visual cortex (V1). It has long been known that this pathway is not homogenous but consists of parallel channels, each carrying specific information. However, it is still debated which RGC types project to the dLGN and how their output is transformed at the level of the dLGN. Here, we started to characterize, in the mouse model, the functional properties of dLGN-projecting RGCs and to compare responses of RGCs and dLGN neurons to the same set of visual stimuli.

We selectively labeled and physiologically characterized dLGN-projecting RGCs. To this end, we injected a retrograde herpes simplex virus (HSV), expressing Cre recombinase, into the dLGN of a Cre/loxP reporter mouse line. The transfection of RGC terminals and the subsequent Cre expression, led to activation of the genetically-encoded calcium indicator, GCaMP6f. This enabled us to selectively record light-evoked calcium responses in dLGN-projecting RGCs. Visual stimuli included frequency/contrast modulated full-field flicker, dense noise, moving bar, and chromatic stimuli, allowing us to match the recorded cells to the functional types identified by Baden, Berens, Franke et al. (2016).

In a separate set of experiments, we characterized the responses of dLGN neurons to the same visual stimuli using in-vivo extracellular multi-electrode recordings in the dLGN of awake, head-fixed mice. We implemented a clustering framework for separating dLGN neurons into functional types, based on features extracted from their responses to the different visual stimuli, and found a much richer representation than previously expected.

In conclusion, this study promises to yield a functional characterization of the population of dLGN-projecting RGCs and the dLGN neurons, and to provide fundamental insights into how the representation of visual information changes along the first stages of the retino-geniculo-cortical pathway.

Funding: This research project/publication was supported by the DFG (EXC 307, CIN), the Bernstein Centre for Computational Neuroscience Tübingen (BMBF FKZ 01GQ1002) and by LMU Munich's Institutional Strategy LMUexcellent within the framework of the German Excellence Initiative.

Functional diversity of mouse retinal ganglion cells in 4096-electrode CMOS array recordings

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The retina is a complex neural network, responsible for breaking down the visual scene into its constitutive features such as color, motion and local contrasts. Retinal ganglion cells, the output layer of the retina, are the only input of visual information to the brain and are responsible for relaying those visual features to specific areas of the brain. Retinal ganglion cells are known to form a diverse population, with more than 30 ganglion cell types currently expected based on anatomical and physiological considerations. Since each type of ganglion cell relays distinct visual information to different regions of the brain, it is important to understand this functional diversity. Previously, much of this functional diversity has been studied by combining ganglion cells' responses across many retinal preparations. Here, we stimulate a mouse retina using a standard battery of light stimuli and simultaneously record the electrical activity from a large population of ganglion cells – in whole-mount retinal preparations – using 4096-electrode CMOS arrays. Using an offline spike-sorting method, we recover spikes from 500 to 1000 ganglion cells in a typical preparation. Analyzing the ganglion cells' response to the stimuli, we measure their receptive field's extent and temporal dynamics, spike-train autocorrelation functions, and direction selectivity. Based on these ganglion cell properties, we assign to each ganglion cell a coordinate in a multi-dimensional space. We then use an unsupervised learning method (spectral clustering) to cluster ganglion cells into groups of functionally-similar cells. Ganglion cells in these functional groups are finally tested for homogeneity in their responses to light stimuli, their axonal conductance speeds and whether a minimum pairwise distance (tiling) is respected between their receptive fields.

Acknowledgments: This work was supported by the Deutsche Forschungsgemeinschaft (GO 1408/2-1 and SFB 889, C1), and by the European Commission (FP7-ICT-2011.9.11/600954, "VISUALISE").

HDAC6 inhibition by tubastatin A protects retinal cells against oxidative stress and induces autophagic clearance

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Retinal degenerative diseases, such as retinitis pigmentosa (RP) and cone dystrophies, are characterized by progressive loss of photoreceptors. Recent studies suggest a role for histone deacetylases (HDACs) in neuro- and retinal degeneration. In a mouse model for RP excessive HDAC activation was observed and HDAC inhibition prevented photoreceptor cell death in mouse models for RP and cone dystrophies. HDAC6 is unique among the 18 members of the HDAC family since it mainly deacetylates non-histone proteins, such as α -tubulin, heat shock protein (HSP) 90 and peroxiredoxin 1 (Prx1), which is involved in the reduction of hydrogen peroxide (H_2O_2). By having these and further substrates, HDAC6 is involved in cellular stress response mechanisms, such as the heat shock response, the ubiquitin proteasome system, autophagy and redox regulation. Inhibition of HDAC6 has been implicated to be protective in models for neurodegeneration. This study aimed to elucidate functions of HDAC6 and the effect of its inhibition on oxidative stress, which is a major cause for retinal degeneration, in cone photoreceptor-like 661W cells. Furthermore, we investigated the influence of HDAC6 inhibition on the autophagic flux and on proteasomal inhibition since defects in the proteolytic machinery have been implicated to be involved in retinal degeneration.

Morphological data and cell viability tests revealed that HDAC6 inhibition, by the specific inhibitor tubastatin A (TST), is protective against H_2O_2 induced oxidative stress in 661W cells. Protection is mediated by the regulation of peroxiredoxin 1 activity rather than by the TST-mediated increased heat shock response. Also, autophagic activity was enhanced in response to HDAC6 inhibition. This was demonstrated by an increase in LC3-positive vesicles, by higher levels of the autophagosome marker LC3-II, by a reduction of p62 and finally by combined treatments using TST and the autophagy inhibitor bafilomycin A or the autophagy inducer rapamycin, respectively. Interestingly, besides the beneficial consequences of TST treatment, HDAC6 inhibition exerts cytotoxic effects in response to proteasomal stress. MG-132, a proteasomal inhibitor, induces protein aggregate formation, which is a common hallmark of neurodegenerative diseases. TST prevents aggregate formation but leads to reduced cell viability when compared to MG-132 treated cells.

Taken together, HDAC6 inhibition by TST seems to be beneficial since it provides a protective means against oxidative stress and since it activates the heat shock response as well as the autophagic process. However, TST is not protective against proteasomal stress, but rather detrimental. Thus, further evaluation of TST induced effects on cellular stress situations, which occur in retinal diseases, has to be conducted to clarify whether HDAC6 inhibition might be a suitable therapeutic tool to fight retinal degeneration. ↪

Linear and nonlinear chromatic integration in the mouse retina.

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In the retina, different photoreceptor types separate the light input into different chromatic signals. The retinal ganglion cells receive these input signals through intermediary bipolar cells and integrate them to form their output signals. Moreover, the retinal ganglion cells integrate chromatic inputs over a wide range of brightness from mesopic (rod-cone mediated) to photopic (cone-mediated) light levels. Here, we studied the neuronal mechanisms of chromatic signal integration in the mouse retina through multi-electrode array recordings. We asked how the retinal ganglion cells integrate their different chromatic inputs and whether the chromatic integration properties of ganglion cells change over different light levels. To do so, we stimulated the retina with visual stimuli that have different combinations of UV and green light with opposing contrast. We found that the majority of recorded mouse ganglion cells integrate chromatic signals in a linear fashion under photopic light levels and a small population of ganglion cells in a nonlinear fashion. For linear ganglion cells we found a combination of UV-green contrasts that causes the ganglion cells to reduce their spiking activity to baseline. For nonlinear ganglion cells, on the other hand no combinations of opposing UV-green stimuli lead to baseline activity. We then categorized ganglion cells based on their chromatic integration properties into groups such as linear ON and OFF, linear-thresholding ON and OFF and linear ON-OFF cells along with nonlinear OFF and nonlinear ON-OFF cells. We further checked the influence of the receptive field center vs. surround for chromatic integration properties of ganglion cells. Using computational models, such as the linear-nonlinear-linear model, we simulated how ganglion cells transform their stimulus-driven activation to spiking activity. Extending these studies to mesopic light levels where the ganglion cells receive rod-mediated inputs lets us investigate whether contributions from rod photoreceptors affect the nature of chromatic signal integration. Together, this helps us obtain a better understanding of the fundamental mechanisms of neuronal signal integration.

Acknowledgments

This work was supported by the DFG (GO 1408/2-1 and SFB 889, C1) and by the European Commission (FP7-ICT-2011.9.11/600954, "VISUALISE").

Local signal processing in horizontal cells

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The mouse retina contains two types of cone photoreceptors (cones), the “true” S-cones (Haverkamp et al., 2005), which exclusively express a short wavelength-sensitive S-opsin, and the M-cones, most of which co-express the medium wavelength-sensitive M-opsin and the S-opsin with a dorso-ventral gradient of S/M opsin ratio increasing towards the ventral edge of the retina (Szél et al., 1992). A single type of GABAergic interneuron, the horizontal cell (HC), samples from all cones within reach and modulates the glutamatergic output from the cone axon terminals by parallel feedback mechanisms (e.g. Kemmler et al., 2014). So far HCs have been considered as an electrically coupled network averaging over many cones. Therefore, HCs are considered to play a major role in global information processing in the outer retina, i.e. with respect to contrast enhancement and light adaptation. However, recent studies suggest that HC feedback also act at the level of a single HC dendrite terminal-to-cone synapse (Jackman et al., 2011; Vroman et al., 2014).

Using two-photon Ca^{2+} imaging in a transgenic mouse line that selectively expresses GCaMP3 in HCs (Zariwala et al., 2012; Ströh et al., 2013), we recorded light stimulus-evoked Ca^{2+} signals in HC dendrites in retinal slices. We used green (~590 nm) and blue (~400 nm) light stimuli and recorded from different retinal regions, thus specifically activating different combinations of S- and M-cones. This approach allowed us to assess if and under which conditions signals from individual cones remain “isolated” within a local dendritic region of a HC, or if (and how) they spread across the entire dendritic tree or in the electrically coupled HC network. In complement, we modelled passive and active cell properties in volume-rendered HC dendrites from a serial block-face electron microscopy dataset (Helmstaedter et al., 2013) and evaluated local signalling in HCs.

Consistent with the dorso-ventral opsin expression gradient, we found that light-evoked Ca^{2+} signals recorded in HC compartments in the dorsal retina were dominated by M-opsin activation, whereas those in the ventral retina were dominated by S-opsin activation. In contrast to what one would expect in a purely globally acting HC, responses measured in neighbouring HC compartments varied markedly in their chromatic preference. Moreover, a binary noise stimulus, which we found to decorrelate Ca^{2+} signals in cone terminals, evoked distinct local signals in neighbouring HC compartments. Together, these findings support the idea that HCs are able to process cone input in a highly local manner. First pharmacological experiments suggest that local signalling in HC dendrites employs a different mechanism compared to that demonstrated in A17 amacrine cells (Grimes et al., 2010), as both voltage-gated Ca^{2+} channels and Ca^{+2} -induced Ca^{2+} release are required for HC dendritic Ca^{2+} signals.

In summary, our data suggest that HCs are able to process cone signals locally, which seems to contradict the “classical” view of HC function.

Localization of the excitatory amino acid transporters EAAT2 and EAAT5 in the neuronal network of the mouse retina

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Glutamate is the major excitatory neurotransmitter in the retina. It is involved in the vertical signaling pathway, where the light signals are transferred from photoreceptors (PR) to the bipolar cells (BC) and from the BCs to the ganglion cells (GC). To enable a short and precise response to light stimuli and since glutamate is also neurotoxic, efficient clearing of the synaptic cleft is provided by excitatory amino acid transporters (EAAT).

The EAAT family consists of five members, which interestingly feature a dual function as both glutamate transporter as well as anion channel. The subtypes differ in their uptake and channel efficiency. While EAAT1-3 show highly efficient glutamate uptake, EAAT4 and EAAT5 display a high glutamate-gated chloride channel conductance, making it even more interesting to understand their putative role. In the retina, EAAT1 is expressed by Müller cells and provides for general glutamate clearance. Much less is known about the localization and function of other EAATs. Therefore, one major goal is to study the expression of EAATs in different retinal cell types. Here, we attempted to localize EAAT2 and EAAT5 in the mouse retina using immunohistochemistry (IHC) and confocal laser scanning microscopy.

Knock out EAAT2 only slightly affected visual responses, therefore, EAAT2 is believed to provide a neuro protective role rather than controlling the visual function. We, as others, found EAAT2 on PRs and BCs. Using cell type specific antibodies we analyzed in detail which of the different BC types in the mouse retina express EAAT2.

EAAT5 was suggested to be exclusively expressed in the retina. We found strong EAAT5 immunoreactivity at synaptic contacts between PRs and BCs as well as at certain ON-BC output synapses. Therefore, EAAT5 might play an important role in light stimulated glutamatergic transmission. In light microscopy, it is difficult to decide whether a punctiform synaptic staining originates from the pre- or from the postsynaptic site. In the future, we will perform studies on the level of electron microscopy to shed more light on the localization of EAAT5.

Morphological and functional implications of the retina in multiple system atrophy

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Neurodegenerative diseases like Parkinson's disease (PD) and Multiple System Atrophy (MSA) have been shown to exhibit physiological and morphological neuronal abnormalities. These abnormalities can result from exceeding aggregation of α -synuclein (α -SYN), a 140aa presynaptic protein. Both PD and MSA are associated with a variety of visual symptoms and a potential role of the retina as a biomarker for progression of Parkinson's disease is recently discussed. Changes in retinal function and alterations in neuroretinal morphology have also been reported in MSA patients. We therefore aimed at investigating the underlying mechanisms in homozygous transgenic mice overexpressing human α -SYN under the proteolipid protein (PLP)-promoter (PLP- α -SYN) compared to wild type (WT) animals of two different age groups (two months, one year). By performing immunohistochemical analyses on vertical retinal sections we discovered that distinct α -SYN signal occurred in inner plexiform and ganglion cell layers of PLP- α -SYN mice, but not in WT mice. This is remarkable because the PLP promoter driving the α -SYN expression in oligodendrocytes was reported to be inactive in the retina. Our PLP-stainings indeed confirmed that the expression stops at the optic nerve/retina junction, where we observed a colocalization with α -SYN. To further investigate on these findings, we performed quantitative real-time PCR using specific TaqMan® probes to detect gene expression levels of PLP and α -SYN as well as of the PLP- α -SYN construct in retinal and optic nerve tissue. To determine neuroinflammation and an effect on dopaminergic neurons, immunohistochemical analyses of the glial fibrillary acidic protein (GFAP), a marker for activation of Müller glia that can indicate neuroinflammatory processes, and tyrosin hydroxylase (TH) that labels dopaminergic neurons, which are primarily affected in α -synucleinopathies. GFAP-positive fibers spanning the peripheral retina were pronounced in aged animals in WT and even more in PLP- α -SYN. In PLP- α -SYN animals, TH-positive processes reached into deeper strata of the inner plexiform layer (IPL), and cell bodies were deformed, but cell numbers were not affected. This is in contrast to a published PD rat model that showed a reduction of TH-positive processes as well as less TH-positive neurons in retinal whole-mount stainings. In addition to that, we performed micro-electrode array (MEA) recordings from retinal wholemounts of 2 month old animals to elucidate potential changes to retinal physiology and light responses that might accompany the morphological phenotype. We found only minor differences in retinal field potentials, indicating unperturbed outer retinal function. The spiking activity of ganglion cells and their responses to visual stimuli was used to assess inner retinal processing and retinal output. Further investigations will be needed to clarify whether defects in retinal morphology could be exploited as differential diagnostic marker in MSA. Our data however clearly implicated an impairment of retinal neurons in the PLP- α -SYN MSA model, which may also underlie visual deficits reported in MSA patients.

Funding: FWF (P26881, AK; F4414, NS), University of Innsbruck, Center of Molecular Biosciences (CMBI).

On the role of common age-related beta-synuclein between visual cortex and neuroretina

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Introduction:

An increase of the protein beta-synuclein (SNCB) has been shown in parts of the aging process of the ascending visual pathway, which were, among others, the neuroretina and the primary visual cortex (V1). SNCB is predominantly found in the brain (e.g. neocortex, hippocampus) but also in the neuroretina of various species. It is a physiological antagonist of alpha-synuclein (SNCA). By inhibition of SNCA-aggregation, neuroprotective characteristics were shown. By oxidative stress and inflammation during the physiological aging process, an increased expression of SNCB was triggered. The role and function of SNCB within the neuroretina, as well as the visually associated parts of the cortex, are not sufficiently known. The aim of this study was the further characterization of SNCB in the neuroretinal and cortical context.

Methods:

To study the influence of SNCB on neuronal and glial cells within the cellular network, samples of retina and cortices from rat (Sprague-Dawley, P5-7) were prepared, dissociated and cultured with different concentrations of SNCB up to 72 h. The influence of the expression of neuronal (beta-III-Tubulin, Neurofilament-200 (NF-200)), glial (glial fibrillary acidic protein (GFAP)) and apoptotic marker (Bax, Bcl-2) was investigated using Western Blot analysis and qRT-PCR. Furthermore, immunohistological stainings were used to investigate the influence of SNCB to the p53-pathway in neuroretina and cortex.

Results:

In contrast to dissociated retinae, the cortex showed neuronal reactions after SNCB-treatment pointing to functional differences between the two brain areas. Thus, a neuroprotective reaction might impact on cortical neurons but not on retinal neurons. On the other hand, in retinal but not in cortical cells, a higher glial activation was detected after SNCB-treatment. This was not observed in the cortex. SNCB appears to transform the retinal but not the cortical cells into a pro-apoptotic state. Immunohistological stainings of dissociated retina and cortex indicate changes in the amount of p53-pathway relevant proteins in glial cells, but only subtle changes in neuronal cells after SNCB treatment. The changes in retinal tissue and cortical tissue are similar.

Conclusion:

The results presented in this study indicate a distinct effect of SNCB on the different neuronal tissues. Further studies are required to investigate the role of SNCB. Furthermore, the effect of SNCB on the essential proteins of the p53 pathway is different in glia cells and neuronal cells. We assume, that further studies on the molecular mechanisms based on age-dependending factors may help to better understand neurodegenerative diseases in retina and cortex.

Optimization of electroretinographic recording from the isolated and superfused murine retina.

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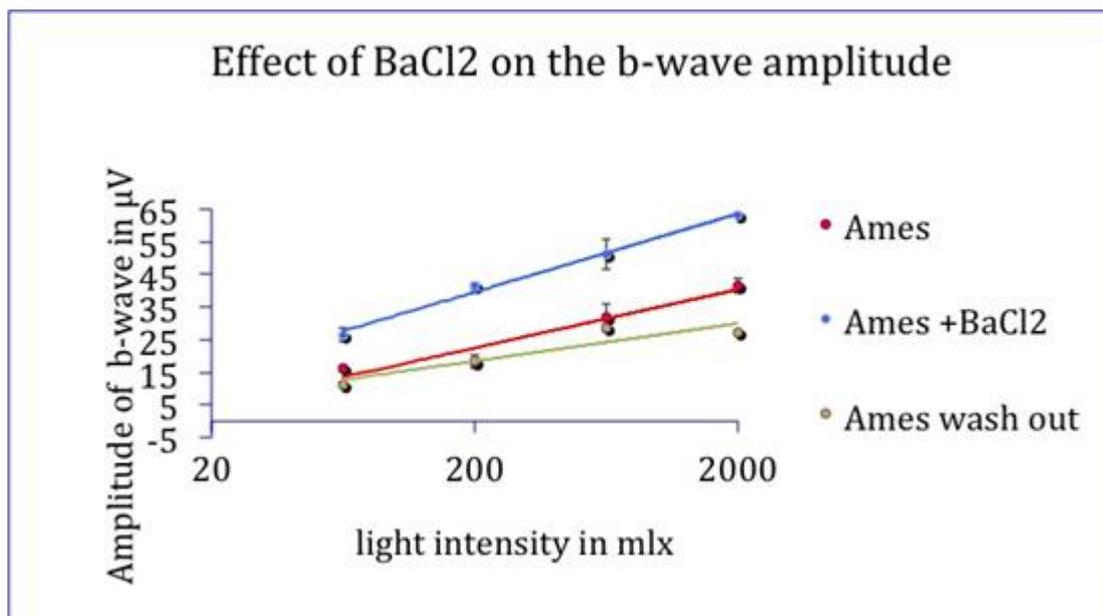
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Analysis of retinal signaling in mutant mice has become a powerful tool for studying retinal function in basic science as well as in translational research. Previously, recording from isolated mouse retina has been extended to about 3 hours by optimizing recording temperature, perfusion velocity, and light intensity. But the reproducibility was limited. Therefore, we performed a series of recordings from isolated mouse retina under different conditions by changing the recording medium and by adding substances for stabilizing the recording of a full electroretinogram (ERG) for this species.

We used a superfused vertebrate retina assay, for which the murine retina had to be isolated with specific tools. Subsequently, the ERG recordings were optimized for the nutrient solution.

To improve the sensitivity and stability of photoreceptor and retinal network responses from the isolated and superfused murine retina, two different nutrient solutions were compared. Furthermore, micromolar concentrations of BaCl₂ were investigated as a means to isolate b-wave responses from the negative-going Kir4.1-mediated slow PIII component, which led to a reversible increase of the apparent b-wave amplitude at different light intensities (see Figure).

In conclusion, the isolated murine retina now can be used as a more reliable and easy to establish pharmacological testing system, which provides the important advantage of being applicable to eyes from animals subjected to selective gene inactivation.



Optogenetics in the eye – development of a light-inducible gene therapy for pathological neovascularization

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Vascular complications due to abnormal growth of vessels or excessive leakage are the most common cause of vision loss. Progress in characterizing the molecular basis of diseases such as age-related macular degeneration (AMD) or diabetic retinopathy has led to major therapeutic advances. Treatments include regular injections of vascularization inhibitors such as antibodies against VEGF, but recently, gene therapies based on viral expression of antiangiogenic proteins are being evaluated in clinical trials. However, in the affected eye, neovascularization occurs not in all retinal regions so that global inhibition also impairs ongoing angiogenesis in the remaining healthy retinal tissue. We sought to overcome this limitation by developing a photoactivated gene expression paradigm for induction of anti-angiogenic transgenes by irradiation with light. This method is based on the Tamoxifen-inducible Cre/lox system and a reversibly inhibited, photo-sensitive ('caged') Tamoxifen analog. The idea is that locally restricted irradiation induces anti-angiogenic transgenes only in the diseased but not healthy regions of the retina. Importantly, after treatment, caged Tamoxifen washes out of the system thereby rendering the eye insensitive to unspecific photoactivation through normal daylight. We could readily induce transgenes with light in vitro and ex vivo while preliminary results also show successful in vivo photoactivation in the illuminated but not the control eye. After further optimization, we plan to use established mouse models of neovascularization to test the effects of localized induction of anti-angiogenic transgenes. Clearly, such an optogenetic approach offers exciting opportunities for gene therapeutic intervention in the eye.

Pericentrin, identified at the basal-body complex in mammalian photoreceptor cells, interacts with Nesprin protein Syne-2 in the retina

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Pericentrin (Pcnt), a highly conserved protein of the pericentriolar material, serves as a multifunctional scaffold for numerous proteins and plays an important role in microtubule organization. Structurally, Pcnt is characterized by coiled-coil domains throughout most of the protein, which mediate interactions between resident structural lattice proteins of the PCM and a number of regulatory centrosomal proteins. Furthermore, Pcnt contains a pericentrosomal matrix targeting motif, called the PACT domain, which is responsible for the targeting of Pcnt and other PCM proteins to the centrosome. Mutations in the human PCNT gene are associated with a range of diseases including primordial dwarfism and ciliopathies. In the mouse retina Pericentrin colocalizes with several proteins responsible for transport processes at the connecting cilium between the two photoreceptor compartments. In order to get more insights on the function of Pericentrin in the retina we try to identify new ciliary as well as centrosomal interaction partners.

We showed that downregulation of Pcnt significantly reduces cilia number and photoreceptor morphology in mouse cell lines and retina cultures. Additionally, we were able to show that Pericentrin interacts with several proteins involved in ciliogenesis, axonemal transport, Meckel-syndrome and primary ciliary dyskinesia. As one of the most promising candidates, we identified the nuclear membrane protein Syne-2, which is responsible for anchoring and positioning the nucleus within the cell. We were able to show a partial colocalization of Pericentrin and Syne-2 at the basal body complex in the inner segment of wildtype mouse photoreceptors. Immunohistochemical stainings and Western blot analysis of Nesprin-2 ABD retinae revealed a strong reduction of Syne-2 splice variants localized in the inner segment. However, Nesprin-2 ABD mice did not display any detectable retinal phenotype regarding the expression and localization of Pericentrin.

An interaction model between a component of the pericentriolar material and a KASH protein in *C. elegans* (Malone *et al.*, 2003) could be indicative for a function of Pericentrin and Syne-2 in nucleus - centrosome association in mammals. The attachment of both organelles seems to play an important role during nuclear migration, required in retinal development, as well as during centrosomal migration at the beginning of ciliogenesis, e.g. the formation of the connecting cilium.

Our findings suggest various functions of different Pcnt splice variants in distinct ciliated tissues and sensory neurons. We assume a Pcnt patchwork composed of specific splice variants in different tissues, which may explain why mutations in the human PCNT gene generate a multitude of different phenotypes.

Support: DFG (GI770/1-1/1-2)-, Universitätsbund Erlangen-Nürnberg e.V.

Response properties in bipolar cells and their impact on ganglion cells in the retina

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Retinal ganglion cells come in about 30 different types, which show a diversity of response properties under visual stimulation (e.g. transient & sustained cells, direction/orientation-selective cells). This diversity appears to evolve along the signal processing stream from photoreceptors to ganglion cells. The contributions of the different intermediate cell types however are not well understood. Bipolar cells are intermediate cells which represent pivotal elements by connecting the outer and inner layers of the retina. We therefore investigate the signal transmission from bipolar to ganglion cells in order to assess bipolar cell contributions to the functional diversity observed in ganglion cells. We combine intracellular recordings from individual bipolar cells and multielectrode-array recordings from multiple ganglion cells in isolated retinas. In a first part, we use visual light stimulation to simultaneously characterize the bipolar and ganglion cells response properties. Here, we found novel bipolar cell response properties: non-linear spatial light integration similar to Y-like cells and increased amplitude to preferred orientations of a bar similar to orientation-selective cells. Furthermore, we use a standard linear-nonlinear (LN) model to study the diversity in the input-output response relationship and to predict bipolar cell's responses to novel stimuli. In a second part, we assess to which ganglion cells an individual bipolar cell is sending its information by inducing electrical current into the bipolar cell while recording the ganglion cell's spiking activity. Here, we find ganglion cells responding already to small current injection (50-80 pA), whereas others only respond to larger currents (300-500 pA) or do not respond at all. Based on this connectivity information, we study the interactions between different response properties in bipolar and ganglion cells to finally better understand how the response diversity observed in ganglion cells evolves.

Acknowledgements

This work was supported by the DFG (GO 1408/2-1 and SFB 889, C1) and by the European Commission (FP7-ICT-2011.9.11/600954, "VISUALISE").

Retinal ganglion cell activity of Cav1.4 mutant mice

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Cav1.4 L-type calcium channels (LTCC) are predominantly expressed in the retina where they are located at the synaptic sites of photoreceptors and most likely also bipolar cells. Heterologously expressed Cav1.4 channels show rapid activation, open at relative negative membrane potential and inactivate slowly. These properties are necessary to sustain the calcium influx at the ribbon synapses to provide a high rate of neurotransmitter release. Mutations in the CACNA1F gene encoding for the alpha1 subunit of Cav1.4 channels are known to cause several congenital retinal diseases, such as incomplete Congenital Stationary Night Blindness Type 2 (CSNB2). One of these mutations, Cav1.4 I745T (IT), has been shown to be a gain-of-function mutation. In heterologous expression systems the IT mutation shifted the voltage-dependence activation of Cav1.4 to more negative voltages. How this abnormal calcium influx affects the retinal circuits is hardly known. Our previous work has demonstrated that the insertion of the IT mutation caused disturbances in the signal transmission of mouse retinas: many ganglion cells (GC) did not respond to full-field stimulation or with a significant delay in multielectrode array recordings of whole-mounted retinas; another significant number of cells showed higher activity in darkness. Aim of this study is to further examine the ganglion cell activity of IT mouse retinas in response to different light stimuli under both scotopic and photopic conditions. The use of two light levels is informative for the overall luminance effect of the retinal function carrying the IT mutation. Whereas the use of multiple light stimuli is aimed to detect specific GC's response pattern. As an example: full-field chirp, consisting in two sinusoidal intensity modulations provides information about GCs preference for temporal frequency and contrast, while the checkerboard stimulus is used to determine the receptive field size. We expect these analyses to deepen our understanding of the mechanisms by which Cav1.4 mutations affect retinal signaling.

Rewiring of bipolar cells in a congenital stationary night blindness type 2 mouse model

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In humans, mutations in the *CACNA1F* gene which encodes Cav1.4 L-type calcium channels are associated with congenital stationary night blindness type 2 (CSNB2). Cav1.4 channels are predominantly expressed at photoreceptor terminals and most likely also in bipolar cells where they serve an important role for synaptic transmission as well as synapse formation and maturation. Our previous work on the retinal morphology in Cav1.4 mutant mice has demonstrated that rod bipolar cells undergo structural remodelling. Dendrites of rod bipolar cells elongate into the outer nuclear layer, the organisation of the outer plexiform layer is disrupted and the synaptic structures of photoreceptors have variable, mostly immature appearance. So far it is unknown whether sprouting rod bipolar cells form new connections in the outer nuclear layer. Preliminary immunohistochemical analyses, using PSD95 as a marker for synaptic endings of rods and cones showed that these terminals were mislocalised to the outer nuclear layer. However, mislocalised terminals contained ribbon structures as seen in co-stainings with the ribbon marker CtBP2. Whether bipolar cells still form contacts with these photoreceptor terminals or whether they try to form new contact still has to be clarified. Our immunohistochemical analysis will elicit further mechanisms by which Cav1.4 channels affect retinal morphology.

Spike correlations indicate electrical coupling between heterotypic ganglion cells

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Fast reciprocal spike correlations between ganglion cells are thought to occur due to electrical coupling via gap junctions, either directly, or through joint coupling with an intermediary amacrine cell. Reciprocal correlations have been reported to occur only between homotypic ganglion cells, in line with the concept of parallel processing in the early visual system. Here, however, we present evidence for reciprocal correlated firing between heterotypic ganglion cells in the guinea pig retina.

We recorded the spike responses of several hundred ganglion cells simultaneously in the isolated retina of pigmented guinea pigs by using a large scale multi-electrode array. Natural-power noise was used to characterize the receptive field properties of the recorded cells. The spatio-temporal profiles of the receptive fields and autocorrelation functions were used to classify distinct cell types. Cross-correlation functions were obtained from pairs of cells of a given cell type combination.

Reciprocal spike correlations indicative of gap junction coupling were observed between two distinct types of sustained ON ganglion cells. In addition, both cell types were homotypically coupled. The first type had large receptive fields and the spike triggered average revealed a triphasic temporal filter. The second type was medium-sized, showed a biphasic filter, and preliminary data suggest that the cells receive S-cone input.

The results show that interactions between pathways in the early visual system are more complex than previously appreciated. The functional role of fast spike synchronization in heterotypic cell types remains to be examined.

Spontaneous emergence of structured responses in a random neural network *in-vitro*

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Neural networks with connections organized by probabilistic rules are conceptually powerful model systems. Among others, random neural networks have been shown to (1) generically exhibit computationally favorable properties for stimulus representation and information processing, (e.g. Lukosevicius & H. Jaeger, 2009), (2) dynamically generate a state of sustained irregular spiking activity (van Vreeswijk & Sompolinsky, 1996) and (3) to account for visual cortical orientation selectivity (Ernst et al., 2001). What is left open in these theoretical studies is the questions whether such ideas are viable in random networks of living cells.

We address this problem using a dissociated culture of rat cortical neurons. The neuronal connection patterns in such cultures are substantially less organized than neural circuits in the brain. We then drive these neurons optogenetically with spatially complex light patterns, generated by a holographic photostimulation system (Golan et al. 2009) and monitor neural responses optically with a redshifted genetically encoded calcium indicator (Dana et al. 2016 bioRxiv) together with ground truth data from a multielectrode array.

Stimulating the cell culture with moving gratings reveals a substantial degree of orientation tuning. We identify at least 33 out of 430 units from 16 cortical cultures as orientation biased, i.e. they show a circular variance < 0.9 . This is highly significant with $p < 0.001$. Notably, the orientation bias described here resembles to some extent cortical orientation selectivity.

Synergy in random motion decoding from a population of direction-selective retinal ganglion cells

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The detection and correct interpretation of motion in visual scenes is important in everyday tasks, e.g., for avoiding cars when crossing the street or for assessing the optic flow when navigating through a room. We study the encoding of visual motion on the first level of visual processing, the retina. There, direction-selective ganglion cells (DSGCs) are known to preferably respond to certain directions of drifting gratings. They are thought to provide important information about the optic flow to higher brain areas. In the salamander, we found three subtypes of DSGCs whose preferred directions are separated by 120°, similar to the directional organization of ON-type DSGCs observed in mouse. Additionally to simple drifting gratings, we probed the motion encoding of salamander DSGCs with more complex motion patterns and projected textures following a 2-dimensional random walk onto the isolated salamander retina. We recorded up to 25 DSGCs simultaneously with multielectrode arrays for performing population analysis.

We investigated which features of the random motion trajectory could be retrieved from the DSGC population code by a downstream neuron. We used a commonly deployed linear multi-cell decoder to reconstruct the motion trajectory from the DSGC responses. These linear reconstructions captured low-frequency features of the actual motion trajectory. For individual DSGCs, motion along their preferred direction was reconstructed best. To compare the decoding from individual and population responses, we derived the mutual information between stimulus and linear reconstruction. Thereby, the decoding of the joint activity of populations with different preferred directions conveyed more information than the summed information of reconstructions from individual DSGCs.

Our findings suggest that this synergy in the motion decoding of DSGC population responses is based on ambiguities in the motion encoding of individual DSGCs. In complex visual scenes, DSGCs simultaneously encode both, motion direction and strong contrast changes in their receptive fields caused by large translational movements of the pattern in either direction. These ambiguities are partly compensated by the concerted signalling of DSGCs with different preferred directions, leading to synergy. This synergy is even stronger, the more the DSGC responses are correlated.

THE DYNAMICS OF ADAPTATION PROCESS TO DIFFERENT LIGHT LEVELS IN THE MOUSE RETINA STUDIED WITH ELECTRORETINOGRAMS

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Purpose: To date, most studies on in vivo electroretinography in mice are performed on steady state light or dark adapted animals. In the present study, we focused on the dynamics of light and dark adaptation processes in the mouse retina.

Methods: The animals were dark adapted overnight and the scotopic flash ERG to a 6.3 cd.s/m² flash was recorded (baseline recording). Then the animals were adapted to 25 cd/m² white light and the responses to 6.3 cd.s/m² flashes were recorded every 60 sec for a 15 min period. Subsequently, the mice were dark adapted again and the ERGs to 6.3 cd.s/m² flashes were recorded every 60 sec for up to 30 min back to baseline. The amplitudes and implicit times of different ERG components (a- and b-wave, oscillatory potentials [OPs] and the photopic negative response [PhNR]) were analyzed. In a second series of experiments, we recorded the changes in the responses to sinusoidal luminance modulation (12 Hz; 100% Michelson contrast) during the adaptation to a 25 cd/m² and a 1 cd/m² mean luminance, respectively. The amplitudes and phases of the 1st harmonic (fundamental) components were extracted through Fourier analysis.

Results: Flash ERGs: Significant increases of the amplitudes of b wave ($p < 0.001$), PhNR ($p < 0.05$) and OPs ($p < 0.05$) were found during light adaptation. Furthermore, the implicit times of b wave ($p < 0.001$) and the 2nd ($p < 0.005$) and 3rd OP peak ($p < 0.001$) decreased significantly. The changes in most parameters were completed after 7 min of light adaptation.

The subsequent reduction of the background illumination led to an instantaneous decline of the ERG signal. Afterwards the amplitudes of a- ($p < 0.001$) and b-waves ($p < 0.001$) and the OPs ($p < 0.001$) increased during dark adaptation. Also the ratio between a- and b-wave amplitude increased strongly ($p < 0.001$) indicating the presence of additional adaptation processes in the signal transmission from photoreceptor to bipolar cells. Furthermore we observed a significant decrease of the implicit times of 2nd ($p < 0.05$) and 3rd ($p < 0.001$) OP peaks.

ERG responses to sinusoidal luminance modulation: We found an interesting difference between light and dark adaptation. The phases of the 1st harmonic components decreased significantly during light adaptation ($p < 0.001$) but were constant during dark adaptation. Their amplitudes decreased during light adaptation ($p < 0.005$) but increased during dark adaptation.

Conclusions: Light and dark adaptation in ERG responses are highly dynamic processes that take several minutes to complete. Furthermore, our data indicate that adaptation can take place at several stages in the retinal circuitry. That the response amplitude decreases to sine-wave modulation after light adaptation is at odds with the changes in the flash ERG components where the component amplitudes increase. Furthermore, in human subjects ERGs also grow during light adaptation after an extended

period in complete darkness. This indicates that adaptation may depend on the signal pathway that is reflected in the ERG and may also be dissimilar in different species.

Two-Photon Calcium Imaging of Dendritic Integration in Mouse Retinal Ganglion Cells

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The retina is a powerful image processor that sequentially decomposes spatiotemporal photoreceptor activation patterns into feature-specific parallel channels. Only a small fraction of the information picked up by the photoreceptor array is sent to the brain by the retinal ganglion cells (RGCs). Key to this refinement process are synaptic interactions in the retina's second synaptic layer, the inner plexiform layer (IPL). Here, individual synaptic connections are subject to powerful modulations by amacrine cells (ACs). As a result, individual synapses may exhibit specific spatiotemporal response properties that differ both pre- and post-synaptically, even for neighboring synapses belonging to the same cell, as previously demonstrated in select types of ACs (e.g. Euler et al., 2002; Grimes et al., 2010).

To study how individual RGC dendrites integrate across a spatiotemporally heterogeneous pool of input streams we perform two-photon calcium imaging at different dendritic sites of the same RGCs. Based on earlier work (Briggman & Euler, 2011; Baden et al., 2016), we sparsely label individual RGCs with a synthetic calcium indicator in the ex-vivo intact retina, preserving full connectivity. Using two-photon imaging we then probe the visual response properties at different sites of RGC dendrites. This allows us to gain detailed insight into how visual signals are processed and integrated within RGCs to ultimately yield the specific response profiles observed at the level of the RGC spike output that is relayed to the brain. Here, we study how the spatiotemporal receptive fields of individual dendritic sites differ depending on their distance to the soma and how changes in the spatial extent, contrast and frequency components of the light stimulus affect dendritic calcium signals. Additionally, we are interested in the impact of dendritic spikes and backpropagating somatic spikes on dendritic integration in different types of RGCs. Using spatial binary noise stimuli, our preliminary data confirm that receptive fields of dendritic sites of RGCs could be reliably estimated using our imaging technique. For some types of RGCs (e.g. the alpha RGCs) dendritic segments closer to the soma systematically exhibits larger receptive fields. Moreover, different dendritic sectors of the same RGC selectively extract visual information present in different retinal positions. Further, first results indicate that different dendritic sites of the same RGC exhibit distinct temporal responses.

In conclusion, spatial and temporal processing can be studied at different dendritic sites of a single RGC using two-photon calcium imaging. This technique will allow the study of synaptic integration in the inner retina in great detail, yielding to a refined understanding of how complex features observed at the level of the RGC spike train sent to the brain are established through synaptic interactions in the retina's IPL.

Visualization of second messengers in the mouse retina using optogenetic sensors

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Second messengers play a major role in cellular signaling pathways and in light adaptation processes in the retina. In the retina, a large number of neurotransmitters is employed by different cell types. Dopamine (DA), adenosine (A), and melatonin (MT) are promising candidates that may control retinal adaptation. These neuromodulators are released at different sites in the retina and under different conditions. They act on two families of G-protein-coupled receptors (GPCRs), leading to either an increase or a decrease in the concentration of cyclic adenosine monophosphate (cAMP), directly coupled to changes in the activity of protein kinase A (PKA). In addition to the classical pathway cAMP/PKA, retinal neurons may use calcium (Ca²⁺) as intracellular messenger. Although much is known about the effects of dopamine and melatonin at cellular and synaptic levels, little is known about the biological effects of adenosine in the retina.

We used the fluorescent genetically encoded A-kinase activity reporter AKAR4 in combination with imaging techniques to examine the effect of dopamine, adenosine, and melatonin on the cAMP metabolism in cultured retinal neurons in real time. AKAR4 also enabled us to investigate the interaction of opposing signaling pathways within the same cell. For the visualization of changes in the internal concentration of Ca²⁺, cultured retinal neurons were loaded with the chemical Ca²⁺-indicator Fluo-4-AM. Changes in intracellular Ca²⁺ were detected in the intact retina in real time using a transgenic mouse line that expresses the FRET-based Ca²⁺-sensor TN-L15 in ganglion cells.

We found that the majority of cultured retinal neurons responded to dopamine leading to changes in internal cAMP and Ca²⁺. Different responses could be observed that were based on different signaling pathways. Only few cells responded to melatonin indicating that it plays a less important role as a neuromodulator in the retina than dopamine. While only few cells responded to adenosine with changes in internal cAMP concentration, calcium imaging experiments indicate that adenosine plays an important role in the modulation of glutamate receptors.

What can a small fish teach us about visual processing?

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Vision can be defined as the process of acquiring knowledge about the environment by extracting information from the light that the objects emit or reflect. To achieve this goal, numerous different visual systems have evolved in the animal kingdom. This brings up the question whether there are universal features to the visual processing solutions we can find in nature. To address this question we use the archerfish as an animal model to study different aspect of visual processing. The selection of this fish species as model animal stems from its remarkable ability to shoot down insects settling on the foliage above the water level, and its ability to learn to distinguish between artificial targets presented on a computer monitor. Thus, the archerfish can provide the fish equivalent of a monkey or a human that can report psychophysical decisions and make controlled and complex experimental procedures possible, yet with a very different brain anatomy. We present our recent findings that show remarkable similarities between the functionality of the visual system of the archerfish and visual systems in mammals and argue that it reflects universal features of visual processing across vertebrates.

Poster Topic

T16: Vision: Striate and Extrastriate Cortex, Eye Movement and Visuomotor Processing

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- [T16-3B](#) Effectiveness of electrically evoked input depends on the gamma-phase of the receiving population in monkey area V4
Eric Drebitz, Heiko Stemmann, Andreas K. Kreiter
- [T16-4B](#) Environmental enrichment accelerates ocular dominance plasticity in mouse visual cortex; putting animals back to a standard cage results in a rapid loss of this plasticity
Evgenia Kalogeraki, Siegrid Löwel
- [T16-5B](#) Imaging of spine dynamics in the visual cortex of awake PSD-95 knockout and wild type mice
Anja Tippmann, Bettina Joachimsthaler, Cornelius Schwarz, Oliver Schlüter, Siegrid Löwel

- [T16-6B](#) Synaptic correlates of the predictive coding of form and motion in V1
Marc Pananceau, Xoana G Troncoso, Benoit Le Bec, Christophe Desbois, Yves Fregnac
- [T16-1C](#) Is the contribution of visual feedback on grasping activity similar in the grasping areas of the dorsal visual stream?
Marina De Vitis, Rossella Breveglieri, Sofia Briganti, Annalisa Bosco, Claudio Galletti, Patrizia Fattori
- [T16-2C](#) Multiple thalamocortical axonal architectures converge in mouse visual cortical areas
Marian Evangelio, Francisco Clasca, Maria Garcia-Amado
- [T16-3C](#) Neuronal response properties during the repetitive presentation of a visual stimulus in mouse V1
Evelyn Dylda, Janelle M.P. Pakan, Scott C. Lowe, Sander W. Keemink, Nathalie L. Rochefort
- [T16-4C](#) Neuronal responses in the upper visual field of the rat
Stefanie Rulla, Benedict Ng, Damian Wallace, Jason Kerr
- [T16-5C](#) Postsynaptic scaffolds and visual stimulation fine-tune the development of glutamatergic synapses in visual cortex.
Plinio D. Favaro, Sophia K. Stodieck, Siegrid Löwel, Oliver M. Schlüter
- [T16-6C](#) Recovery from vision loss in subacute stroke following tDCS treatment
Younes Adam Tabi, Raimund Alber, Hermann Moser, Carolin Gall, Moritz Dannheimer, Bernhard A. Sabel
- [T16-1D](#) Response modulation by spatial attention in area MT of primate visual cortex is not mediated by the cholinergic system.
Jordi Aguila, Vera Veith, Clíodhna Quigley, Stefan Treue
- [T16-2D](#) Response properties of neurons in the binocular visual cortex of PSD95 knockout mice *in vivo*
Susanne Dehmel, Kanishka Waghmare, Michael Weick, Xiaojie Huang, Man Ho Wong, Tim Golisch, Oliver M. Schlüter, Siegrid Löwel
- [T16-3D](#) The potentials of the methanolic leaves extract of *Lannea schimperi* (HOSCHST. EX RICH) ENG. Aas a surface anaesthetic agent.
Hudu Mikail Garba, Akumka David Dezi, Muhammed Adamu
- [T16-4D](#) The role of postsynaptic density protein 93 for visual cortical plasticity
Siegrid Löwel, Sophia S. Stodieck, Leon Hosang, Plinio D. Favaro, Oliver M. Schlüter
- [T16-5D](#) Towards no-report readouts of conscious visual perception
Eva Poland, Iris Steinmann, Albert Lehr, Annekathrin Schacht, Arezoo Pooresmaeili, Melanie Wilke
- [T16-6D](#) Visual pop-out in barn owls: From behavior to neural correlate
Julius Orłowski, Hermann Wagner

Behavioural state modulation of inhibition is context-dependent and cell-type specific in mouse primary visual cortex

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Neurons in primary sensory areas not only respond to sensory stimuli but also to parameters reporting the behavioural state of the animal. The increased gain of visual responses during locomotion provides a model to elucidate the circuit mechanisms underlying behavioural state-dependent changes of sensory responses. Recent *in vivo* studies suggest that this gain control in primary visual cortex (V1) is mediated through inhibitory interneurons resulting in the disinhibition of pyramidal neurons during locomotion. We tested this model using two-photon calcium imaging to record the activity of three non-overlapping populations of inhibitory neurons (expressing either parvalbumin [PV], somatostatin [SST] or vasointestinal peptide [VIP]) in layers 2/3 and 4 in awake-behaving mice. We characterized changes in activity during locomotion in darkness as well as during visual stimulation. We found that the three main classes of interneurons increase their activity with locomotion during visual stimulation, in contradiction with the disinhibition model. In addition, we found that responses of inhibitory neurons to locomotion strongly differed in darkness and during visual stimulation, revealing context-dependent cell-type specific locomotion responses in V1. This context-dependent modulation of activity by locomotion was particularly prominent in SST neurons. In contrast, VIP neurons remained strongly responsive to locomotion both in darkness and during visual stimulation. Finally, on the population level, PV neurons were similarly responsive to locomotion in both contexts, however, on the single cell level they also show a diversity of responses. We suggest alternative mechanisms of how locomotion modulates cell-specific neuronal activity in V1, highlighting the context-dependent, dynamic nature of interneuron function.

Binocular integration and disparity sensitivity in mouse visual cortex

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Binocular neurons in the visual cortex combine signals from left and right eye images. The small differences between these images, called binocular disparities, provide the visual system with critical information for depth perception. This form of binocular integration appears to exist in several species, but it has only been extensively investigated in primates and cats. In these species, individual neurons sensitive to binocular disparities are found in almost all regions of the visual cortex, with somewhat different disparity tuning properties across primary and higher visual areas.

The mouse is emerging as a key model for understanding visual perception. Despite lacking the elaborate functional architecture typically found in primates and carnivores, mouse visual cortex as a whole is highly organized. It consists of the primary visual cortex (V1) and about a dozen extrastriate areas, with supposedly distinct roles in visual information processing, which are only partially understood, however. Mouse V1 has been reported to contain disparity tuned neurons similar to those found in other mammals. However, binocular disparity is still poorly studied in the mouse, and it has not been investigated whether it is differentially represented among higher visual areas. Comparison of disparity tuning across different areas might help delineating the functional specialization of mouse visual areas.

Therefore, our aim is to characterize binocular disparity in V1 and in two higher visual areas, RL and LM, of anesthetized mice. Visual areas were first identified with intrinsic optical imaging. To record the activity of neurons in the selected regions, we then performed two-photon imaging using the genetically encoded calcium indicator GCaMP6s. To measure disparity tunings, binocular drifting gratings at eight different interocular disparities were presented using a haploscope, allowing for independent stimulation of each eye.

We found that a large fraction of neurons in all areas studied are modulated by disparity. Many neurons exhibit a strong response enhancement after binocular stimulation, even when classified as monocular by conventional ocular dominance measurements. In fact, binocular disparity selectivity and ocular dominance appeared to be unrelated in all three areas. Moreover, we found no evidence for a spatial organization for binocular disparity, in agreement with the salt-and-pepper organization typical of mouse visual cortex. Overall, through the analysis so far performed, we find no major differences in binocular integration across V1, RL and LM.

Biologically-inspired neural model for the adaptation of neurons in area IT

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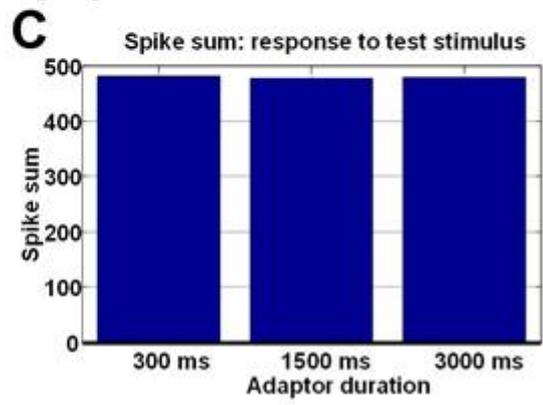
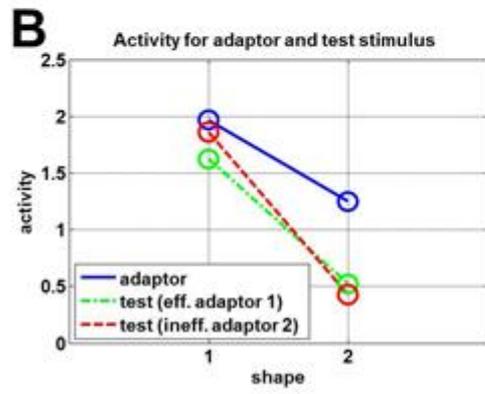
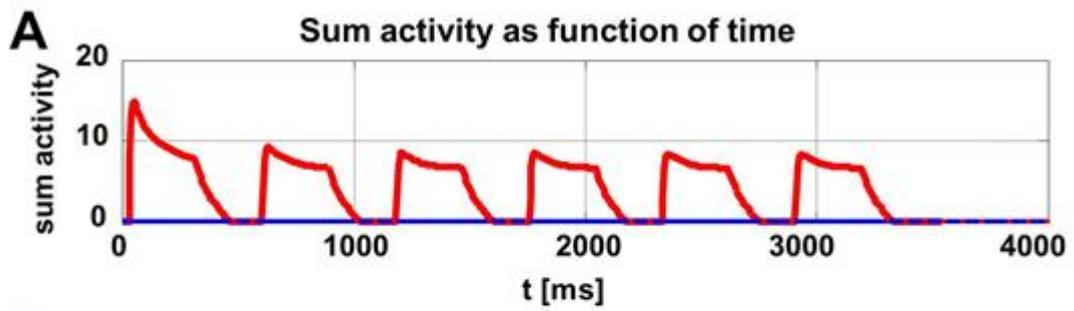
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For repeated stimulation neurons in inferotemporal cortex (area IT) show adaptation effects. Such effects at the single-cell level are consistent with the observation of repetition suppression in fMRI and of high-level after-effects, e.g. for face perception. A variety of theoretical explanations has been discussed, which are difficult to distinguish without detailed electrophysiological data. Meanwhile, several detailed physiological experiments on the adaptation of shape-selective neurons in inferotemporal cortex (area IT) have provided constraints that help to narrow down the possible computational neural mechanisms. We propose a neurodynamical model that reproduces these experimental observations by biophysically plausible neural circuits. We model the activation dynamics of recurrently connected shape-selective neurons in area IT using a mean-field limit. The consistent modelling of the observed adaptation effects requires the inclusion of multiple adaptation mechanisms: (i) spike-rate adaptation; (ii) an input fatigue adaptation process, modeling adaptation in earlier hierarchy levels and of afferent synapses; (iii) a firing-rate fatigue adaptation process that models adaptation dependent on the output firing rates of the neurons. A single model with a fixed set of parameters accounts jointly for a spectrum of recent electrophysiological results on adaptation of IT neurons, which are highly constraining for possible underlying computational mechanisms: (i) Shape of the typical PSTHs of IT neurons; (ii) temporal decay for repeated stimulation of the same neurons with many repetitions of the same shape stimulus [1] (Fig. A); (iii) relative strength of adaptation effects obtained with efficient and ineffective adaptor stimuli, which stimulate the neuron strongly or only moderately, used either for adaptation or testing [2] (Fig. B); (iv) independence of the strength of the adaptation effect on the duration of the adaptor stimulus (Fig. C). All proposed adaptation processes turned out to be necessary in order to account jointly for all experimental results. The result in Fig. B cannot be reproduced without an appropriate mixture of input fatigue and a firing-rate fatigue. The independence of adaptation strength on adaptor duration cannot be reproduced by most popular models for adaptation at the single-cell level. This suggests that adaptation in IT neurons is significantly influenced by several biophysical processes with different spatial and temporal scales.

References:

1. Sawamura H, Orban GA, Vogels R: Selectivity of neuronal adaptation does not match response selectivity: a single-cell study of the fMRI adaptation paradigm. *Neuron* 2006, 49(2):307-318.
2. De Baene W, Vogels R: Effects of adaptation on the stimulus selectivity of macaque inferior temporal spiking activity and local field potentials. *Cereb Cortex* 2010, 20(9):2145-2165.

Acknowledgements: Supported by EC Fp7-PEOPLE-2011-ITN PITN-GA-011-290011 (ABC), FP7-ICT-2013-FET-F/604102 (HBP), FP7-ICT-2013-10/611909 (Koroibot), DFG GI 305/4-1 + KA 1258/15-1, HFSP RGP0036/2016.



Changes in the spine density during the maturation of neural circuits in the visual cortex of wild-type and PSD-95 knockout mice

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Initial brain wiring is accomplished primarily under genetic control, but experience-dependent activity is needed to fine-tune the function and synaptic connectivity of neural circuits. The peak of these processes is observed early in development, during the critical period (CP), a restricted time window when synaptic connections get refined and are still very plastic and modifiable depending on the external input. Maturation of AMPA-silent synapses has been shown to be one of the mechanisms mediating the strengthening of neural circuitry. We recently observed that postsynaptic density protein-95 (PSD-95) is necessary for synaptic maturation and the closure of the CP for ocular dominance plasticity in the primary visual cortex (V1) of mice¹. PSD-95 KO mice exhibit lifelong ocular dominance plasticity.

In addition, electrophysiological recordings have shown that in both knockout (KO) and wild-type (WT) mice, ~50% of the synapses in layers 2/3 of V1 are AMPA-silent before eye opening. While in WT mice only <5% remain silent by the end of the CP, in KO mice, silent synapse numbers stay elevated throughout development. Further inspection of electrophysiological properties of the neurons in KO and WT animals has shown that the frequency of miniature excitatory postsynaptic currents (mEPSCs) increases after eye opening and plateaus before the CP ends. Considering the differences in maturation of silent synapses in WT and KO animals, we reasoned that there must be a third parameter to explain the parallel increase in mEPSC frequencies in both genotypes. We have postulated that there might be changes in the spine density of visual cortical neurons to compensate for the differences in silent synapse numbers between KO and WT animals. To test this, we have established an *in vitro* electroporation protocol to fluorescently label spines of layer 2/3 pyramidal neurons with Lucifer Yellow. We have chosen to analyze spine density at four different time points during early postnatal development: before eye opening (P10-P12), after eye opening (P12-P14), during early (P19-P21) and late critical periods (P29-P32). Our preliminary data suggest that in both genotypes, there is an increase in spine density after eye opening and also throughout the critical period.

In conclusion, this work will provide critical insight into the morphological changes that accompany the maturation of neural circuitry and that might underlie the modified development and increased plasticity of PSD-95 KO mice.

Reference:

¹Huang et al. (2015) PNAS 112 (24): E3131-E3140

Circuit analysis of layer 2/3 pyramidal cells in mouse visual cortex

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The function of neural circuits is determined mainly by the specific connectivity between individual cells. Likewise, the response and tuning properties of single neurons arise generally from the information carried by their synaptic inputs. However, little is known about the detailed relationship between the organization of synaptic connections and neural response properties at the level of single cells. Our goal is to understand the principal local excitatory and inhibitory connections of individual layer 2/3 pyramidal cells and relate these to their functional response properties.

To address this question, we first characterize the principal laminar excitatory and inhibitory synaptic inputs to layer 2/3 pyramidal cells of mouse primary visual cortex (V1) using laser scanning photostimulation (LSPS). In acute coronal slices, we record electrophysiologically from a target neuron and map its cortical inputs by systematically stimulating its potential presynaptic partners using UV glutamate uncaging (e.g. Callaway and Katz, 1993; Yoshimura, Dantzker and Callaway, 2005).

In order to correlate the response properties of neurons with their input pattern, we follow an *in vivo/in vitro* approach. To this end we first characterize the visual response properties of individual neurons with *in vivo* 2-photon calcium imaging, and subsequently find back the same neurons in acute coronal slices for circuit analysis with LSPS. In order to characterize the response properties with *in vivo* 2-photon calcium imaging, we express genetically encoded calcium indicators (GECIs) in layer 2/3 pyramidal cells in the binocular zone of the visual cortex. This combined approach promises new and deeper insights into the relationship between neural response properties and neural circuit structure.

Callaway EM, Katz LC (1993) Photostimulation using caged glutamate reveals functional circuitry in living brain slices. *Proc Natl Acad Sci U S A* 90:7661–7665

Yoshimura Y, Dantzker JL, Callaway EM (2005) Excitatory cortical neurons form fine-scale functional networks. *Nature* 433:868–873

Darpp-32 - a marker for principal neurons in teleosts

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Dopamine is a key modulator of locomotor circuits in invertebrates and vertebrates, including zebrafish. The neuronal phosphoprotein Darpp-32 is a potent inhibitor of protein phosphatase 1 and a key integrator of dopaminergic signaling on the cellular level. We looked at Darpp-32 expression and putative dopaminergic input in the zebrafish brain to identify neural circuits that are receptive to dopamine modulation. We found three different isoforms of Darpp-32 at the mRNA level, two of them being expressed throughout the course of development into adult stages. On the protein level we found Darpp-32 expression in the Purkinje neurons of the developing cerebellum as well as in the developing optic tectum. We then established the distribution of Darpp-32 protein in the adult zebrafish brain. Immunofluorescence staining on coronal and sagittal sections of the adult brain showed Darpp-32 protein expression in the principal cells of all cerebellum-like structures known in teleosts additional to the expression in Purkinje neurons. From this data we conclude, that Darpp-32 is a novel marker for principal neurons in the cerebellum and cerebellum-like structures in teleosts. We are now in the process of generating a Darpp-32 knock-out transgenic zebrafish line, using CRISPR/Cas9 mediated genome editing. We will use this knock-out line to investigate the physiological function of Darpp-32 in intact neural circuits.

Determining complex receptive field motion preferences in primate cortex area MSTd

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Area MSTd in primate extrastriate visual cortex is assumed to play a central role in the encoding of optic flow stimuli, i.e. large-scale motion patterns on the retina, caused by the movement of large parts of the visual environment relative to the organism. This assumption is partly based on the observation that neurons in MSTd show tuned responses to linear motion patterns, as well as to 'spiral motion stimuli', a class of complex motion stimuli creating a circular continuity including expanding, contracting and rotating patterns as well as combinations of them.

Here we report a study aimed to determine the exact motion patterns MSTd neurons are most responsive to, and whether these patterns are more appropriate 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 single cells in two rhesus monkeys, trained to foveate a fixation point of a large back-projection screen. The MSTd receptive fields were mapped using a reverse correlation approach. Here a large complex random dot pattern motion stimulus covered the receptive field, formed by the smooth variation of local dot direction and speed between a grid of positions in the stimulus where the local speed was chosen randomly from all possible directions and a large range of speeds. These local motion vectors changed randomly every 100ms, resulting in a rapidly changing complex white-noise motion stimulus.

This approach has allowed us to recover a detailed motion preference pattern for every recorded MSTd cell. We observe that these preference patterns are generally more complex than can be assumed based a given neuron's linear and spiral motion direction tuning. As a population MSTd neurons are able to provide a detailed representation of complex motion patterns observed.

Developmental synapse refinement in mouse visual cortex

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Fast excitatory synaptic transmission in the brain primarily involves AMPA receptors (AMPA) at glutamatergic synapses. However, during early developmental stages, many glutamatergic synapses are postsynaptically silent at resting membrane potential and hence are non-transmitting. The molecular events how the majority of synapses become transmitting during maturation remain elusive. While many studies indicate that these silent synapses are inactive due to the lack of AMPARs, some studies show that they contain labile AMPARs but gain silence during synaptic transmission. Both concepts hint that postsynaptic insertion of AMPARs occurs during development to convert silent synapses into mature transmitting ones. By analyzing the failure rate of excitatory postsynaptic currents (EPSCs) evoked with minimal stimulation protocol, we showed previously that there is a developmental decrease in the silent synapse ratio of layer 2/3 pyramidal neurons in mouse visual cortex (from about 55% at age P10 to less than 10% after P60). There is no decrease when PSD-95 (a postsynaptic density protein involved in AMPAR trafficking) is knocked out.

In this study, we observed an acute increase in miniature EPSC (mEPSC) frequency (which likely reflects the number of transmitting synapses) after eye opening (P13) but no significant change during the critical period (P20-30). These results are apparently at odds with the decline of the silent synapse ratio. Presynaptic mechanisms are unlikely to mediate this change, because release probability as assessed by paired-pulse ratio did not change. Our findings suggest that developmental synaptic refinement is not simply a conversion of silent synapses to transmitting synapses, but additional mechanisms, such as synaptic pruning, may be involved in the maintenance of mEPSC frequency (hence a homeostasis of basal synaptic transmission) after eye opening.

Effectiveness of electrically evoked input depends on the gamma-phase of the receiving population in monkey area V4

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Attention-dependent selective signal routing between cortical areas has been proposed to depend on gamma-band phase synchronization between downstream neurons and their afferent input from upstream areas. In support of this hypothesis, previous work has shown that populations of V1 neurons delivering afferent input signals of the attended stimulus and the V4 neurons receiving this input synchronize their gamma-band activity. At the same time, V1 neurons delivering converging afferent input signals of a nearby distractor to the same V4 neurons show almost no gamma-band synchronization. This raises the question whether there is a causal relation between the instantaneous phase of the receiving neurons' gamma cycle and the effectiveness of afferent signal transfer.

To investigate this question, we trained a macaque monkey (*Macaca mulatta*) to a demanding shape-tracking task. The task requires attending one of two closely spaced and continuously deforming shapes to identify reappearance of the target's initial shape. We recorded multi-unit activity (MUA) and local field potential (LFP) in cortical area V4 while the two stimuli were both placed within the population receptive field (pRF) of the neurons at the recording site in area V4. Another micro-electrode was placed in area V2 for electrical stimulation at a site, which represents one of the two stimuli. At different times during the behavioral trials the recording site in V2 was stimulated by a single, bipolar electrical pulse.

We found electrophysiological and behavioral effects of weak electrical single-pulse stimulation, which depended on the phase of the stimulus induced gamma-band activity at the V4 recording site. If the electrically stimulated V2-site represented the distractor stimulus, the height of MUA-response peaks for the V4 recording site strongly depended on the phase of the gamma-band activity of the V4-LFP. Correspondingly, the power of the LFP was selectively enhanced in the gamma-band after stimulation in the most effective phase but mostly unaffected, when the electrical pulse was delivered at an opposite phase. To investigate behavioral effects of the same weak electrical pulses, we compared the 25% fastest with the 25% slowest correct behavioral responses when electrically stimulating the V2 recording site representing the distractor. If pulses arriving in V4 at a specific phase caused a retardation of responses, the distribution of the phase of arrival should differ between the slow and the fast responses. Indeed, we found that for slow responses a significant peak occurred at the same gamma-band phase, which showed the strongest neurophysiological effects (Rayleigh's test, $p < 0.05$). No such phase dependence appeared for the fast responses. None of the phase-specific effects was observed for stimulating V2 sites representing the target.

In summary, the results demonstrate a dependence between the phase of gamma-band activity at which input signals arrive in V4 and the strength of the resulting neuronal responses and behavioral effects. This suggests a causal relation between the phase of gamma- oscillations and the effectiveness of afferent synaptic input in cortical neurons.

This research was supported by funding from the DFG (SPP 1665).

Environmental enrichment accelerates ocular dominance plasticity in mouse visual cortex; putting animals back to a standard cage results in a rapid loss of this plasticity

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While ocular dominance (OD) plasticity in the primary visual cortex (V1) is age-dependent in standard cage (SC) raised mice, plasticity of mice raised in or moved to a so-called enriched environment (EE) continues lifelong (Greifzu et al., 2014 PNAS; Greifzu et al., 2016 Neurobiology of Aging): In SC-mice, OD-plasticity is maximal during the critical period (postnatal day (PD) 25-40), reduced in young adults and absent in mice older than PD 110 (e.g. Espinosa and Stryker 2012 Neuron). In contrast, mice raised in EE-cages providing more voluntary physical exercise (running wheels), social interactions (larger animal groups) and cognitive stimulation (regularly changed maze) display lifelong OD-plasticity. In addition, EE housing can restore OD-plasticity in adult mice transferred from SC to EE after PD 110.

Given the strong plasticity-promoting effect of EE, we wondered whether shorter visual deprivation periods are already sufficient to induce OD-shifts in EE- compared to WT-mice of various age groups. To this end, we used optical imaging of intrinsic signals to analyze V1-activation after 2 or 4 days of monocular deprivation (MD) in PD 27-34, PD 80-101 and PD 121-183 mice. Indeed, 2 days of MD were already sufficient to induce OD-shifts in both young and old EE-mice, while this was not the case in age-matched WT animals. OD-shifts were, however, stronger after longer MD-periods. Since OD-plasticity could be restored by transferring mice from SC- to EE-cages, we next wondered how long OD-plasticity of adult EE-mice (>PD 130) would persist after moving them from EE- to a SC-cage. Surprisingly, OD-plasticity was rapidly abolished: already after 1 week of SC-housing OD-plasticity could no longer be induced by a 7-day MD in mice born and raised in EE. OD-plasticity after MD was, however, rescued when we supplied the SC-cages with a running wheel. These data strongly indicate that the inability to run (by e.g. putting mice in SC-cages) has clear plasticity-preventing effects.

In summary, our results demonstrate that raising mice in less deprived environments with access to running wheels (e.g. in EE) strongly accelerates plasticity of cortical networks and extends it into older age. In contrast, preventing voluntary physical exercise in adulthood immediately precludes experience-dependent plastic changes.

Imaging of spine dynamics in the visual cortex of awake PSD-95 knockout and wild type mice

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The postsynaptic scaffolding protein PSD-95 is present in the majority of excitatory synapses and modulates their postsynaptic function and maturation (Cane et al., 2014). We recently showed that adult PSD-95 knockout (KO) mice have 9x more AMPA-silent synapses in the primary visual cortex (V1) than wildtype littermates (WT) and retained a lifelong juvenile-like ocular dominance plasticity (Huang et al., 2015). In addition, experience-induced network changes happened faster in PSD-95 KO compared to WT mice. Thus, PSD-95 KO mice displayed enhanced cortical plasticity but the neuronal circuits were less stable suggesting that dendritic spines may be more dynamic in V1 of the KOs.

To this end, we used two-photon microscopy through a chronic cranial window to repeatedly image spines in awake head-fixed mice (Joachimsthaler et al., 2015). Spines were visualized by injecting a LifeAct-GFP virus into V1 of WT and PSD-95 KO mice, which labels the F-actin of neurons, which is the major cytoskeletal component of dendritic spines (Hotulainen and Hoogenraad, 2010).

We were able to follow up the same dendrites and spines over extended periods of time (up to 13 days) to record changes of dendritic spine numbers and dynamics. Preliminary results indicate that our adult WT mice have similar values of spine dynamics as previously published for mouse visual cortex (Grutzendler et al., 2005); in contrast, PSD-95 KO mice have a higher spine turnover rate (reduced number of stable spines, and higher number of eliminated and new spines) and higher spine density compared to wild type mice.

Cane M, Maco B, Knott G & Holtmaat A (2014) *J Neurosci* 34: 2075-2086.

Grutzendler J, Kasthuri N & Gan W-B (2005) *Nature* 420: 812-816

Hotulainen P & Hoogenraad CC (2010) *J Cell Biol* 189: 619-629.

Huang X, Stodieck SK, Goetze B, Cui L, Wong MH, Wenzel C, Hosang L, Dong Y, Löwel S & Schlüter OM (2015) *Proc Natl Acad Sci USA* 112: E3131-E3140.

Joachimsthaler B, Brugger D, Skodras A & Schwarz C (2015) *J Neurosci* 35: 3772-3781.

Synaptic correlates of the predictive coding of form and motion in V1

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Long distance horizontal connections, intrinsic to primary visual cortex (V1), have been hypothesized to play a role in binding cells with identical functional preferences across the visual field, irrespective of their receptive field (RF) location. Combined intracellular and imaging techniques have shown that this binding requires stimulus-induced cooperativity to enhance the long-range orientation-selective spread beyond the feedforward imprint (Chavane et al, 2011). Using test stimuli allowing spatial summation within the aggregate RF of the cortical hypercolumn, we have recently reexamined the spatio-temporal features of the synaptic subthreshold receptive field of V1 cells (Gerard- Mercier et al. J. Neurosci. 2016). Our results showed that synaptic responses to flashed 3-4° Gabor patches can be elicited from the far periphery (up to 15°) and, most remarkably, exhibit a coherent organization, reflecting the grouping bias of the "perceptual association field" for collinear contours (Field et al, 1993).

The present new intracellular experiment carried on the cat V1 was designed to characterize the spatial synergy and temporal coherence requirements. We used 6-stroke apparent motion (AM) concentric sequences of Gabor patches at saccadic speeds (~200°/s), centered on the subthreshold RF (with the distance between strokes scaled to the diameter of the RF) extending the motion path up to 25° into the periphery. The response to stimulation of the RF center alone was compared to the response to the AM sequence, which was either centripetal or centrifugal with the orientation of the individual elements either collinear or cross-oriented to the motion path. Control conditions included randomized order of the Gabor patches presentation and the change of the AM speeds. We also included sequences restricted to the silent surround of the RF to infer the filling-in responses induced by the periphery alone.

Our results show a supra-linear subthreshold input from the far periphery, and a non-linear boosting of the neuronal discharge resulting in a significant phase advance (5-20 ms) in the spiking response. Summation processes during the AM sequence show de novo emergence of significant responses for stimuli flashed as far as 10-15° away from the classical RF. The boosting effect is specific to centripetal AM at saccadic speeds and could not be induced by centrifugal AM, by the random Gabor sequence or by AM at lower speeds. Collinear movement was also more effective than cross-oriented movement. All these results are consistent with our hypothesis that cooperative "Gestalt-like" interactions are triggered when the visual input carries a sufficient level of spatial and temporal coherence matching the properties of the underlying V1 connectivity.

Is the contribution of visual feedback on grasping activity similar in the grasping areas of the dorsal visual stream?

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One of the major functions of vision is to enable an efficient and active interaction with the surrounding environment. The execution of complex movements, such as grasping objects, requires the integration of multisensory inputs. Area V6A, located in the medial posterior parietal cortex of the macaque brain, is centrally involved in sensorimotor transformations and contains neurons responsive to visual stimuli as well as cells modulated by movements requiring different wrist orientations and grip types performed in darkness (Fattori et al., 2015). The present study aims at understanding the relative contribution of visual information and of hand shape for grasps in the dorsomedial area V6A. The use of the same experimental setup employed in the dorsolateral grasping area AIP, known to be involved in the control of grasping actions directed towards different objects in light and in dark (Baumann et al., 2009; Schaffelhofer and Scherberger 2016; Murata et al., 2000), allowed us to make a comparison between the grasp-related properties of V6A and AIP.

Two male *Macaca fascicularis* were trained to grasp three-dimensional objects of different shapes. The animals employed different grips to grasp them: whole-hand prehension, hook grip, finger prehension, primitive precision grip, and advanced precision grip. The spatial location of the object to be grasped was kept constant. The reach-to-grasp task was performed both in dark and in light. The same objects were observed, without performing grasping, in a third task. We performed extracellular recordings from 317 V6A neurons. We quantified the neural activity during grasping preparation, execution and object holding. We found that the overwhelming majority of neurons (94%) was task-related, that is, was modulated by at least one grip type in at least one visual background with respect to the baseline activity (Student's t-test, $p < 0.02$, corrected for multiple comparison). Most of task-related cells were found to be influenced by both grip type and visual information, but in a different way: almost half of task-related cells were excited by visual input, whereas half were inhibited. We also quantified the grip sensitivity and we compared it in the two visual conditions. In most cases, grip sensitivity was poorly influenced by visual feedback. This result was also confirmed by the demixed Principal component analysis (dPCA), that reduces the dimensionality of the data taking task parameters into account. dPCA demonstrated that grip components were prominent with respect to visual ones (35% vs. 14%), surprisingly for a visual area like V6A. These results confirm the role of V6A in integrating visual and motor-related signals. It also emerges that both parietal grasping areas, V6A and AIP, update and control the configuration and orientation of the hand as it approaches the object to be grasped, although with a different relative weight of motor-related (grip type) and sensory (visual) signals.

Multiple thalamocortical axonal architectures converge in mouse visual cortical areas

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In addition to lateral geniculate (dLGN) axons, rodent visual cortex receives innervation from several additional thalamic nuclei. How these multiple thalamocortical (TC) pathways distribute and/or overlap in the visual cortical areas is not yet well understood. Here, we have investigated TC pathways innervating mice visual areas (namely V1, LM-V2, P, PM, AM, MM, A, RL, AL, LI, POR) at the micropopulation and single-cell level.

To identify TC inputs, first we examined cells retrogradely labeled in thalamus by small Fast Blue (FB) or Cholera Toxin Subunit B (CTB-Alexa 488, CTB-Alexa 596) deposits in cortex. In other experiments, we made iontophoretic microinjections of biotinylated dextranamine 10KDa (BDA) in thalamus, and examined the distribution of labeled axons in cortex. Tracer injections and labeled axons were plotted onto bidimensional maps of cortex. Axonal length and varicosity numbers were stereologically measured. Axonal varicosity size was measured in selected areas and layers. In addition, to elucidate the presence of branched axons simultaneously innervating separate areas, we virally transfected (Sindbis-Pal-GFP or AAV1.hSyn.eGFP.WPRE.bGH) individual dLGN or lateral posterior (LP) neurons, and 3D reconstructed-measured their entire dendritic and axonic arbors. Our data show that only ~60% of thalamic cells labeled by V1 tracer deposits are located in dLGN, 30% are located in LP, and smaller populations are labeled in the laterodorsal, centrolateral, posterior and ventromedial nuclei. dLGN sends few projections to extrastriate areas (mainly to LM-V2); in these areas, diversity of thalamic input sources is even broader.

Supported by European Union 7th Framework Programme -Human Brain Project and MICINN.

Neuronal response properties during the repetitive presentation of a visual stimulus in mouse V1

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Our ability to learn relies on the potential of neuronal circuits to change through experience. Previous studies have shown that daily brief presentations of a visual stimulus over 5 consecutive days induces long-lasting potentiation of electrophysiological responses in mouse primary visual cortex (V1). This population response potentiation is stimulus specific, depends on cortical mechanisms and manifests behaviourally as long-term habituation to the familiar stimulus. However, another study showed that there is a decrease in overall population response after daily brief presentation of a visual stimulus. This decrease was due to a decrease in the number of visually responsive neurons, while there was no change in the mean activity of excitatory neurons during stimulus presentation.

In this study, we used chronic *in vivo* two-photon calcium imaging in awake-behaving mice to determine changes in neuronal activity and orientation-selectivity at the single cell level in V1 after daily brief presentations of a visual stimulus. We monitored the activity of excitatory neurons as well as of three non-overlapping populations of inhibitory neurons (vasoactive intestinal peptide (VIP), somatostatin (SST) and parvalbumin (PV)-positive interneurons) in layer 2/3 of mouse V1 during five consecutive days: before, during and after a 5-minute daily presentation of a sinusoidal phase-reversing grating. To image the activity of interneuron subtypes, we used Cre-driver lines (PV-, SST-, or VIP-Cre mice) cross-bred with a Cre-reporter tdTomato line, in which we injected adeno-associated viruses (AAV1.Syn.GCaMP6s.WPRE.SV40) into V1 for the expression of GCaMP6s in all neurons. Furthermore, behavioural state was monitored during each experiment (stationary vs locomotion).

We recorded the GCaMP6s signal and running speed simultaneously, both in darkness and during presentation of the familiar stimulus, over 5 consecutive days. In addition, the orientation-selectivity of imaged neurons was determined on day 1 (before) and day 5 (after) the presentation of the familiar stimulus. Orientation selectivity was also determined one day after the familiar stimulus presentation was finished (day 6) and 7 days later (day 14). Using this protocol, we characterize the stability and plasticity of visual and locomotion responses in V1 layer 2/3, during the brief presentation of a repetitive visual stimulus.

Neuronal responses in the upper visual field of the rat

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While rats move in space, they maintain a constant overlap of their left and right eye visual fields in the space above their heads. These eye movements are most likely a strategy to detect predators coming from above. This statement is supported by a strong shelter-seeking response of rats exposed to visual stimuli above their heads. However, if the same stimuli are displayed in frontal or lateral parts of the animal's visual field, the animal's behavior doesn't change. Since the primary visual cortex of rats has a retinotopic organization, meaning that input from different areas of the animal's visual field is processed in different parts of its visual cortex, we would like to assess whether the neuronal responses to stimuli presented at different angles in the visual field differ. To test this we modified a conventional two-photon calcium imaging setup to allow for direct optical recording from neurons in the overhead visual field. The microscope was frontally extended using a periscope coupled to a miniature objective to create access above the head of the rat. In addition custom software was developed to deliver visual stimuli using a lightweight OLED display on a tablet running the Android OS, as well as a technique to calibrate a mounted camera in order to triangulate its position in space in relation to head coordinates. We used two different stimuli which were previously shown to elicit a flight response: a dot moving in multiple lines over the stimulus screen and an expanding or contracting looming dot. In both moving and looming dot stimulus types, a black on white and a white on black setup were used. We also presented moving gratings at different angles to identify orientation responsive cells.

We recorded 654 cells from 17 animals of which ~22% were responsive to a looming dot stimulus, ~19% were activated by a moving dot stimulus and ~21% showed activity to moving gratings at different angles. When changing the location of the visual stimulation from frontal to overhead, the percentage of active cells increased (26% at 8°, 41% at 77° visual angle from the horizon). Neurons stimulated at around 65-80° with a moving dot stimulus preferentially responded to a black on white contrast while cells presented with a frontal stimulus responded equally to both black on white and white on black. In the looming dot condition there were more responsive cells in the overhead region compared to the frontal one but we couldn't show a clear preference for a specific contrast condition. The amount of cells showing responses to moving gratings at different angles was roughly the same in all recorded areas. Our preliminary results indicate that there are different response characteristics for neurons in specific parts of the primary visual cortex depending on the stimulus and contrast parameters used.

Postsynaptic scaffolds and visual stimulation fine-tune the development of glutamatergic synapses in visual cortex.

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AMPAreceptor-lacking glutamatergic synapses (silent synapses) are a feature of immature brain circuits. Through *ex vivo* whole-cell patch-clamp electrophysiology in mouse primary visual cortex (V1), we observed that normal maturation of glutamatergic neurotransmission was characterized by a robust reduction in the fraction of silent synapses: from about 80% at postnatal days (PD) 3-5, to 50% at PD10-12, 30% at PD19-21 and further to 20% at PD25-30 (Huang et al., 2015; Favaro, 2014).

We further identified two postsynaptic scaffolds, which antagonistically fine-tune the time-course of silent synapse development in the visual cortex: While PSD-95 deletion prevented proper synaptic maturation, PSD-93 deletion accelerated it. This functional antagonism was further confirmed by analysis of double-KO synapses, which were indistinguishable from WT at PD25-30.

Furthermore, we confirmed that dark rearing prevents synaptic development following eye opening at PD13-14, as previously reported (Funahashi et al., 2013), reinforcing the role of visual stimulation leading to experience-induced circuit refinement in V1. However, even in the absence of visual input, PSD-93 KO circuits still presented accelerated unsilencing of silent synapses. Endogenous PSD-93 apparently acts as a "molecular brake" preventing precocious maturation of neurotransmission in V1.

Our results evidence a functional interplay between postsynaptic scaffolds and visual stimulation, fine-tuning early development of glutamatergic neurotransmission in V1. As PSD-93 and PSD-95 have antagonizing roles on neuronal communication, the consequences of their action on V1-dependent functions are, presumably, also opposite to each other.

Recovery from vision loss in subacute stroke following tDCS treatment

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Background

There is some potential for recovery after visual system damage which is mediated by plastic reorganisation of neural tissue after occipital stroke as seen in fMRI (Brodthmann et al., 2007, 2008, 2015, Dilks et al., 2007). Bola et al.(2014) investigated the importance of fronto-occipital network dynamics in visual processing and the effect of transcranial alternating current stimulation on those networks and on the restoration of blindness after optic nerve damage and first investigations are now available regarding stroke-treatment in visual system damage in chronic phase (Alber et al. 2015) But studies on early intervention in the early post-lesion recovery phase are not yet available Since transcranial direct current stimulation (tDCS) is considered to be safe even in acute and sub-acute phase, we now used tDCS to study its impact on behavioural and neurophysiological correlates in terms of dynamics in the time course of vision recovery during the first 6 months after stroke.

Aim:

Uncover the effects of tDCS in subacute stroke patients to improve vision.

Question:

Does tDCS enhance vision recovery in an earlier stage after stroke?

Methods:

In a randomized, sham-controlled, double-blind clinical trial, nineteen sub-acute stroke patients diagnosed with unilateral posterior cerebral artery ischemia were enrolled. Patients were randomized in a verum and a sham group controlled for age, gender, and level of vision as measured by the mean sensitivity (MS) in threshold perimetry. The groups underwent a baseline resting-state and checkerboard-VEP EEG recording and were then treated with tDCS sham or verum. After 10 days of treatment, another EEG post-treatment recording session was carried out with a final follow-up recording after three months.

Results:

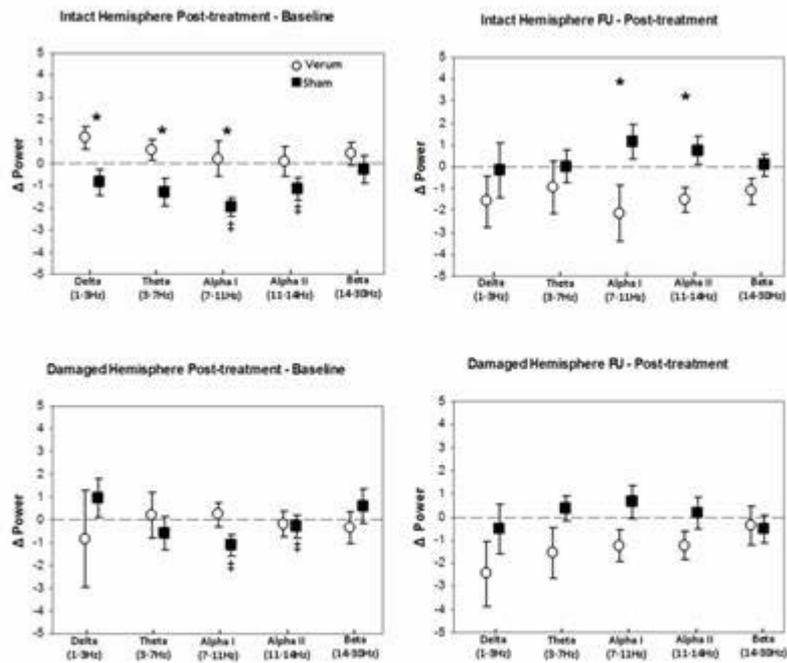
Mean sensitivity increased significantly after treatment but this was not found to be stable after 3 months in both groups (see chart) as it dropped back towards baseline levels. We calculated absolute change of power (post-treatment – baseline, follow-up – post-treatment; see graph) in all frequency bands to compare the change between hemispheres, groups and again link them with the change in our outcome variables. The verum and sham group both showed significant differences in absolute change over baseline to post-treatment in Delta ($t_{15}=2.615$, $p=.020$), Theta ($t_{15}=2.483$, $p=.025$) and low Alpha ($t_{15}=2.457$, $p=.027$), and from post-treatment to follow-up in low Alpha ($t_{12}=-2.297$, $p=.040$) and high Alpha ($t_{12}=-2.449$, $p=.031$), but only in the intact hemisphere. Whereas the verum group increased in power, the sham group decreased in power. When we compared both hemispheres, only in the sham

group low Alpha ($t8=-2.809$, $p=.023$) and high Alpha ($t8=-2.891$, $p=.020$) decreased significantly more in the intact than in the damaged hemisphere.

Conclusion: Resting state EEG power analyses revealed that neurophysiological changes appear some time after the stroke. Possibly, specific training induces physiological changes which could be interpreted as compensational mechanisms of the intact hemisphere and tDCS could have some “protective” effect in stabilizing alpha power. But these changes were not maintained at follow up. Probably longer stimulation might stabilize the visual field improvements effect (mean sensitivity and alpha drop to follow up stronger in verum than in sham). Thus, though tDCS enhanced both vision recovery and modulated EEG power, but the effects were not stable at follow-up.

Group Statistics				
	Gruppe	N	Mean	Std. Deviation
MSDiff_t1_t2_Percentage	Verum	8	19,70	16,09
	Sham	9	11,69	6,95
MSDiff_t1_t3_Percentage	Verum	7	18,40	30,20
	Sham	8	9,57	8,58
MSDiff_t2_t3_Percentage	Verum	7	-4,29	13,40
	Sham	8	-1,42	10,13

Chart: This chart shows visual field mean sensitivity changes in percentage between the three different timepoints (t1 – baseline; t2 – post-treatment; t3 – follow-up).



Graph: This graph shows absolute change values ("Post-treatment - Baseline" and "Follow-up - Post-treatment") for the different frequency power bands in verum and sham group, separated for intact and damaged occipital ROIs (OI, OC).
 *Significant group differences at $p < 0.05$;
 #Significant differences between intact and damaged lobe at $p < 0.05$.

Response modulation by spatial attention in area MT of primate visual cortex is not mediated by the cholinergic system.

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The aim of this study was to determine whether the enhancement of sensory responses in the middle temporal area MT of primate visual cortex by spatial attention is mediated by the cholinergic system.

We recorded single unit neuronal activity in MT of two rhesus monkeys, trained to maintain their gaze on a central point on a computer screen while performing a spatial attention task. A cue indicated which one of two random dot patterns (RDP) should be attended. One was placed inside the receptive field of the recorded cell and the other outside, diametrically opposite. Attentional modulation was quantified by comparing visual responses when the RDP inside the RF was attended (attend-in condition) vs. unattended (attend-out condition). A sensory condition required a luminance change detection at fixation while ignoring both RDPs. After acquisition of control block data, the task was repeated during pressure injection [Veith et al., 2016] of a cholinergic agent, followed by a recovery period after the injection. The antagonist scopolamine (n=97) was used to block muscarinic receptors, or the endogenous extracellular concentration of cholinergic neurotransmitters was increased with the agonist acetylcholine (n=38).

Of the recorded single units with sufficient trial repetitions and response strength, approximately 30% showed a significant difference in firing rate between control and injection blocks of the sensory condition (scopolamine: 36/97, acetylcholine: 12/38). Most cells increased their response with acetylcholine (9/12), while scopolamine induced a heterogeneous effect (19/36 showed an increase, 17/36 a decrease). The strength of effect did not differ for attend-in and attend-out conditions.

The attentional effect was studied for the two subsets of cells that were significantly manipulated by scopolamine or acetylcholine. We observed a significant and strong influence of spatial attention on our cell population in absence of a cholinergic agent. Acetylcholine injection did not induce a significant change on attentional modulation though, independently whether a given cell showed an increase or a decrease in its response during the fixation condition. For scopolamine, the subpopulation showing an increase in firing rate also showed a significant increase in attentional modulation during both control and injection blocks. The subpopulation showing a decrease in firing rate interestingly did not show a significant influence by attention neither in control nor injection blocks.

Local application of scopolamine or acetylcholine on average did not lead to any change in attentional enhancement, so we cannot support mediation by the cholinergic system in attentional modulation in macaque area MT.

Supported by DFG-CRC-889.

Response properties of neurons in the binocular visual cortex of PSD95 knockout mice *in vivo*

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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) and retain a lifelong juvenile-like ocular dominance plasticity (Huang et al., 2015, PNAS 112 (24): E3131-E3140). 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 used extracellular multielectrode recordings to investigate response properties of neurons in the binocular part of V1 of isoflurane-anesthetized PSD-95 KO and WT mice. Visual stimuli consisted of moving sine wave gratings of 8 different orientations (2 directions each) and seven spatial frequencies (0.05-0.32 cycles/degree), presented with 2 Hz temporal frequency at full contrast (42.7 cd/m² maximum luminance) to either the ipsi- or contralateral eye. We recorded both evoked and spontaneous spike rates, and quantified the orientation selectivity of the evoked responses (calculated as the orientation selectivity index = OSI), the preferred spatial frequency, and preferred direction of all recorded units. Two of the investigated response properties yielded significant differences between WT and KO mice: Compared to the WT mice, PSD-95 KO mice showed elevated response rates to contralaterally presented gratings of preferred orientation and spatial frequency (mean±SEM: WT=26±2 spikes/sec, KO=38±4 sp/s, Man Whitney rank sum test: p=0.035). In addition, the orientation selectivity index of PSD-95 KO mice was less well matched between contralateral and ipsilateral eye responses (orientation selectivity difference between ipsi and contra eye stimulation, mean±SEM: WT=0.13±0.04, KO=0.20±0.03, Man Whitney rank sum test: p=0.02). Ongoing behavioral experiments investigate what consequences these differences might have for visual perception.

The potentials of the methanolic leaves extract of *Lannea schimperi* (HOSCHST. EX RICH) ENG. Aas a surface anaesthetic agent.

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Local anaesthetics provide relief from pain when applied locally to nerve tissue by blocking conduction of sensory nerve impulse from the receptor to the brain cortex. This study aimed at evaluating surface anaesthetic activity of the methanolic leaves extract of *Lannea schimperi* using guinea pig corneal reflex method. Ten healthy guinea pigs of both sexes were randomly separated into two groups of five animals each for guinea pig cornea method of surface anaesthesia. Group I received 24 mg/ml of the methanolic leaves extract of *Lannea schimperi* while group II received 0.2 % lidocaine. Two drops of the test drug solutions were instilled into the right conjunctival sac, the left eye was taken as control. The plant extracts at 24 mg/ml exhibited a good surface anaesthetic activity by significantly inhibiting response to corneal reflex for a period of 25 minutes. Both the standard drug lidocaine and the plant extract recorded their highest surface anaesthetic activity at 15 minutes with percentage inhibition of 100 % and 92.3 % respectively. The result therefore suggests that methanolic leaves extract of *Lannea schimperi* possess surface anaesthetic principles that may require further scientific elucidation.

The role of postsynaptic density protein 93 for visual cortical plasticity

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Activity in the binocular region of the mouse primary visual cortex (V1) is dominated by input from the contralateral eye. Monocular deprivation (MD) of this eye results in an ocular dominance (OD) shift towards the open eye. During a critical period (CP) in early life of mammals, V1 is particularly susceptible to plastic changes: In C57Bl6/J mice, the CP opens around postnatal day (PD) 20 and closes around PD 35. While 4 days of MD are sufficient to induce plasticity during the CP, "adult" OD-plasticity up to an age of PD 110 needs 7 days of MD. Beyond PD 110, OD-plasticity is absent in standard cage raised mice¹. We recently observed that knockout (KO) mice for postsynaptic density-95 (PSD-95) retain a lifelong and juvenile OD-plasticity and have 9x more AMPA-silent synapses in V1 than wildtype littermates². In contrast, the percentage of silent synapses dropped from ~55% (PD 10-12) to almost 0% already during the CP (P21-30) in PSD-93 KO mice and thus faster than in WT mice, indicating that PSD-93 and PSD-95 might have opposing functions for cortical network maturation and stabilization.

We therefore tested whether OD-plasticity declines more rapidly in PSD-93 KO mice, in line with the reduced number of silent synapses. To this end, we visualized V1-activity after MD in 3-4-week-old PSD-93 KO mice using optical imaging of intrinsic signals³. Indeed, PSD-93 KO mice showed an earlier closure of the CP: While during mid-CP (PD 24-27), PSD-93 KO animals showed an OD-shift after 4 days of MD (OD-index (ODI) declined from 0.37 ± 0.04 to -0.03 ± 0.06 after MD, $n=5/6$; $p < 0.001$; unpaired t-test), OD-plasticity was already absent during late-CP (PD 28-35; ODI decreased from 0.32 ± 0.02 to 0.28 ± 0.02 , $n=5/6$; $p=0.17$, t-test), while WT mice of the same age still showed OD-plasticity. To clarify the locus underlying the decreased plasticity in PSD-93 KO mice, we tested the effect of time and region specific silencing of PSD-93 expression with viral vectors (AAV-sh93). Knock-down (KD) of PSD-93 in V1 at P0 indeed completely phenocopied the KO-effect: OD-plasticity was already absent in late-CP PSD-93 KD but not in control-KD mice.

Since dark rearing (DR) has recently been shown to delay silent synapse maturation in mouse V1⁴ we tested whether DR might prevent the early decline of OD-plasticity in PSD-93 KO mice. This was not the case. Finally, we tested whether giving PSD-93 KO mice access to running wheels (which boost OD-plasticity in WT-mice⁵) would delay their early CP-closure. This was also not the case. In summary, the data clearly show that the CP for OD-plasticity closes precociously in both PSD-93 KO and KD-mice and that neither DR nor voluntary physical exercise can prevent the early decline of plasticity.

In conclusion, while PSD-95 is necessary for synaptic maturation in visual cortex, PSD-93 seems to prevent a precocious network stabilization thus ensuring that experience can optimize network function. AMPA-silent synapses are therefore critically important for neural network refinement and their conversion into transmitting synapses presumably is the terminating event for critical periods.

Supported by the DFG SFB889 (project B5 to S.L. and Project B3 to O.S.)

¹Lehmann & Löwel (2008) PLoSOne 3: e3120.

²Huang et al (2015) PNAS 112(24): E3131-40.

³Kalatsky & Stryker (2003) Neuron 38: 529-45; Cang et al (2005) Vis Neurosci 22: 685-91.

⁴Funahashi et al (2013) J Neurophysiol 109: 2064-76.

⁵Kalogeraki et al (2015) J Neurosci 34: 15476-81.

Towards no-report readouts of conscious visual perception

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To study the neural basis of conscious visual perception experimenters seem obliged to resort to overt behavioral reports from their subjects such as button presses or eye movements. However, relying on overt responses confounds the neural measures of conscious perception with many other cognitive variables such as decision making, motor planning and performance monitoring. In addition, those behavioral measures might be difficult to instruct and obtain in certain subject groups such as animals, infants and neurological patients (Tsuchiya et al., 2015). Thus, having no-report (i.e. implicit) measures for consciousness perception would be of great benefit. In the current study we tested several physiological parameters in monkeys (N = 2) or humans (N = 17) that have been shown in previous studies to correlate with reported subjective visibility: low (9-12 Hz) and high (30-80 Hz) frequency power in local field potentials (LFP) (Wilke et al., 2006) as well as pupil diameter (de Gee et al., 2014). To this end we employed a visual illusion that renders salient visual stimuli subjectively invisible upon appearance of a moving surround pattern (Maier et al., 2008). In the 'report' condition, stimuli were adjusted to be ambiguous, leading to target disappearance in about 50% of trials. In the no-report condition disappearance was determined by the stimulus configuration, i.e. presentation of target and surround either to the same ('visible') or to different eyes ('invisible') while keeping overall luminance levels constant. Physical target removal and delayed report served as experimental controls. We found that 1) Subjective visibility in extrastriate visual cortex (i.e. V4) was reliably reflected in gamma power even without report, while perceptual modulation in lower frequency bands was contingent upon the report. 2) Pupil diameter primarily depended on whether the percept was overtly reported. Here, already a short delay between stimulus events and report rendered pupil modulation insignificant. Our results suggest gamma power in visual cortices as the most promising marker for conscious visibility, while low frequency power LFP and pupil diameter seem heavily confounded by report-related cognitive and motor variables.

de Gee, J.W., Knapen, T., and Donner, T.H. (2014). Decision-related pupil dilation reflects upcoming choice and individual bias. Proc Natl Acad Sci U S A 111, E618-625.

Maier, A., Wilke, M., Aura, C., Zhu, C., Ye, F.Q., and Leopold, D.A. (2008). Divergence of fMRI and neural signals in V1 during perceptual suppression in the awake monkey. Nat Neurosci 11, 1193-1200.

Tsuchiya, N., Wilke, M., Frassle, S., and Lamme, V.A. (2015). No-Report Paradigms: Extracting the True

Neural Correlates of Consciousness. Trends Cogn Sci 19, 757-770.

Wilke, M., Logothetis, N.K., and Leopold, D.A. (2006). Local field potential reflects perceptual suppression in monkey visual cortex. Proc Natl Acad Sci U S A 103, 17507-17512.

Visual pop-out in barn owls: From behavior to neural correlate

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To us humans, an item pops out if it differs from its surroundings in one feature, such as motion or orientation. In that case it, the target, is detected effortlessly and independently of the number of other items, the distracters. We want to know, to what extent such pop-out perception - and possible neural correlates - exist in barn owls. Barn owls lack significant eye movements which is compensated by rapid head saccades. Therefore, their gaze can be tracked with a head mounted microcamera, the OwlCam. In a set of behavioral experiments we confronted three barn owls carrying OwlCams with search arrays containing one target and 15 – 63 distracters. We tested 3 features that are known to pop out to primates: luminance, motion and orientation. Thus, the target was either oriented differently, brighter, or moving in a different direction from the uniform distracters. When trained to search and fixate the targets, search times did not depend on the number of distracters. Thus, we conclude that these features pop out for barn owls, too. In a second experiment, we searched for neural correlates in the visual wulst, considered the homologue to the mammalian striate cortex. There, we recorded the responses of neurons to the same three features with singleton presentations, pop-out arrays, and uniform arrays. Our data shows that responses to all array types were suppressed compared to the singleton responses (surround suppression). For motion, neural responses were stronger for the pop-out condition than for the uniform condition. This effect was weaker for orientation and luminance. Our findings demonstrate pop-out perception in an avian species and suggest that some of its neural correlates can be found in the avian homologue to the visual cortex.

Poster Topic

T17: Auditory Mechanoreceptors, Vestibular, Cochlea, Lateral Line and Active Sensing

- [T17-1A](#) Absence of the NO-sensitive guanylate cyclase isoform NO-GC1 or NO-GC2 protects cochlear inner hair cells and their synapses
Dorit Möhrle, Katrin Reimann, Nicole Eichert, Steffen Wolter, Markus Wolters, Evanthia Mergia, Doris Koesling, Andreas Friebe, Michaela Kuhn, Frank Schweda, Robert Feil, Marlies Knipper, Lukas Rüttiger
- [T17-2A](#) Avoidance Behavior triggered by Cochlear Optogenetics
Alexander Dieter, Christian Wrobel, Gerhard Hoch, Marcus Jeschke, Tobias Moser
- [T17-1B](#) Cochlear BDNF improves hearing acuity with sensory experience. Is this a prerequisite for adaptive homeostatic plasticity?
Marie Manthey, Dario Campanelli, Wibke Singer, Lukas Rüttiger, Marlies Knipper
- [T17-1C](#) Sexual dimorphism in the auditory fovea of the duetting bushcricket *Ancylecha fenestrata*: anatomical basis and behavioral relevance
Jan Scherberich, Jennifer Hummel, Stefan Schöneich, Manuela Nowotny
- [T17-1D](#) Stochastic resonance in an acoustically communicating insect
Zainab Ali Saad Abdelatti, Manfred Hartbauer
- [T17-2D](#) Unravelling mechanotransduction in the locust ear: Evidence in favour of Inactive-Nanchung as the primary mechanotransduction ion channel.
Ben Warren, Tom Matheson

Absence of the NO-sensitive guanylate cyclase isoform NO-GC1 or NO-GC2 protects cochlear inner hair cells and their synapses

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In the inner ear, the cyclic guanosine 3'-5'-monophosphate (cGMP) signaling pathway has been described to facilitate protective processes for cochlear hair cells and hearing function in response to traumatic noise exposure [1]. However, it is unclear how the cGMP generators contribute to this protective effect. In mammals, cGMP can be generated from guanosine triphosphate by two families of guanylate cyclases (GCs): the soluble, NO-responsive GC (NO-GC) and the transmembrane, particulate GCs [2].

The aim of this study was to investigate the roles of NO-GC and of the particulate guanylate cyclase-A (GC-A) in hearing function, vulnerability to noise exposure and recovery from acoustic trauma. Hearing threshold, supra-threshold auditory processing at sensation level, and outer hair cell function were measured by the auditory brainstem response and the distortion product otoacoustic emission in mice lacking either one of the two isoforms of NO-GC (NO-GC1 KO or NO-GC2 KO) or GC-A. Differences in hearing function were correlated to changes in the hair cell molecular phenotype and cochlear expression of NO-GC or GC-A, visualized by immunofluorescence microscopy and PCR. Intracellular cGMP was quantified by FRET measurement.

The results indicate that absence of either isoform of NO-GC has a protective effect on the inner hair cells and their synapses. In contrast, GC-A KO mice showed distinct vulnerability of outer hair cells after acoustic trauma, suggesting a protective effect of the GC-A/cGMP pathway. The results can be explained by different cGMP generators in functionally distinct parts of the auditory pathway and will be discussed considering NO-GC or GC-A in cGMP-signaling as an otoprotective cascade after noise-induced damage of the ear.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (FOR 2060 project FE 438/5-1 and RU 713/3-2) and by Fortüne 2339-0-0 (University of Tübingen, Tübingen, Germany).

[1] M Jaumann et al.: Nat Med. 2012, 18.2: 252-259

[2] T Chrisman et al.: J Biol Chem. 1975, 250:374-381

Avoidance Behavior triggered by Cochlear Optogenetics

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Direct electrical stimulation of spiral ganglion neurons (SGNs) via cochlear implants represents the state of the art treatment for hearing restoration in profoundly hearing impaired, even enabling speech recognition for many users. However, the electric current spreads in the saline environment of the cochlea, which in turn leads to activation of rather large subsets of SGNs. Hence, the number of independently usable stimulation channels of cochlear implants is restricted and frequency resolution of the user is limited. This inherent limitation of cochlear implants could be overcome by using Optogenetics. Here, SGNs are genetically modified to express Channelrhodopsins (light-gated ion channels) and subsequently stimulated with light. Focusing light to activate small subsets of SGNs would then allow to overcome the current limitation of independent stimulation channels and thus increase frequency resolution of artificial sound encoding.

In this study the channelrhodopsin-2 variant CatCh was injected into the spiral ganglion of adult Mongolian gerbils, leading to high light sensitivity of spiral ganglion neurons. After an expression time of several weeks, an optical fiber was implanted into the round window of the cochlea. Using the Shuttlebox paradigm, animals were then trained in a detection task where they learned to indicate the perception of a stimulus (blue laser pulse delivered through the optical fiber) via locomotion. In parallel, optically driven auditory brainstem responses (oABRs) were measured during the period of behavioral testing to monitor the physiological response to optogenetic stimulation of SGNs.

We found that amplitudes, latencies and thresholds of oABRs stayed stable for several weeks. During this period gerbils learned to avoid electric aversive stimuli cued by optogenetic SGN stimulation within a few days and obtained response rates of up to 95%. Behavioral thresholds of light amplitude were found to be below physiological thresholds (< 3mW) and thresholds of light pulse duration were as short as 0.1ms.

In conclusion, this study demonstrates that stimulation of channelrhodopsin-expressing spiral ganglion neurons with blue light over the course of several weeks creates both a stable physiological response and a percept strong enough to cue behavior.

Cochlear BDNF improves hearing acuity with sensory experience. Is this a prerequisite for adaptive homeostatic plasticity?

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In the adult system, brain derived neurotrophic factor (BDNF) has beneficial roles for memory, cognitive function and plasticity [1- 6] but BDNF can also have potentially harmful effects as described for neuropathic pain [7] or psychiatric and neurodegenerative disorders [8- 12]. During development of the central nervous system (CNS), BDNF was described at first as a survival and growth factor. During development of the auditory system first sensory experience driven activity has been suggested to trigger intracortical inhibition towards shaping the central sound resolution in dependency of BDNF. The experience dependent driving force for this process has so far not been specified further. We demonstrated previously that BDNF (studied in mice with floxed BDNF genes crossed with tissue specifically expressing Cre transgenic mice, BDNF^{fl/fl} Pax2 Cre) in the cochlea and partially in lower brainstem regions but not in the higher frontal or cortical brain regions improves auditory fidelity with sensory experience [13]. This auditory fidelity includes the improved sensitivity of auditory fibers, the lowering of hearing thresholds, the enlarged dynamic range, shortening of latency and altered inhibitory strength. The changes of markers for inhibitory neuronal connections spread along the entire auditory pathway as well as hippocampal circuits [1]. Here we asked, to what extent the failure in BDNF Pax2 Cre ko mice to develop appropriate auditory acuity and fidelity would influence the capability of the central auditory pathway to compensate for injury (noise trauma) induced cochlear deprivations [14,15]. We exposed BDNF Pax2 Cre KO and control mice to enriching (80 dB SPL), mildly traumatic (100 dB SPL), and traumatic (120 dB SPL) sound and measured various functional (ABR) and molecular markers (plasticity genes, markers for inhibition and excitation) along the auditory pathway. The findings are presented and discussed in the context of sensory experience-induced maturation of auditory fidelity as potential prerequisite for adaptive homeostatic plasticity.

[1] Bramham et al., 2005. *Prog Neurobiol* 76, 99-125. [2] Egan et al., 2003. *Cell* 112, 257-269. [3] Lee et al., 2012. *Hippocampus*. [4] Minichiello, L., 2009. *Nat Rev Neurosci* 10, 850-860. [5] Rauskolb et al., 2010. *Journal of Neuroscience* 30, 1739-1749. [6] Thoenen, 2000. *Prog Brain Res* 128, 183-191. [7] Biggs et al., 2010. *Mol Pain* 6, 44. [8] Aznar et al., 2011. *J Alzheimers Dis* 23, 177-193. [9] Hu et al., 2008. *J Neurochem* 105, 1-17. [10] Krishnan et al. 2010. *Am J Psychiatry* 167, 1305-1320. [11] Lu et al. 2008. *Novartis Found Symp* 289, 119-129; discussion 129-135, 193-115. [12] Pardon, 2010. *Vitam Horm* 82, 185-200. [13] Chumak et al. 2015. *Mol. Neurobiol.* [14] Singer et al., 2013. *Mol Neurobiol.* Feb;47(1):261-79. [15] Rüttiger et al., 2013, *PLoS One.*;8(3):e572472013.

Sexual dimorphism in the auditory fovea of the duetting bushcricket *Ancylecha fenestrata*: anatomical basis and behavioral relevance

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In duetting katydids, the mutual signaling between males and females is asymmetrical, which poses the intriguing question how signal disparities are reflected in the ears of the opposite sex. Therefore we investigated sexual dimorphism in the sound producing and hearing organs in the duetting katydid *Ancylecha fenestrata* (Tettigoniidae: Phaneropterinae). For mate attraction, males and females independently evolved file and scraper structures on their forewings to produce short sound pulses with sex-specific dominant frequencies of about 30 and 10 kHz, respectively. At nighttime males produce a mating call that consist of a 60-70 ms sound pulse repeated every 3-6 s in average using typical file and scraper structures. Nearby females occasionally respond to the male calls by a single 40 ms sound pulse with a dominant frequency of about 10 kHz. For stridulation, females use several toothed veins on their right forewing and a long scraper at the side of their left forewing. During playback we discovered that males were significantly ($p < 0.002$) more likely to approach the sound source by phonotaxis during the presentation of the female calls and were generally more active than vice versa. In most cases females did not show any locomotory responses to the playback of male calls.

Both sexes have tonotopically organized hearing organs in the tibia of their forelegs with low frequencies represented in the proximal part and high frequencies represented in the distal part of the *crista acustica*. Males possess a specialized inner ear region with a high number (about 50% of total number) of receptor cells tuned to a narrow frequency range of about 10 kHz. The male *crista acustica* length (male: 1.9 mm, female: 1.6 mm; $p < 0.005$) and the number of sensory units (male: 115, female: 86; $p < 0.001$) is significantly larger than in females. With a combination of anatomical, biomechanical, neurophysiological and behavioral measurements we revealed that these additional auditory receptors elongate a male-specifically extended auditory fovea in the *crista acustica* promoting high sensitivity to the carrier frequency of the female mating call. The shape of the *crista acustica* changes dramatically along its proximo-distal (longitudinal) length. However, in the medial part, where the auditory fovea is located, the height of the hearing organ remains rather constant. Especially in the *crista acustica* of the males, the organ height does not change along about 50% of the organ's length and provides the morphological basis of the auditory fovea.

Similar overrepresentation of a narrow and behaviorally relevant frequency range (auditory fovea) had been extensively studied in bats and barn owls in the context of echolocation and auditory prey localization, respectively. Here, we provide evidence for sex-specific adaptation of an auditory fovea in an insect ear that reflects the asymmetric signaling in reciprocal acoustic communication. The population coding by similarly tuned afferents allow for hyperacute temporal signal processing, which help male *A. fenestrata* to precisely localize acoustically responding females. This seems to be necessary because the risk to be detected by a predator due to the tasks of repeated and intensive stridulation, as well as phonotaxis during the night is high for the males.

We like to thank Manfred Kössl for his support and fruitful discussions. This work is supported by the DFG (NO 841/1-2).

Ancylecha fenestrata



2 cm

Stochastic resonance in an acoustically communicating insect

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Stochastic resonance (SR) is a paradox phenomenon that describes an increased detectability of a subthreshold signal when noise is added to a non-linear system. This may seem paradoxical, since noise is usually considered as something detrimental to the receiver. The term "stochastic resonance" was first used by Benzi et al. (1981) to describe the periodic recurrence of ice ages. In the early 1990, SR was also discovered in sensory neurons using external noise. We investigated the existence of SR in the auditory system of a chirping katydid species of the genus *Mecopoda* to study the influence of white noise and the trill generated by a sympatric katydid species on the detectability of periodic pure tone signals presented one dB below hearing threshold. We evaluated the spiking response of an ascending auditory interneuron (TN1) to pulsed 2, 8 and 20 kHz signals at increasing levels of noise. Results obtained from more than 10 individuals demonstrate the existence of SR when 20 kHz signals were presented simultaneously with moderate white noise. This was evident from the average TN1 response which remained above the detection limit of 50% responded signals at signal-to-noise-ratios (SNRs) between 13 dB and -2.3 dB. SR was also found for the 2 kHz signal when white noise was broadcast at the SNRs 26 and 17 dB. The detectability of these signals strongly decreased at lower SNRs. Periodic 2 kHz signals broadcast simultaneously with the trill caused strong between-individual variability. Nevertheless, the median percentage of TN1 response exceeded the 50% detection limit at a SNR of 10 dB and the maximum percentage of signals eliciting a TN1 response was found at SNRs between 35.5 dB and -18.5 dB. At the SNR of -1.2 dB, the 8 kHz signal broadcast together with the trill resulted in an average TN1 response to more than 50% of signals. In some individuals, we also quantified the variability of TN1 response at various SNRs by repeating the stimulation 10 times. Within individual variability was high when the trill was broadcast, which can be explained by the syllable pattern of this calling song consisting of soft and loud syllables. These results demonstrate the existence of SR in acoustically communicating insects and suggest that under certain SNRs the calling song of a heterospecific species may facilitate the detection of subthreshold conspecific signals containing a prominent 2 kHz frequency band.

Unravelling mechanotransduction in the locust ear: Evidence in favour of Inactive-Nanchung as the primary mechanotransduction ion channel.

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Insects are powerful model organisms to understand auditory transduction because their ears share common principles of operation across phyla. Despite significant headway in unravelling insect hearing, our understanding of the fundamental process of mechano-electrical transduction is hampered by a lack of intracellular recordings from the auditory neurons. We have pioneered patch-clamp recordings from the bipolar auditory neurons of the locust ear, and recorded their *ex vivo* responses to acoustic stimulation in order to investigate the mechanotransduction currents underpinning hearing.

In agreement with Hill's (1983) observations, we recorded two spike types in auditory neurons: (1) small spikes that are elicited by depolarisation of the receptor potential in the distal cilium and propagate along the dendrite towards the soma; and (2) larger classical spikes that are triggered by the small spikes and which propagate along the axon to the metathoracic ganglion. Both spike types are TTX-sensitive, abolished in low external Na⁺ and are voltage-gated. Using voltage steps we estimated the small-spike initiation site in the distal section of the dendrite close to the sensory cilium, where mechanotransduction is believed to occur.

To isolate and maximise the transduction current we blocked active conductances using TTX and TEA, and voltage-clamped neurons at hyperpolarised potentials. The transduction current varied in a sigmoidal manner with sound intensity and was well fitted with a Boltzmann function (Fig. 1A), as is also the case also for the mammalian hair cell transduction current. We measured the dendritic length constant which allowed us to then determine the number and conductance of the mechanosensory ion channels. We pharmacologically characterised the channels using an array of mechanotransduction ion channel blockers. Amiloride, a blocker of Piezo ion channels, failed to block the transduction current. Gadolinium and SKF-96365 which block the transduction current flowing through heterologously expressed NompC channels, failed to reduce the transduction current *ex vivo*, whereas the general TRP channel inhibitor 2-APB blocked the transduction current. Application of the insecticide pymetrozine, which opens the candidate mechanotransduction ion channel Inactive-Nanchung, elicited a baseline current with a magnitude that was very similar to that of the maximal sound-evoked transduction current (Fig. 1B,C). We conclude that the Inactive-Nanchung mechanotransduction ion channel can account for all of the sound-evoked transduction current in locust auditory sensory neurons.

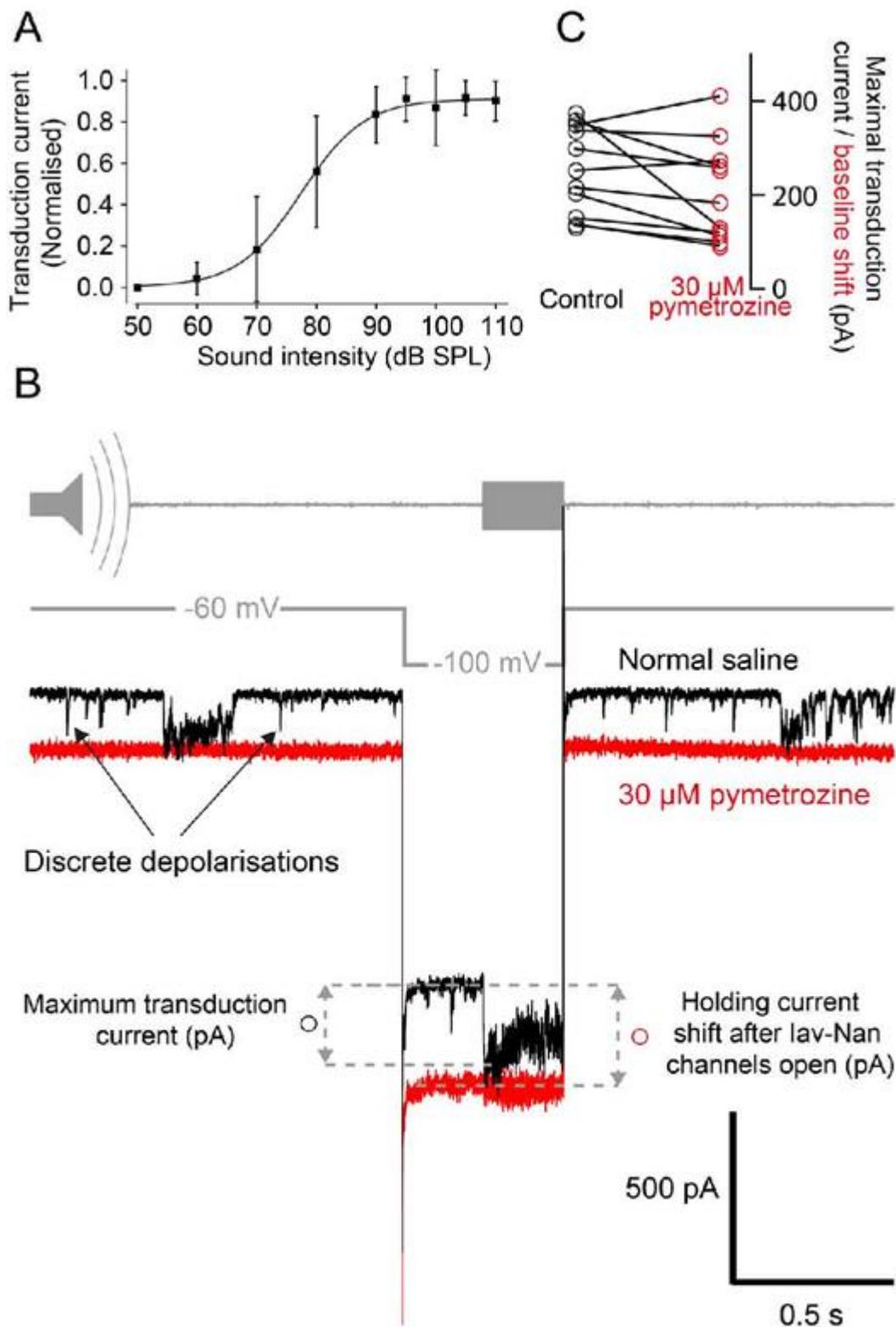


Figure 1. A, The transduction current varies sigmoidally with sound intensity. B, Auditory neurons exhibit depolarising currents in response to 30 μ M pymetrozine which is an agonist of the mechanotransduction ion channel Inactive-Nunchung (compare black trace (control) with red (pymetrozine)). C, This depolarisation current (red) is similar in magnitude to the maximal transduction current (black).

Poster Topic

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[T18-2D](#) Pressure Difference Receiving Ears Influence ITD Detection In The Auditory Brainstem Of Alligators. (*A. Missispiensis*)
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[T18-3D](#) Processing spatial depth in the auditory cortex of the fruit-eating bat *Carollia perspicillata* in the presence of natural acoustic jamming noise
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[T18-6D](#) Temporal sound processing in the auditory cortex depends on both myelin integrity and oligodendrocyte-dependent metabolic support
Sharlen Moore, Wiebke Möbius, Iva Tzvetanova, Klaus-Armin Nave, Livia de Hoz

[T18-7D](#) The impact of impaired brainstem bifurcation on hearing
Steffen Wolter, Dorit Möhrle, Dennis Zelle, Marlies Knipper, Hannes Schmidt, Lukas Rüttiger

[T18-8D](#) Time scale of adaptation to tonal sequences in mouse auditory midbrain neurons
Marina Alexandrovna Egorova, Eugenia Sergeevna Malinina, Gleb Dmitrievich Khorunzhii, Guenter Ehret

A new model for the development of tinnitus-related hyperactivity based on adaptive stochastic resonance

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Subjective tinnitus is generally assumed to be a consequence of hearing loss. In animal studies it has been demonstrated that acoustic trauma induced cochlear damage can lead to behavioral signs of tinnitus. In addition it was shown that noise trauma may lead to deafferentation of cochlear inner hair cells even in the absence of elevated hearing thresholds, and it seems conceivable that such hidden hearing loss may be sufficient to cause tinnitus.

Numerous studies have indicated that tinnitus is correlated with pathologically increased spontaneous firing rates and hyperactivity of neurons along the auditory pathway. It has been proposed that this hyperactivity is the consequence of a mechanism aiming to compensate for reduced input to the auditory system by increasing central neuronal gain, a mechanism referred to as homeostatic plasticity (HP) maintaining mean firing rates over longer timescales for stabilization of neuronal processing.

Here we propose an alternative, new interpretation of tinnitus-related development of neuronal hyperactivity in terms of information theory. In particular, we suggest that stochastic resonance (SR) plays a key role in both short- and long-term plasticity within the auditory system and that SR is the primary cause of neuronal hyperactivity and tinnitus. We argue that following hearing loss SR serves to lift signals above the increased hearing threshold, thereby partly compensating for the hearing loss. With this approach we follow the idea that any signal first has to be detected by a sensor before it could be amplified by increased neuronal gain. In our model, the increased amount of internal 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. We demonstrate the plausibility of our hypothesis using a computational model and provide exemplary findings in human patients that are consistent with that model. Finally we discuss the observed asymmetry in human tinnitus pitch distribution as a consequence of asymmetry of the distribution of auditory nerve type I fibers along the cochlea in the context of our model.

Activation of the deaf auditory system triggers remodeling of the GABAergic but not the glutamatergic network

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During ontogeny, excitatory and inhibitory networks mature and tonotopic maps develop in auditory brain regions depending on sensory activity during early sensitive periods. However, following early-onset deafness, systemic changes to synaptic and membrane properties of central auditory neurons were identified. For cortical and subcortical auditory regions, like the central inferior colliculus (CIC), changes of inhibitory and excitatory synaptic strength were verified. It was found that synaptic mechanisms supporting long-term potentiation do not mature properly in the cortex and cellular deficits occur in subcortical auditory regions. We raised the question if activation of the adult deaf brain via cochlear implant (CI) may trigger remodeling of excitatory and inhibitory networks.

To answer this question, we studied neurons in the mature midbrain of hearing-experienced and neonatally-deafened rats 1 and 7 days after monaural CI stimulation in wakefulness. For intracochlear stimulation, an electrode array was inserted into the medial turn of the left cochlea corresponding to the 8-16 kHz area under normal conditions. Plasticity of the excitatory and inhibitory pathways were analyzed histochemically by focusing on expression of glutamate decarboxylase (GAD) 65 and 67, and vesicular glutamate transporter 1 (VGLUT1), markers for inhibitory (GABAergic) and excitatory (glutamatergic) circuitries, respectively. In addition, Fos was used to identify activated and plastic neurons. Stimulation induced changes in excitatory and inhibitory circuitries were evaluated in brain sections of the CIC using immunohistochemistry.

Compared to hearing-experienced rats, sustained CI stimulation of deaf rats resulted in a high number of Fos positive CIC neurons spread over the dorso-lateral part of this nucleus, indicating a loss of tonotopic order. This response was accompanied by a massive increase of size and number of GAD65 and GAD67 positive synaptic terminals, but not of the number of neuronal somata. In contrast, the level of VGLUT1 positive synaptic terminals remained unchanged. In conclusion, the initial question must be answered affirmatively: ongoing CI stimulation modulates the GABAergic but not the glutamatergic network of CIC in a hearing-inexperienced auditory system.

Characteristic molecular and functional biomarkers for tinnitus in humans

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Tinnitus is a widespread auditory disorder affecting approximately 10-15% of the population, often with debilitating consequences. The exact neurophysiological basis of chronic tinnitus remains unknown and in various aspects highly controversial. While some studies link a pathological increase in central responsiveness subsequent to cochlear damage with tinnitus other suggest that hearing loss is not necessarily causally involved in tinnitus and a failure to generate central hyperactivity is rather associated with tinnitus. We here present the results from preclinical pilot studies that aimed to investigate characteristic functional and molecular biomarkers in homogenous matched hearing impaired human subjects with and without tinnitus. Using a combination of investigations from biomarkers in body fluids, from audiometry fine structure analysis and resting or evoked fMRI in distinguished patient and control groups we present new characteristic features for the tinnitus group. We will discuss the findings in the context of urgent needs for using objective biomarkers for future therapeutic interventions in tinnitus patients

Acknowledgments:

This study was supported by grants from the Deutsche Forschungsgemeinschaft DFG-Kni-316-11-1;12-1, DFG RU713/3-1

Contralateral/ipsilateral postsynaptic potentials and binaural integration in midbrain single neurons

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The central auditory system is capable of integrating information from both ears through a process of neural integration. This fundamental capacity improves sound discrimination and hearing quality. The central nucleus of the inferior colliculus (ICc) in the midbrain is a convergent center, receiving excitatory and inhibitory inputs from all subcollicular nuclei on both sides of the ascending pathways. Although binaural integration for sound localization (eg., interaural time and level differences) has been well investigated in the past decades, our understanding of the synaptic mechanisms of binaural integration in the ICc is unclear. This study aimed to identify the various types of binaural inputs that impact ICc neurons and then to characterize the integrations of these binaural inputs.

Anesthetized C57 mice were employed in this study. The postsynaptic potentials (PSPs) of ICc neurons in response to contralateral, ipsilateral and bilateral acoustic stimulation were recorded by using in-vivo whole-cell patch-clamp. A total of 122 ICc neurons were sampled. We observed that monaural (contralateral or ipsilateral) tone could induce excitatory (E), inhibitory (I) PSPs (EPSP/IPSP) or no change in membrane potential (O). According to the PSPs of ICc neurons to contralateral and ipsilateral stimulation, we sorted ICc neurons into 7 categories. Out of 122 neurons, 81 (66.4%) were EPSP-EPSP (EE), 5 (4.1%) were E-IPSP (EI), 20 (16.4%) were E-no response (EO), 7 (5.7%) were II, 2 (1.6%) were IE, 2 (1.6%) were IO and 5 (4.1%) were complex-mode (CM) neurons. The CM neurons were able to show EPSPs to some frequencies/amplitudes of monaural tone stimulation while IPSPs to others. These data suggest that the synaptic profiles of binaural inputs to a given ICc neuron are complex. We then examined the PSPs of ICc neurons to binaural tone stimulation. The tones delivered to contralateral and ipsilateral ears had identical onset, identical frequency and equivalent amplitude in order to mimic the sound source directly in front of the mouse (zero-degree azimuth), a common direction of attended sound source. The binaural PSPs could be larger than, smaller than or similar to the contralateral PSPs as summarized in Table 1. These data showed that the PSPs of ICc neurons to binaural stimulation are dominated by the contralateral inputs when subjected to the modulation of ipsilateral inputs. Most types of neurons with contralateral EPSPs exhibited more than one binaural outcome (facilitative, ineffective or suppressive). The types associated with contralateral IPSPs mostly exhibited ineffective binaural outcomes. In CM types, different combinations of contralateral and ipsilateral stimulus had different binaural outcomes. Our data strongly suggest that single ICc neurons are involved in different neural circuits required for binaural integration.

The ICc is a critical processing center for binaural integration involving diverse tasks such as sound localization, redundancy and squelch. Our findings testify to the complexity of the neural processing in the midbrain required for binaural hearing.

Contra- Ipsilateral		Binaural	Facilitative	Suppressive	Ineffective
EE	81		20 (24.7%)	25 (30.9%)	36 (44.4%)
EI	5			5 (100%)	
EO	20			11 (55.0%)	9 (45.0%)
II	7			2 (28.6%)	5 (71.4%)
IE	2				2 (100%)
IO	2				2 (100%)
CM	5			2/2 EI pair 3/4 EE pair	3/3 II pair 1/4 EE pair

Distinct frequency-specific myelination patterns in gerbil, but not in mouse, adjust conduction velocity and synaptic transmission delay of action potentials in auditory brainstem neurons as an adaptation for ITD processing

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The auditory system of mammals relies on two different cues for sound localization in the horizontal plane: Interaural level differences (ILDs) and interaural time differences (ITDs). ILDs are used for localizing high frequency sounds by all mammals including mice and gerbils. In contrast, ITDs are used for the localization of low frequency sounds, which is only done by animals with low frequency hearing such as gerbils, but not mice. Despite their distinct functionality, both processing circuits contain the medial nucleus of the trapezoid body (MNTB), a brainstem nucleus, that receives excitatory input from the globular bushy cells (GBC) of the contralateral antero-ventral cochlear nucleus (AVCN). Recent data from our lab has revealed that the axonal myelination pattern of GBC axons and consequently the action potential conduction velocity varies in a frequency-dependent manner in mongolian gerbils (Ford et al., 2015). Particularly, axons tuned to low frequencies exhibited non-canonical myelination patterns and faster conduction velocities. Nevertheless, the functional role of this anatomical specialization remain obscure.

Here, we investigated to what extent the morphological specializations of GBC axons are associated with the hearing range of the animal and consequently the use of ITD and ILD processing circuits. To this end, we compared the axon morphology of laterally and medially terminating GBC axons between gerbil and mice. Additionally, we investigated the effect of GBC axon morphology and frequency tuning on synaptic transmission at the Calyx of Held synapse *in vivo*.

We show that the non-canonical myelination pattern that was found in low-frequency-tuned GBC axons of gerbil (Ford et al., 2015) is not present in mice, suggesting that this anatomical specialization is associated with the processing of ITDs. In accordance with this notion, the synaptic delay of GBC axons tuned to low frequencies were less modulated by activity compared to high frequency fibers, a prerequisite for the high temporal accuracy of ITD processing.

Together, these findings suggest that frequency-specific morphological and physiological specializations of GBC axons represent a functional adaptation for ITD processing.

Echo-acoustic flow determines object representation in complex spatial layouts

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Echolocating bats use the echoes of their sonar emissions to determine position and distance of objects or prey. Target distance is represented in a chronotopically organized map of echo delay in the auditory cortex (AC) of bats. During flight in complex environments streams of echoes are reflected from multiple objects along the flight path. To separate sounds from specific objects in such streams is a challenging task for the auditory system of bats as well as many other animals.

We combined naturalistic call/echo sequences simulating a bat's flight in virtual acoustic space with extracellular recordings in the AC of anaesthetized bats (*Phyllostomus discolor*). We found neurons that selectively focused to echoes from only one object in a complex stream of echoes originating from two different objects along a virtual flight path. The objects were processed sequentially in the order of object approach. We further varied the temporal pattern of sonar emission during the simulated flight sequences to test its influence on cortical object representation. The detailed representation of an object in the cortical target range map was not fixed but could be dynamically adapted depending on temporal patterning of call/echo pairs during target approach within the flight sequence.

Our results show that neurons in the AC of bats can separate different streams of echoes and focus their response to specific objects in a complex naturalistic flight sequence, depending on the dynamic variation of sonar information (i.e. echo-acoustic flow) during flight. Therefore, stream segregation in mammals can be based on the integration of multiple dynamically changing acoustic parameters.

This work was supported by a DFG-Grant (FI 1546/4-1) to U. Firzlaff.

Effects of Early Hearing Experience on Functional Connectivity in Primary and Higher-Order Cortical Field

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Neuronal activations and interactions are often manifested as oscillations in extracellular recordings. Here we evaluated time-frequency representations (TFR) of local field potentials (LFP) recorded simultaneously from the primary auditory cortex (A1) and posterior auditory field (PAF). Responses elicited with acoustic (through loudspeakers) in hearing animals and electric stimuli (through cochlear implants) in hearing and deaf animals were compared to see the effect of stimulation mode and experience. LFP signals were recorded in isoflurane-anaesthetized cats using two multi-electrode arrays. The animals were grouped into acoustically stimulated hearing, electrically stimulated hearing, and electrically stimulated congenitally deaf. Total, evoked, induced TFR power, and inter-trial phase locking factor (PLF) were calculated from bipolar derivation signal between neighbouring channels using Morlet wavelet analysis. Using debiased weighted phase-lag index (WPLI_d), the coupling within different channels within the same recording field (A1 to A1, PAF to PAF) as well as across recording fields (A1 to PAF) are quantized.

The TFR grand mean for each group and site showed that evoked (phase locked) responses appeared mainly at early latency (<100ms) while induced (non-phase locked) responses appeared at both early and long latencies (>100ms). Hearing experience affected both evoked and induced responses in PAF to a greater extent than in A1. In the hearing animals, PAF showed stronger and longer responses by acoustical stimulation. In contrast, there were very weak and brief induced responses at longer latency range in PAF of congenitally deaf. This also resulting weaker couplings across two recorded fields in the deaf compared to the hearing groups. This finding indicates that hearing experience is essential for corticocortical A1-PAF interactions.

Acknowledgments: Supported by DFG (Hearing4All Excellence Cluster) and DAAD – Indonesian German Scholarship Program (IGSP).

Effects of early sensory deprivation on the development of multisensory thalamocortical and intracortical connections

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The nervous system integrates information from multiple senses. This multisensory integration already occurs in primary sensory cortices like A1 (auditory), S1 (somatosensory), and V1 (visual) via direct cross-modal thalamocortical and corticocortical connections. Sensory loss from birth in humans results in functional recruitment of the deprived cortical territory by the spared senses during subsequent development but the underlying circuit changes are poorly understood. Using anatomical tracer injections into three primary sensory cortices (A1, S1, V1) within the first postnatal month of life in a rodent model (Mongolian gerbil) we show that multisensory connections are initially established on the thalamocortical level and only later emerge intracortically and that most thalamocortical connections are eliminated during the formation and refinement of intracortical connections. Early auditory, somatosensory, or visual deprivation increases multisensory connections encompassing lemniscal, non-lemniscal, and multisensory pathways via axonal reorganization processes but not apoptosis or neurogenesis of projection neurons. Thereby, the axonal remodeling is mediated by non-lemniscal thalamic nuclei and the primary areas themselves. Functional single-photon emission computed tomography imaging (SPECT) of regional cerebral blood flow reveals an altered stimulus-induced activity and a higher functional connectivity specifically between primary areas in deprived animals. Together, we show that intracortical multisensory connections are formed as a consequence of sensory driven multisensory thalamocortical activity and that spared senses functionally recruit deprived cortical areas by an altered development of sensory thalamocortical and corticocortical connections. The functional-anatomical changes after early sensory deprivation have translational implications for the therapy of developmental hearing loss, blindness, and sensory paralysis and might also underlie developmental synesthesia.

Electrical Stimulation of the Mouse Auditory Midbrain

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Electrical stimulation of the inferior colliculus (IC) has been shown to elicit cortical responses in anesthetized guinea pigs and rats and has recently been used to modulate perception of tones in non-human primates. So far, it has proven to be difficult to elicit a frequency selective tone perception by stimulation of one frequency lamina in the IC. In order to investigate the characteristics of the elicited cortical activity, we electrically stimulated the IC of 7 anaesthetized mice using monopolar (MP), bipolar (BP), and tripolar (TP) configurations while recording evoked potentials in the primary auditory cortex (AC).

We used a 1x16 PtIrO Neuronexus electrode array inserted parallel to the tonotopy axis in the IC to deliver electric single pulses of 400 μ s duration (200 μ s/phase, biphasic, charge-balanced) at various current intensities in MP, BP, or TP configuration in seven mice, BP and TP spanning multiple isofrequency laminae. Simultaneously, we recorded LFPs and spikes from the AC using a 4x8 PtIr Neuronexus array. We first recorded tone- and click evoked potentials to characterize the characteristic frequency (CF) of the IC and AC units. Subsequently we recorded electrically evoked responses in the AC.

Comparing all electrically evoked responses, the mean lowest response thresholds did not significantly differ between MP, BP, and TP stimulation. The number of spikes evoked per stimulus peaked near 80 μ A for all three configurations. Rates were highest for MP, followed by BP and TP stimulation. We found a trend in all configurations for first spike latencies to decrease with increasing stimulation currents, while response durations increased.

In order to estimate the spread of activation, we compared AC units that were aligned in CF to the unit electrically stimulated in the IC with those that were had different CFs. The evoked spike rate increased with decreasing CF difference. Highest rates for MP and BP stimulation were achieved at perfect alignment. However, the spread of activation was broader in MP configuration. We found an asymmetry bias towards a CF difference of up to +1 octave (AC-IC). Evoked cortical activation was significantly stronger in units tuned to frequencies between 0 and +1 octave higher than the stimulated IC unit, than in units tuned to frequencies between 0 and -1 octave lower. This bias was strongest for TP stimulation and weakest for BP stimulation, while MP evoked activity spread in the positive direction across one octave almost equally. At negative CF differences, all three behaved similarly, showing weak or no activation at all. Not a single response was recorded for a CF difference greater than three octaves.

Our results suggest that electrical stimulation of the IC leads to activation that is frequency- and intensity-dependent, and is strongest at moderately strong intensities. Also, the stimulation is strongest at the place where the stimulation electrode is located. While MP stimulation leads to highest rates in general, the spread of activation into the AC is broadest. BP stimulation shows the narrowest functional spread of activation and the highest rates at perfect CF alignment. Thus, it can be hypothesized that BP stimulation is better suited for spectrally more simple perceptions than MP stimulation. Given the absence of a threshold difference, this makes BP stimulation better suitable for clinical application.

Supported by Deutsche Forschungsgemeinschaft (Cluster of Excellence Hearing4All).

Electrophysiological and behavioral characterization of mice missing the auditory midbrain

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A way to characterize brain structures and their function is to generate mouse models with specific genetic mutations that lead to well defined phenotypes. Ap-2 transcription factors comprise a family of 5 closely related sequence-specific DNA binding proteins that play pivotal and non-redundant roles in embryonic organogenesis. The transcription factor Ap-2 is exclusively expressed in the central nervous system. Interestingly, gross anatomical inspection of HE-stained brain section revealed a complete lack of the auditory midbrain, the inferior colliculus (IC). Despite this lack Ap-2 -deficient mice showed no obvious behavioral deficits.

We examined auditory function of the Ap-2 -deficient mice by behavioral and electrophysiological methods. In the behavioral experiment, an auditory discrimination paradigm was used (pure tone discrimination, 7kHz vs. 12 kHz, 75 dB SPL). Multiunit recordings were performed in the caudal part of the temporal cortex with pure tone stimulation. All experiments were performed in Ap-2 -deficient mice and wildtypes.

Ap-2 -deficient mice showed no discrimination learning performance at all compared to wildtype mice. Despite the missing IC, the presence of neuronal responses to sounds in the auditory cortex (AC) indicated that auditory information still reached the neocortex. Neuronal responses of Ap-2 -deficient mice showed changes in response characteristics, such as shorter latencies and lower evoked rates. The temporal cortex area, where neuronal responses were recorded, was significantly smaller in size compared to wildtype mice.

These changes may reflect that auditory function in Ap-2 -deficient mice can be mediated via a possible rewiring within the auditory pathway with partial restoration of inferior colliculus function. Further studies are required to clarify the ascending connections to the AC in Ap-2 knockout mice.

In summary, our data define Ap-2 -deficient mice as a new and valuable animal model to study auditory functions in case of auditory midbrain loss.

Functional specialization of mouse auditory midbrain neurons with different response patterns in processing of communication calls

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Neurons in the main auditory midbrain nucleus, the central nucleus of the inferior colliculus (ICC) respond with various temporal patterns to tone bursts (review in Ehret, 1997). Generally, ICC neurons may be divided up into two groups based on their response patterns. The first is characterized by tonic activity. Neurons have tonic, phasic-tonic, pauser and long-latency responses which may last as long as the sound continues. Neurons of the second group respond with phasic onset discharges. We studied the response patterns of ICC neurons with regard to possible functional specialization to process wriggling calls, one type of mouse communication calls with three harmonics in the low-frequency range of mouse hearing (Ehret & Riecke, 2002).

Extracellular recordings under ketamine/xylazine anesthesia were obtained from 122 neurons in response to 18 models of wriggling calls (single tones, two-tone and three-tone complexes of 5+15 kHz plus a middle frequency variable between 5.4 and 13.6 kHz) and a natural call of 5+10+15 kHz formants. Data showed non-linear spectral summation of responses to sound frequency components and spectral facilitation to various sounds with two or three frequency components. Spectral facilitation means that given combinations of frequency components in certain sounds elicit higher response rates than the rate predicted by summation of response rates to the same single frequencies. Neurons with such facilitated responses have been called combination-sensitive (Mittmann & Wenstrup, 1995; Portfors & Felix, 2005). Spectral facilitation was present in on-responses of 25% and off-responses of 38% of our ICC neurons. Facilitation (on- and off-responses) in more than 80% of the neurons was associated with primary-like (class I) and inhibition-dominated (class II) shapes of frequency response areas (Egorova et al., 2001) and tonic, phasic-tonic, pauser and long-latency responses. Only about 15% of combination-sensitive neurons showed V-shaped frequency tuning (class III) and phasic responses. The effectiveness of facilitation increased from sounds with two frequencies to three-component wriggling call models to the natural wriggling call and its harmonic model.

Thus, processing of multi-frequency sounds, especially communication calls with several format frequencies, is enhanced by spectral facilitation of responses of neurons with various tonic response patterns in the ICC. Phasic on-response neurons have more constant latencies in their frequency receptive fields to tone onsets than neurons with tonic responses (Khorunzhii & Egorova, 2014; Ehret et al., 2015) making them ideal candidates for coding the onset of sounds with high temporal precision.

Supported by the Russian Foundation of Basic Research (projects 09-04-00656 and 15-04-05234).

Egorova, M., Ehret, G., Vartanian, I. & Esser, K.-H. *Exp Brain Res*, 140, 145-161, 2001.

Ehret, G. In: *The Central Auditory System* (Ehret G, Romand R, eds), Oxford University Press, New York, pp. 259-316, 1997.

Ehret, G., Egorova, M.A. & Khorunzhii, G.D. *Proc. 35th Göttingen Neurobiology Conference*, T18-6B, 2015.

Ehret, G. & Riecke, *PNAS*, 99(1), 479-482, 2002.

Khorunzhii, G.D. & Egorova, M.A. *J. Evol. Biochem. Physiol.*, 50(4), 357-361, 2014.

Mittmann, D.H. & Wenstrup, J.J. *Hear. Res.*, 90, 185-191, 1995.

Portfors, C.V. & Felix II, R.A. *Neuroscience*, 136, 1159-1170, 2005.

Hearing dysfunction in otoferlin Ile515Thr mutant mice.

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Mutations in the multi-C2-domain protein otoferlin lead to hearing impairment or deafness. Otoferlin is an inner hair cell specific protein, which is essential for hearing. In its absence neurotransmitter exocytosis is abolished. In human patients the Ile515Thr point mutation in the C2C-Domain of the protein causes only a mild increase in hearing thresholds, but a severe speech perception deficit, an impaired adaptation to continuous sounds and auditory fatigue. Mice which are homozygous for the same mutation reflect this auditory synaptopathy by exhibiting a phenotype of moderate hearing impairment involving enhanced adaptation to continuous or repetitive sound stimulation. In the *Otof*^{I515T/I515T} inner hair cells the amount of otoferlin at the membrane of the active zones is reduced and synaptic vesicles are enlarged. This indicates that otoferlin is critical for the reformation of properly sized and fusion-competent synaptic vesicles.

We now analyze central sound encoding in *Otof*^{I515T/I515T} mice *in vivo* to investigate how the peripheral deficit affects sound encoding in higher brain areas of the auditory pathway, particularly the inferior colliculus. Specifically, we analyze responses to pure tones and amplitude modulated tones in consideration of sound thresholds, frequency tuning, rate-intensity-functions, phase locking and responses to paired stimuli.

Otof^{I515T/I515T} mice have elevated ABR thresholds and a reduced amplitude of ABR wave I, while subsequent peaks are better preserved. Single units from the auditory nerve fibers show normal spontaneous spiking, frequency tuning and thresholds, but reduced spike rates and increased jitter with a striking dependence on the repetition rate and duration of the stimulus. In response to paired stimuli, the response to the second pulse is reduced and longer silent intervals are required for recovery.

In the inferior colliculus similar changes could be observed. Its neurons show normal spontaneous spiking, frequency tuning and thresholds, but a decreased spike rate when challenged with long stimuli. The phase locking to amplitude modulated tones is impaired and a longer silent interval between paired tones was required to detect the second tone.

Consistently, experiments using prepulse inhibition of the startle response and operant conditioning show an impairment of the perception of silent gaps in noise.

Single unit recordings from *Otof*^{I515T/I515T} mice indicate an unusual sound encoding deficit with a use-dependent reduction of spike rates. We believe that this reflects an impaired vesicle reformation at the inner hair cell ribbon synapse due to reduced levels of functional otoferlin at the membrane. The peripheral deficit in the encoding of amplitude modulated and paired tones cannot be fully compensated at least up to the level of the inferior colliculus, although the defect seems not to be as severe as in the auditory nerve. The resulting gap detection deficit likely contributes to the communication problems of human patients with otoferlin mutations.

Impact of optogenetically released dopamine on cortical processing in the *Mongolian Gerbil*

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The close relationship of dopamine (DA) and learning has been investigated in different behavioral paradigms in both humans and animals. For instance, previous studies revealed that increase of DA levels in auditory cortex (AI) precede performance improvements in cortical learning tasks. Yet, the precise mechanism of DA influence on cortical processing during learning is still elusive. Recently, we have shown that the systemic application of the dopaminergic agonist SKF 38393 influences cortical processing of gerbil AI in a layer-dependent manner and improves detection of behaviorally relevant stimuli. In order to more precisely pinpoint the mechanisms behind these effects, we have optogenetically stimulated the VTA of gerbils and recorded current source density profiles across cortical layers. We found that, compared to initial baseline measurements, overall tone-evoked spiking rates after VTA stimulation are increased. This was accompanied by an increase of infra- and supragranular sink activity particularly for non-best frequencies (non-BFs). We found that dopamine promoted thalamo-cortical input activity in layers Va/b (bottom-up processing) and prolonged the duration of tone-evoked cortical activity. Hence, stimulation of VTA dopaminergic neurons eventually promoted cortico-cortical interaction with neighboring non-BF columns in layers I/II, which, according to our hypothesis, might yield the integration of behavioral relevant input in AI (top-down processing).

Impaired topographic map refinement and synaptic strengthening of an inhibitory auditory microcircuit in deaf mice

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Neural circuits of sensory systems are initially broadly established in early development and become subsequently refined in an activity-dependent manner. Often, the refinement occurs prior to sensory input under the influence of spontaneously generated activity (Kirkby et al., 2013, Neuron). The importance of spontaneous activity has been well documented in the visual system (Burbridge et al., 2014, Neuron). For the auditory system, however, the impact of spontaneous activity on circuit refinement is much less described, especially at inhibitory projections. Spontaneous prehearing activity is generated in the cochlea (Tritsch et al., 2007, Nature; Wang et al., 2015, Cell) and the activity pattern is conserved in downstream targets (Tritsch et al., 2010, Nature Neuroscience). To analyze the role of spontaneous activity on circuit refinement of the inhibitory, glycinergic projection from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO), we studied mice at postnatal day 10-12 of the *Otoferlin*^{deaf5/deaf5} line, which show a strongly reduced vesicle exocytosis from inner hair cells (Heidrych et al., 2009, Human Molecular Genetics). Consistently, in-vivo recordings of the MNTB confirmed strongly reduced spontaneous activity levels. To determine the MNTB input area, we performed focal flash photolysis of caged glutamate in the MNTB with simultaneous recording from LSO principal neurons in acute brainstem slices of *Otoferlin*^{deaf5/deaf5} mice. This revealed a 2-fold broader extent in both the mediolateral and dorsoventral direction, which strongly suggests impaired synapse elimination. This impaired elimination resulted in a distorted and imprecise topographic map. Besides synapse elimination, synaptic strengthening is another hallmark of circuit refinement. Therefore, we recorded from LSO neurons in acute brain slices, while electrically stimulating MNTB fibers with gradually increasing stimulation intensities, which resulted in a gradual recruitment of converging axons. The number of converging axons was increased by 50% in *Otoferlin*^{deaf5/deaf5} mice, confirming the impaired elimination found in the glutamate uncaging experiments. Furthermore, the single fiber strength was reduced by 40% in *Otoferlin*^{deaf5/deaf5} mice, suggesting an impaired synaptic strengthening. To reveal the functional basis of the impaired strengthening, we determined the single fiber quantal content. This analysis revealed a 40% reduced quantal content, suggesting that the impaired synaptic strengthening is due to a reduced quantal content. In summary, we conclude that spontaneous prehearing activity is required for the topographic map refinement of the MNTB-LSO microcircuit.

Intracortical Microstimulation Modulates Oscillatory Responses to Concurrent Acoustical Stimulation in the Auditory Cortex

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The primary task of cortical columns is the integration of different information streams from many cortical and subcortical sources. Measuring low-frequency intracortical oscillations provide a means of assessing these processes. The present study investigated whether the concurrent application of focused, electrical intracortical microstimulation (ICMS) together with physiological, acoustical stimuli is able to differentially modulate low-frequency oscillations in the auditory cortex.

Building on previous work where we combined ICMS with extracellular recordings on the same shank of a linear multi-electrode array *in vivo* to show that a focused activation of the cortical network is possible with single ICMS pulses of sufficiently low current, we examined the modulating effects of ICMS pulses on cortical responses to acoustic stimuli.

Responses were recorded from the auditory cortex of Ketamine/Xylazine anaesthetized guinea pigs using 15 electrodes of a linear Neuronexus electrode array. ICMS was performed with single current pulses (monopolar, biphasic, charge-balanced, cathodic-leading, 0.1-45 μ A, 200 μ s/phase) on the remaining 16th electrode of the shank. This electrical ICMS was combined with acoustic click stimulation (50 μ s condensation clicks, 40 dB above ABR threshold) with varying time delays between ICMS and acoustic click onset. For time-frequency decomposition of the low-frequency oscillatory activity a Morlet wavelet analysis was performed.

Local field event-related potentials showed nonlinear interactions when combining auditory stimuli with focused electric activation in different depths and delays. A current-source density analysis revealed complex interferences in the underlying cortical activations, depending among others on the delay between auditory and electric stimulation. Although important in the electric only condition, the depth of ICMS had less of an influence on the local field potential response during combined stimulation. The strongest effect of combining ICMS and acoustic clicks was found to be a pronounced gamma band activation several 100 ms post stimulus which was completely absent in the auditory only condition.

Together this shows that applying short electrical ICMS currents is not only able to directly activate cortical networks, but also to modulate cortical oscillations, and therefore intracortical processing, on longer timescales than would be expected from such focused sub-millisecond pulses.

Supported by Deutsche Forschungsgemeinschaft (Cluster of Excellence Hearing4all)

Judgments of perceptual distance in the behaving mouse: physical properties versus valence of acoustic stimuli.

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Behavioral responses to current sensory stimuli depend on associations learned during past experiences with the same or similar sensory stimuli. The aim of this study was to understand how animals extract and apply these associations depending on the acoustic stimulus physical properties and valence. For this purpose we investigated mouse frequency discrimination of pure tones in an enriched behavioral paradigm and tested how the physical properties of the used stimuli affected discrimination learning and generalization.

C57BL/6J adult female mice lived in an Audiobox (TSE) that allows continuous monitoring of individual behavior via an implanted subcutaneous transponder. The Audiobox consists of a home-cage connected by a long corridor to a specialized sound-attenuated corner. Food was available ad libitum in the home-cage and water was available in the corner. Visits to the corner were accompanied by the presentation of auditory stimuli. Mice were trained to discriminate between one tone frequency (safe tone) that was associated with access to the water and another tone (conditioned tone) associated with an air-puff. Following assessment of the speed and degree of discrimination learning, we estimated generalization gradients through the subsequent exposure to novel tones in the corner. Novel tones had frequencies between, below or above the safe and conditioned tones. Although novel tones were never paired with an air-puff we found robust differences in the responses to these stimuli. As has been shown before, mice were able to separate their responses to novel tones into two categories with a sharp boundary in between. Sounds that were closer to the safe tone in frequency were associated with reward and those closer to the conditioned tone were treated as dangerous. The shape of the psychometric curve varied with the distance between the safe and conditioned tone such that when these were close to each other (F of 40% and 20%) the curves became steeper with less generalization. This effect was particularly evident at the safe end. It also took more time for mice to reach the same levels of discrimination than mice trained with F above 40%. The shape of the psychometric curve was the same independently of whether the safe tone was of a frequency above or below the conditioned tone but discrimination levels, measured with d' values, were lower when the conditioned tone was below the safe.

We tested the stability of the generalization curves through subsequent conditioning of a sound that was previously safe. When a tone in-between the safe and conditioned tones became conditioned, the new psychometric curve was not simply shifted sideways to respect the new boundaries but also slightly downwards with respect to the previous curve (less dangerous), indicating that the new conditioned sound maintained its valence below the previous conditioned tone. Mice did, however, learn to discriminate the new conditioned tone faster than they learnt the first.

Overall, the data revealed that the behavioral judgement of perceptual distance depends strongly on the relative physical distance of the trained stimuli, and less on experience. Some aspects of generalization do, nonetheless, depend on the specifics of the training protocol. One example is the asymmetry in the generalization around the safe and conditioned tone which suggests that the generalization of positive and negative stimuli may be two independent processes.

Long lasting cellular adaptation in the medial superior olive induced by continuous noise exposure

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To preserve sound localization acuity following changes in the acoustic environment, the processing of auditory cues needs neuronal adaptation. This adaptation is important for the developing and the matured system. Recent studies have shown mechanism of how binaural neurons in the adult brainstem adapt to short-term stimulation history within milliseconds. Further in-vivo studies in the binaural brainstem showed long-term reversible effects on the processing of binaural cues to moderate long lasting noise exposure. However, the cellular mechanisms in the medial superior olive (MSO) underlying this long-term adult adaptation remain unclear.

Here we used intracellular in-vitro electrophysiology from MSO neurons in brainstem slices of adult (>P60) Mongolian gerbils. The long-term effects were induced by exposing adult gerbils for 2 weeks to moderate acoustic noise with no stable binaural cues.

We found intrinsic as well as synaptic changes after noise exposure. In terms of intrinsic adaptation we observed a decrease of the membrane input resistance combined with a decrease of the time constant. Incorporating these two cellular changes into a computational model of binaural sensitivity we can explain parts of the previous neuronal adaptation of the binaural responses after long-lasting noise exposure observed in-vivo. Furthermore on the synaptic level we found an increased frequency of miniature excitatory and inhibitory currents (mEPSC and mIPSC) after noise exposure. A more detailed investigation of the excitatory and inhibitory inputs showed clear differences: While for the excitation the kinetics were changed, the amplitude of stimulated inhibitory current was significantly decreased after noise exposure.

Taken together these data focusing on cellular long-term adaptation of single MSO neurons provide insight into the role of brainstem neurons in binaural adaptation to the acoustic environment of adult mammals.

Long-term dynamics of sensory representations in mouse auditory cortex

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We recently found that the activity of local neuronal ensembles of the auditory cortex encode broad sound categories into non-linear population response modes (Bathellier et al., *Neuron* 2012; 76, 435–449). The discrete responses of these ensembles generate a basis set of perceptual categories that can be used to drive behavioral decisions. Additionally, chronic in vivo imaging approaches have demonstrated that the structural correlate of the postsynaptic component of excitatory synapses, dendritic spines, are highly dynamic throughout the life time of an animal (Loewenstein et al., *J. Neurosci.* 2011; 31(26):9481–9488). We are intrigued by the interplay of these two very distinct, but inherently interconnected types of dynamics: how are sensory representations formed and maintained in light of dynamic connectivity? Classical neurophysiological approaches, like extracellular recordings of action potentials, have largely not been able to provide chronic observations of activity dynamics due to technical limitations. Towards this goal, we established a procedure to monitor the activity of local neuronal ensembles of the auditory cortex in response to sounds over prolonged periods of time in the awake animal. Using chronic two-photon imaging, we have recorded sound-evoked activity patterns of large populations of neurons over the course of more than a week. We have gathered an extensive dataset including chronic single cell activity of tens of thousands of cortical neurons. The application of intrinsic signal optical imaging allows us to combine data from multiple animals and demonstrates broad sampling of major auditory cortical fields. We aim to reveal principles of functional reorganization of cortical circuits to answer the fundamental question how the sensory neocortex provides stable sensory percepts while enabling constant flexible integration of novel experiences.

Stability of sensory representations in the presence of synaptic turnover

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Synapses of cortical neurons display ongoing changes, even in the absence of an explicit learning paradigm (e.g. Loewenstein Y, Kuras A, Rumpel S. "Multiplicative dynamics underlie the emergence of the log-normal distribution of spine sizes in the neocortex in vivo", *J Neurosci*, 2011). How does this volatility in structural connections affect the functional properties of cortical circuits? Here, we address this question in a model of population activity in mouse auditory cortex, to interpret ongoing parallel experiments employing chronic two-photon imaging in the auditory cortex of awake mice. Previous experiments found that neural population responses to brief complex sounds often cluster into a discrete set of activity patterns, which were predictive of discrimination performance and spontaneous categorization of sounds in behaving mice (Bathellier B, Ushakova L, Rumpel S. "Discrete neocortical dynamics predict behavioral categorization of sounds", *Neuron*, 2012). Such discrete representations could provide a high degree of robustness against fluctuations in synaptic strengths. Here, we use a circuit model to study the emergence of activity cluster states and their structural stability in the presence of synaptic turnover. We show that the model displays basic response properties of population activity in mouse auditory cortex, including a skewed distribution of neural firing rates and a clustering of broad sound categories into non-linear population responses. This holds over a parameter regime where recurrent connections are sufficiently heterogeneous and the network is dominated by inhibition. We use this model to study systematically the impact of synaptic turnover on collective response properties. We find that a similar response stability as observed in experiment requires changes of inhibitory synapses to be orders of magnitude slower than changes of excitatory synapses. Furthermore we find that gradual changes in the circuitry result in plateaus of stable collective response modes with abrupt transitions between them. Our model suggests that synaptic volatility may affect sensory representations in a highly nonlinear fashion.

Membrane resonance phenomena in neurons of the superior olive complex

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Neurons in the medial and lateral superior olive (MSO and LSO) receive temporally well tuned synaptic excitatory and inhibitory inputs. These inputs can generate sub-threshold membrane potential oscillations (MPOs). Neuronal sub-threshold MPOs can be amplified by resonance phenomena. The resonance properties of neurons are governed by active ionic conductances and the passive membrane properties that give rise to bandpass filter-like impedance profiles. Membrane resonance enhanced MPOs can reduce action potential current thresholds and therefore crucially shape processing of auditory information.

To investigate the existence and mechanisms of sub-threshold resonance profiles in LSO and MSO neurons we used frequency modulated sinusoidal current injections (ZAP-stimulus) in in-vitro whole-cell recordings of neurons in acute slice preparation. As a model for high frequency hearing we recorded from postnatal day (P) ~22 old mouse LSO neurons and as a model for low frequency hearing we used P16 and P60 gerbil MSO neurons.

Nearly every recorded neuron in mouse LSO and gerbil MSO showed resonance properties in response to ZAP stimulation. In both cell types, the resonance showed a frequency dependent separation between the hyperpolarizing and the depolarizing part of the voltage response. The hyperpolarizing part of the voltage response occurred at low ZAP frequencies and was sensitive to blockage of HCN channels. Depolarizing resonance was present at high stimulation frequencies and lowered action potential current thresholds. The resonance frequency (R_f) of the depolarizing part of the voltage response increased from mouse LSO to MSO cells in gerbils of P16 to P60 as did the resting membrane resistance. In addition, R_f of the depolarizing part was increased by both increasing stimulation amplitude and adding a depolarizing constant holding current. However, R_f was best correlated with the dynamically changing initial membrane resistance at ZAP-stimulation onset. Decreases in this initial input resistance increased R_f . Conductance-clamp recordings verified the importance of membrane resistance in determining R_f . Adding or subtracting a constant leak conductance increased or decreased the depolarizing R_f respectively. Taken together, mouse LSO and gerbil MSO neurons appear biophysically similar mostly differing in input resistance. In response to a ZAP-stimulation input resistance appears to be the major determinant for the frequency at which LSO and MSO neurons resonate with a depolarizing voltage response.

Neurodegeneration and Cell Death Mechanisms in the Mouse Central Auditory System after Single or Repeated Noise Trauma

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Beside peripheral pathologies of neuronal structures, noise trauma leads to changes in central neuroanatomy and neurophysiology. Several studies have shown an impact on the auditory pathway either by acoustic deprivation or acoustic overstimulation. Our group has found a significant loss of cells in central structures of the ascending auditory pathway after a noise trauma (Gröschel et al., JNeurotrauma 2010). Moreover, we were able to detect cell death mechanisms after traumatizing single noise exposure, particularly in the auditory brainstem (Coordes et al., JNeurotrauma 2012), which started immediately postexposure and lasted for several days. Therefore, recent data indicate that apoptosis seem to play a key role in the underlying pathologies. Here, we describe the time course of neurodegeneration and cell death mechanisms in the central auditory system after a single or repeated noise trauma using histological and immunohistochemical techniques. Normal hearing mice (NMRI strain) were exposed to a broadband noise (5-20 kHz) at a sound pressure level of 115 dB for 3 hours. Cell densities were measured in brain slices after hemalum eosin (HE) staining. Further, cell death mechanisms were visualized via TUNEL-staining (terminal deoxynucleotidyl transferase dUTP nick-end labelling). Data have been analysed at different time points (1, 7 or 14 days) after a first or a second noise exposure. The focus was put on the ventral and dorsal cochlear nucleus (VCN, DCN), inferior colliculus (IC), medial geniculate body (MGB) and primary auditory cortex (AI). Data showed a strong decrease in cell densities within the central auditory system as well as a significant increase of TUNEL-positive cells. The effects were particularly present in the brainstem and midbrain structures (VCN, DCN, IC) and, to a lesser extent, in the MGB and AI after a single noise trauma compared to normal hearing controls. In contrast, a second noise trauma had a much stronger impact on higher auditory brain structures compared to a single exposure, whereas the lower pathway was less affected. A single noise trauma seems to induce early apoptosis mainly in the basal structures of the central auditory system, whereby the amount of cell death mechanisms in MGB and AI was somewhat weaker. Possibly, afferent inhibitory projections from hierarchically lower auditory areas protect higher structures from a direct acute noise impact during the first exposure. However, the effects after a second trauma could be due to a reduction of protective mechanisms as a result of pre-existing damage as well as deprivation-induced neurodegeneration.

The present work was supported by the Deutsche Forschungsgemeinschaft DFG (grant number: GR 3519/3-1).

Neuronal responses to amplitude modulation in the bat auditory cortex

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Amplitude modulation (AM) is a widely distributed feature of natural vocalisations. Among other animals, bats and humans use sequences of amplitude modulated acoustic information to communicate with each other. Amplitude modulated sounds are thought to carry ethologically important information for the listeners, and this information needs to be extracted by neurons in the auditory pathway. The aim of this study was to investigate how neurons in the auditory cortex (AC) of the bat species *Carollia perspicillata* extract AM information. To that end, artificially generated acoustic stimuli with different modulation frequencies were presented to anaesthetised animals and neuronal activity was measured with carbon fibre microelectrodes. In total, data from 80 neurons was collected. The results show that neurons in the bat AC can follow AM frequencies up to 8.57 Hz by phase-locking to every cycle of the modulation envelope. In response to higher modulation frequencies (MFs) up to 2 kHz, a majority of the neurons generated a burst of spiking activity at the offset of the stimulus. Our data suggests that bat cortical neurons are rather slow regarding their temporal following capacity, even though these animals do have to cope with fast time-varying acoustic information on a regular basis.

Pre- and Post-Synaptic Cholinergic Modulation on Endbulbs of Held in the AVCN of Gerbils

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Endbulbs of Held giant synaptic terminals transmit precise temporal information from the auditory nerve to low frequency spherical bushy cells (SBC) in the anteroventral cochlear nucleus. Furthermore, cholinergic axons of the olivo-cochlear bundle and from neurons in the pontomesencephalic tegmentum enter the cochlear nucleus. The function of this top-down innervation in temporal coding in the cochlear nucleus is not well understood. Recently, Goyer et al. (2016) have demonstrated the presence of nicotinic receptors in the neuropil of the anteroventral cochlear nucleus. Physiologically, SBC respond to cholinergic agonists with a depolarization of the resting membrane potential (RMP) on two time scales: transiently for milliseconds to seconds through the activation of $\alpha 7$ -nicotinic receptors and over a range of minutes via muscarinic receptor signaling. Here we begin to investigate the underlying mechanisms of the long-lasting RMP modulation mediated by muscarinic receptors and we test whether acetylcholine also has a presynaptic effect on the endbulb of Held synaptic terminals.

Immunohistochemical staining against m3-muscarinic receptors and against calretinin were performed in parasagittal auditory brainstem slices of P31 gerbils (*Meriones unguiculatus*). Whole-cell patch clamp recordings from SBC were obtained in frontal slices of P14-20 gerbils. First, 500 μ M carbachol was applied in presence of strychnine (1 μ M) and recordings were obtained in voltage-clamp to assess the frequency and the amplitude of putative synaptic mini currents. In a different set of experiments we monitored the RMP and the action potential (AP) threshold in current clamp mode upon bath application of different pharmacological agents acting on the cholinergic system (carbachol 500 μ M, 4-DAMP 1 μ M, oxotremorine 1 μ M)

We found that the application of carbachol significantly increased the frequency of synaptic mini inward currents (to 115%) but did not cause any consistent changes in their amplitude. Immunohistochemical data revealed the presence of m3-muscarinic receptors in the dendritic area of SBC. Accordingly, wash-in of a cholinergic agonist (carbachol) depolarized the RMP (+4.6 \pm 0.6mV) and showed an influence on the AP threshold (+6.1 \pm 0.6mV). Surprisingly, the application of an agonist of muscarinic receptors (oxotremorine) affected the AP threshold (+3.4 \pm 0.7mV) but did not induce any noteworthy change in RMP. Conversely, wash-in of an antagonist of m3 receptor (4-DAMP) impacted the AP threshold (-5.5 \pm 1.3mV) but also hyperpolarized the SBC (-3.8 \pm 1.6mV). Our results thus indicate a complex pre- and post-synaptic effect of acetylcholine on SBC. The increase of mini current frequency suggests an increase in the release probability. On the postsynaptic side, the m3 muscarinic receptor was clearly involved in setting the RMP and the action potential threshold of SBC, thus substantially influencing their excitability.

In summary we find the general effect of cholinergic modulation of SBC to be an increase in excitability and efficacy of excitatory transmission. Thus, top-down projecting cholinergic centers can profoundly influence temporally precise information processing already in the early stages of the auditory pathway.

Precise inhibition in the auditory brainstem fine tunes and facilitates action potential firing

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The temporal integration of excitatory and inhibitory synaptic inputs constitutes a basic principle of sensory processing in neuronal circuits. Typically, inhibition not only lowers neuronal excitability, but differences in its arrival time relative to excitatory inputs also establish a time window for action potential (AP) generation. In most sensory circuits, inhibitory inputs are considered to be the limiting factor for temporal resolution of this integration process due to slower time courses compared to excitatory inputs. In the auditory sound localization system, however, the time course of both excitatory and inhibitory inputs has been demonstrated to be exceptionally short. However, the functional role of inhibition for the temporal processing and its resolution in these brainstem circuits is unclear. To examine these questions, we studied the temporal integration of inhibition and excitation in the lateral superior olive (LSO) *in vitro* and *in vivo*. Neurons in the LSO gauge differences in sound amplitudes between the two ears by integrating glutamatergic excitatory and glycinergic inhibitory inputs from the ipsi- and contralateral ear, respectively. To determine the temporal resolution of this integration process, we presented click-train stimuli of varying inter-click-intervals (ICI) and assessed changes in AP rate and AP timing. By modulating the relative onset times of the stimuli between the two ears (interaural time difference, ITD), we show microsecond precise sensitivity which remains functionally resolved for ICIs > 1 ms. Moreover, AP times shifted considerably as a function of ITD. Surprisingly, at specific ITDs with inhibition leading excitation, we observed a significant facilitation of AP rates compared to stimulation without inhibition. Thus, the precise arrival of inhibition relative to excitation tunes ITD processing with microsecond sensitivity, and can promote AP generation.

Pressure Difference Receiving Ears Influence ITD Detection In The Auditory Brainstem Of Alligators. (A. Missispiensis)

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The difference between the timing of sounds at both ears (interaural time difference, ITD) is a key feature for sound source localization. In archosaurs, the detection of ITD is assumed to be consistent with the Jeffress model, which consists of coincidence detectors and delay lines. Coincidence detectors receive input from both ears and respond maximally if the inputs converge simultaneously. Delay lines compensate for the range of external delays and innervate arrays of coincidence detectors, leading to the formation of a map or place code of ITD. A structure that resembles the Jeffress model has been found in the nucleus laminaris (NL) of archosaurs, including alligators and birds. Alligators, like most birds, are sensitive to ITDs at low frequencies, where coupled ears allow transmission of sounds through the dorsal and ventral sinuses that connect both middle ears. This internal coupling could increase the range of interaural time differences, and thus compensate for small head sizes. It also opens an additional way to detect ITDs, since the eardrum vibration in response to sound becomes directional, which may lead to spatial tuning in monaural nuclei, as for example in lizards. We hypothesized that alligator first order nuclei might be spatially tuned, since their eardrum responses have been shown to be directional. We therefore performed in vivo extracellular recordings in the auditory brainstem in American alligators. Neurons in the monaural nucleus magnocellularis (NM), which carry precise information about the stimulus phase, were tuned to interaural time difference. However, the amplitude of NM tuning curves was much smaller than the amplitude in NL. ITD tuning in NL also deviates from a classic delay line model, because we found many units with characteristic phases around 0.5, which means that the characteristic delay lies in the trough of the ITD tuning. Additionally, some recording sites within NL had characteristic delays that differed for low and high frequencies, with a cutoff frequency around the resonance frequency of the interaural canal, above which ear drum directionality drops. Thus, directional sensitivity in nucleus magnocellularis might influence ITD detection in NL.

Processing spatial depth in the auditory cortex of the fruit-eating bat *Carollia perspicillata* in the presence of natural acoustic jamming noise

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For proper spatial orientation, animals have to compute their own location in relation to surrounding objects (egocentric representation). Vision, audition and somatosensation represent only few sensory modalities on which the animals can potentially rely on for quantifying their egocentric representation. Echolocating bats primarily use their highly developed auditory system for short range orientation. Behaviorally, bats are suitable animal models to investigate spatial orientation as they usually move along three dimensions and spatial analysis has to be done rapidly for obstacle avoidance or hunting flying insects. They use the echo delay, defined as the time interval from emitting a biosonar call until the arrival of the corresponding echo, for calculating the distance between them and surrounding objects. Neurons tuned to specific echo delays are topographically organized in the dorsal auditory cortex forming a target distance map. These neurons are highly specialized to detect a wide range of different echo delays ranging from 2 to 24 ms, that correspond to object-distances between 0.3 to 4 m.

For creating a correct acoustic image from the surrounding, bats have to distinguish their own echoes from echoes of conspecifics. During echolocation, bats never encounter an acoustic silent environment. Although high frequency sounds are sparsely represented in the wild, there are still high frequency sound sources (jamming noise) that could potentially mask echo perception. The present study addresses the question of how acoustic noise from conspecifics, different bat species, or locusts affects the echolocation behaviour and the neuronal encoding of distance information. In a behavioral paradigm echolocation performance of the fruit-eating bat *Carollia perspicillata* was assessed in the presence and absence of jamming noise. Our results show that echolocation behaviour does not seem to be tremendously affected by jamming noise. Multielectrode recordings from the target distance map of awake bats show that delay tuned neurons can encode target distance information although responding additionally to jamming noise.

Spectrotemporal plasticity of receptive fields by parvalbumin-positive interneurons in auditory cortex

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The interplay of excitatory and inhibitory signals forms the basis for the processing of neuronal information at different levels of mammalian pathways up to the cortex. However, how exactly different types of inhibitory cells shape receptive fields within the complex networks of sensory cortices remains largely elusive.

In this study we asked how an important population of inhibitory interneurons (parvalbumin-positive – PV+) shape spectrotemporal receptive fields (STRF) of the primary auditory cortex (A1). To this end, the activity of the PV+ neurons was increased by prolonged, low-level optogenetic depolarization, using a stable step-function opsin (SSFO) variant of ChR2. For this purpose, an adeno-associated virus containing the gene of SSFO was injected into the primary auditory cortex of a mouse (B6 Cast/PVALB-Cre). The illumination of A1 with blue light (wavelength = 470nm) depolarized the PV+ cells, whereas orange light (wavelength = 590nm) closed the opsin channels again. This optogenetic manipulation was performed during extracellular recordings from A1 of an awake animal while DRC stimuli (dynamic random chords) were presented.

The effect of the activated SSFO varied between the types of neurons. While the optogenetic manipulation increased the firing rate on inhibitory neurons about 10%, the firing rate on excitatory neurons decreased about 50%. This means that small changes in the activity of PV+ neurons have a large impact on local excitatory cells.

Surprisingly, despite the large decrease in excitatory activity, there were no major differences found in the STRF between the state of optical activation of ChR2 and optical deactivation, but only slight changes in spectral and temporal profiles. Neurons affected by an increased PV+ activity tended to develop a more selective frequency tuning and enhanced temporal precision. Further analysis demonstrated that optical activation of PV+ neurons primarily caused a reduction of the excitatory areas in the STRF and a little change to inhibitory bands. The reduction was highest at the spectral and temporal region of the neuron's best frequency and became weaker at its surrounding areas. This observation is consistent with a network model with a co-tuning of excitation and feed-forward inhibition provided by nearby PV+ interneurons (Seybold et al., 2015).

SPON receives excitatory input from Octopus cells and responds better to the onset of broad-band sounds *in vivo*

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All mammals have a remarkable capacity for extracting relevant acoustic information from a complex acoustic environment. To achieve this automated processing of complex sounds, the mammalian brain uses parallel neuronal pathways. How the coarse temporal information contained in communication sounds, such as vocalizations, is analyzed by the brain is a fundamental for understanding auditory perception of human speech. The superior paraolivary nucleus (SPON) is a prominent group neurons in the auditory brainstem of mammals, which responds selectively to rhythmic sound information of relevance for speech or other species specific communication. Recently, we demonstrated that the SPON fires to the onset of a tone burst *in vivo* (Felix et al. 2013 J Neurophys. 109:2691-704), which is matched by strong synaptic excitation from few fibers with high release probability *in vitro* (Felix and Magnusson, 2016 Neurosci. 334:1-12). Since auditory parallel processing builds on specialized neurons conveying information about distinct acoustic features, a clarification of the exact source of excitation to SPON is warranted. To this end, we have used an interdisciplinary approach that combines anatomical and physiological experiments.

Anatomically, injections of the bidirectional tracer biotinylated dextran amine were made into SPON of mice to retrogradely label possible sources of excitatory inputs in the brainstem. In all cases, we found only a discrete (and excitatory) population of labeled neurons on the contralateral side to the injection site: octopus cells in the posteroventral cochlear nucleus (PVCN). On the ipsilateral side, the labeled cells were glycinergic principal cells of the nucleus of the trapezoid body (MNTB), and multipolar neurons of the lateral nucleus of the trapezoid body (LNTB), both of which presumably corresponding to inhibitory inputs.

Physiologically, single units were isolated in SPON of anesthetized mice. The response evoked by broad-band or narrow-band sound stimuli presented at the contralateral ear were investigated. The center frequency was set to the neuron's characteristic frequency for both stimuli. Virtually all SPON neurons fired action potentials (AP) at the onset of both type of stimuli. However the, the onset was much more robust (on average three times as many APs) for the broad-band compared to the narrow-band frequency stimulation.

Taken together, these results provide evidence for a specific and prominent Octopus cell input from the PVCN to the SPON, which triggers a strong onset response to sounds. The higher selectivity to broadband sounds of the excitatory drive of SPON strengthens the hypothesis that the output of this brainstem area may be important for segmenting complex sounds into meaningful units in higher order auditory areas.

Temporal sound processing in the auditory cortex depends on both myelin integrity and oligodendrocyte-dependent metabolic support

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Our understanding of the roles that myelin and oligodendrocytes (OLs) play in information processing in the brain has widened in the past years. The static view of myelin as an insulator is changing. For example, the capability of OLs to transport glycolytic end-products directly to the axon confers them an important metabolic support role, which might be essential during periods of high frequency firing. With the aim to dissect the partially independent roles of myelin and OLs in supporting the reliable firing of axons, we studied sound processing in the auditory cortex in mice with either different degrees of dysmyelination, or an OL-specific metabolic impairment.

We performed *in vivo* multiunit recordings in the auditory cortex of anesthetized mice, using sounds protocols that aimed to test both spectral and temporal auditory processing. To test temporal acuity, we used a gap-in-noise detection protocol. This protocol was paralleled by comparable behavioral tests, in order to understand the relationship between the neuronal responses and perception.

We used four mouse models with either 1) complete (*shiverer*^{-/-} mice) and 2) partial (MPB-hypomorph^{-/-} mice) dysmyelination, 3) cortical-specific dysmyelination (*Emx1-cre::MBP^{fl/fl}*) and 4) OL-specific inducible glycolytic dysfunction (*Plp1-CreERT2::Hif1^{fl/fl}*).

As expected, we found that dysmyelination caused a major increase in response latencies, whose magnitude was correlated with the extent of dysmyelination. In addition, complete dysmyelination caused hyperexcitable responses, which might have been caused by the mislocalization of Na²⁺ and K⁺ channels in naked axons. Both complete dysmyelination and OL-metabolic dysfunction caused neuronal-population fatigue and temporal acuity deficits, which could not be explained simply by a delay in conduction velocity. Strikingly, all aspects specific to spectral processing were normal.

Likewise, partial dysmyelination caused an increase in the behavioral gap detection threshold, a common measure of temporal acuity, in the absence of a frequency discrimination deficit.

In summary, we found that both, complete dysmyelination and OL-specific metabolic dysfunction without dysmyelination, generated deficits in auditory temporal processing while leaving spectral processing intact. This suggests that specifically temporal-detection mechanisms require axons that are not only fast and reliable, but also metabolically stable. These characteristics are given by the structure of myelin and the OLs metabolic capacities, respectively.

The impact of impaired brainstem bifurcation on hearing

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cGMP signaling triggered by the binding of C-type natriuretic peptide (CNP) to its receptor guanylyl cyclase B (GC-B; NPR2; NPRB) has been linked by genetic evidence to a remarkable variety of physiological functions like skeletal bone growth, female fertility, cardiac growth, fat metabolism and gastrointestinal function. For the nervous system it has been recently demonstrated that the CNP/GC-B/cGMP/cGMP-dependent protein kinase type I (cGKI) signaling pathway is essential for sensory axon branching at the dorsal root entry zone of the spinal cord and at the rhombomeres of the hindbrain during embryonic development. Also in the cochlear nucleus (CN), distinct auditory nerve fiber (ANF) types that differ in their discharge rate and sound sensitivity bifurcate, sending collaterals to the anteroventral, posteroventral, and dorsal subdivisions. The lack of GC-B has been shown to lead to a loss of bifurcation in the CN without obvious functional deficits. Here, we describe the hearing function of adult GC-B knock-out mice and their wild-type littermates in detail, using evoked auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE) and auditory steady state response (ASSR). Histological correlates of the auditory phenotype were verified by applying immunohistochemistry on fixed sections of cochlea and brain tissue and high-resolution fluorescence microscopy. We discuss the results in the context of previous findings.

Time scale of adaptation to tonal sequences in mouse auditory midbrain neurons

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Human speech and communication sounds of animals consist of temporal sequences of elements that may be perceived as single auditory objects. Psychophysical studies demonstrated that the temporal sound context (temporal grouping and/or separating elements) strongly affects the perceptual quality of sound streams, such as syllables in human speech (Bregman, 1990). Similarly, streams of mouse pup wriggling calls are perceived by mothers as important only if single calls are at least 100ms (Geissler & Ehret, 2002) and inter-call intervals 100-400ms long (Gaub & Ehret, 2005). Neural codes for auditory streams and their perceptual boundaries are largely unknown. Neuronal adaptation may be one mechanism for auditory stream coding and segregation (Ulanovsky et al., 2004). Here, we test the hypothesis that adaptation in inferior colliculus neurons contributes to coding the temporal structure of sequences of mouse pups wriggling calls.

In anesthetized (ketamine/xylazine) mice, single-unit responses to series of four 100ms tones at the unit's characteristic frequency (40dB above threshold) with varying inter-tone intervals were recorded and spike rates analyzed. On average, equally strong adaptation from the first to the next (second, third, fourth) tones was noted for inter-tone intervals <200ms. For intervals longer than 500ms, spike rates to the tones in the series did not differ significantly, i.e. adaptation was largely absent. Inter-tone intervals of 100-500ms produced similar adaptation among the neurons. Inter-tone intervals of 4-50 ms produced neuron-specific adaptation including different shapes of recovery functions from adaptation. Two thirds of the units had monotonic rising recovery functions. At inter-tone intervals of 4-50 ms these units either responded only to the first tone or responses to the second, third and fourth tones were gradually reduced. Neurons with totally suppressed responses after the first tone, i.e. strongest adaptation, had phasic discharges and broad frequency receptive fields (class III, or V-shaped neurons, Egorova et al., 2001). One third of the units had non-monotonic recovery functions with response rates to the second, third and/or fourth tones in a series exceeding the response to the first tone in several units. Typically, these neurons responded with tonic, phasic-tonic, pauser or long-latency discharges, often with an off-response present. Frequency receptive fields of these neurons were sharply tuned (class I, primary-like or class II, inhibition-dominated, Egorova et al., 2001).

Thus, neuronal adaptation in the inferior colliculus differs among neurons with different shapes of frequency receptive fields and temporal response patterns. On average, the perceptual properties of wriggling calls in the time domain were most closely reproduced by the adaptation behavior of phasically responding, broadly tuned class III neurons.

Supported by the Russian Foundation of Basic Research (15-04-05234) and the Deutsche Forschungsgemeinschaft (EH53/22-1).

Bregman, A.S. Auditory scene analysis. The Perceptual Organization of Sound. Cambridge, MA: MIT Press, 1990.

Egorova, M., Ehret, G., Vartanian, I. & Esser, K.-H. Exp Brain Res, 140, 145-161, 2001.

Gaub, S. & Ehret, G., J Comp Physiol A, 191, 1131-1135, 2005.

Geissler, D.B. & Ehret, G., PNAS, 99, 9021-9025, 2002.

Ulanovsky, N., Las, L., Farkas, D., Nelken, I. J Neurosci, 24, 10440 – 10453, 2004.

Poster Topic

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A calcium signaling 'fingerprint' in vomeronasal sensory neurons

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In recent years, a growing number of chemosensory signals were discovered that are detected by the mammalian vomeronasal organ (VNO). These complex chemical cues regulate social behavior and carry information about sexual, social and reproductive status of both con- and heterospecific individuals. To date, however, little is known about both the sensory coding strategies implemented by the VNO and the stimulation-dependent activity patterns in single vomeronasal sensory neurons (VSNs).

In this study, we used Ca^{2+} imaging in acute mouse VNO slices to determine the activity of VSNs both on the individual neuron and the population level. Slices were loaded with the Ca^{2+} -sensitive dye Cal-520/AM in a custom-made circulating oxygenation chamber. Precise focal perfusion of gender-specific pooled urine samples from wild as well as inbred lab strain mice allowed us to begin to analyze the neural code of vomeronasal information inherent to both sets of stimuli. Comparative analysis of response patterns, kinetics, adaptation and robustness provides novel insights into information differences and commonalities conveyed by wild *versus* lab strain urine.

Together, we present an improved *in situ* Ca^{2+} imaging approach that will allow effective VNO ligand screening, characterization of population response patterns, as well as single VSN analysis of individual Ca^{2+} transients. Thus, on-going experiments aim to provide a quantitative perspective on vomeronasal coding at the VSN population level as well as a detailed analysis of Ca^{2+} signaling events in single neurons.

Activation of the mouse OR37 subsystem coincides with an attenuation of activity in the paraventricular nucleus of the hypothalamus

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Within the main olfactory system of mammals, a unique subsystem exists comprised of sensory neurons expressing odorant receptors of the OR37 subfamily. These receptors are exclusive for mammals and highly conserved across species. The mouse OR37 receptor subtypes A, B and C were shown to be activated by the long-chain aliphatic aldehydes penta-, hexa- and heptadecanal, respectively. The search for biological sources of these compounds now shows that bodily secretions from conspecifics activate the OR37A, B and C glomerulus. At the same time the activity of cells in a target region of projection neurons from OR37 glomeruli, the paraventricular nucleus of the hypothalamus (PVN), is reduced compared to controls (clean test box). The large number of activated cells in the PVN of mice that are placed into a clean test box are corticotropin-releasing hormone cells, indicating an induction of the stress axis due to the novel environment. The much lower number of activated cells of mice in a box enriched with bodily secretions from conspecifics indicates a reduced stress response. Since bodily secretions from conspecifics activate the OR37 system and simultaneously reduce stress-induced activation of the PVN, it was tested whether the ligands for OR37 receptors can induce this effect. Indeed, a similarly reduced activity in the PVN is found in mice kept in a clean test box and exposed to a mixture of the OR37 ligands delivered via an air stream. These data indicate that the OR37 system may play a role in mediating a phenomenon called social buffering.

This work was supported by the Deutsche Forschungsgemeinschaft (SPP1392).

Ancestral amphibian V2R expression during metamorphosis

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The sense of smell helps animal species to evade predators, localize prey and recognize viable mates. In mammals olfactory receptor families are segregated into different olfactory organs, main olfactory epithelium (MOE) and vomeronasal organ (VNO). In contrast, teleost fish olfactory receptor families are intermingled in a single sensory surface. To what extent such differences influence the coding and discrimination abilities of the respective olfactory systems is unclear, and the evolutionary path toward such segregation is unknown. The analysis of amphibians, which are early diverging tetrapods compared with mammals, may shed light on this transition from shared sensory surface to segregated subsystems.

Recently, our phylogenetic analysis of the V2R (vomeronasal type 2 receptor) family led to the identification of three distinct subdivisions in the largest group of v2r genes (A1, A2, A3). We used this sequence information to clone several *Xenopus laevis* v2r genes representative of the above mentioned subdivisions. Expression analysis revealed the V2R family in transition between a fish-like and a mammalian-like mode of expression, where later diverging V2Rs (A2, A3) are expressed in the vomeronasal organ and early diverging V2Rs (A1, C) are expressed exclusively in the main olfactory epithelium. Moreover, within the MOE v2r genes are expressed in a basal zone, partially overlapping, but clearly distinct from an apical zone of OMP and odorant receptor-expressing cells. Furthermore correlation analysis suggested early diverging V2R receptors may carry the functional response to amino acid odors.

In this study, we investigate in parallel the expression of the v2r gene family and the localisation of amino acid responses during metamorphosis. During metamorphosis the main olfactory epithelium of *Xenopus* tadpoles transforms into an air-filled cavity (principal cavity, air nose), whereas a newly formed cavity (middle cavity) takes over the function of a water nose.

Acknowledgement:

This work is funded by: DFG Schwerpunktprogramm 1392

References:

Syed AS, Sansone A, Nadler W, Manzini I, Korsching SI. Ancestral amphibian v2rs are expressed in the main olfactory epithelium. PNAS 2013

Sansone A, Syed AS, Tantalaki E, Korsching SI, Manzini I. Trpc2 is expressed in two olfactory subsystems, the main and the vomeronasal system of larval *Xenopus laevis*. J Exp Biol. 2014

Automated Operant Olfactory Conditioning of Group-Housed Mice

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Despite the staggering increase in specific as well as efficient techniques for generating transgenic mouse lines the behavioural analysis of these strains still relies heavily on manual characterisation of individual animals. Not only is this approach labour and cost-intensive but also highly prone to experimenter-induced errors and variations. Additionally, most tests are conducted on single-housed animals during the daytime – a suboptimal setting for natively social and nocturnal animals.

To circumvent these limitations, we used an automated operant olfactory conditioning setup that allows for group-housing of even large cohorts of animals (up to 25 subjects) while simultaneously training these animals on a go/no-go odour discrimination task.

Animals, identified via an implanted RFID-tag, could initiate trials themselves allowing for generation of unique training protocols specifically tailored to each animal. The animals reached >95% correct performance as quickly as during manual training (Nixon et. al 2004, Shimshek et. al 2005, Nixon et. al 2010). Simultaneously we could monitor key additional parameters like the licking-patterns, air flow, air pressure, temperature and humidity with millisecond precision.

Using this setup we were able to simultaneously train up to 25 male mice from different genetic backgrounds and with different ages on our go/no-go paradigm using multiple pure odours as well as the more complex binary mixtures of previously learned pure odours. For instance even completely naïve animals were able to reach our criterion of 95% performance in less than 400 trials (321 trials \pm 43 SEM) for the first odour pair and less than 250 trials (226 trials \pm 25 SEM) for the second odour pair. Regardless of odours used, this performance criterion was usually reached within less than 5 days (1,7 days \pm 0,4 SEM) and subsequently remained constantly high even over weeks of training using the same odour pair.

In summary, this setup enables automated training of socially housed mice while minimizing experimenter interaction with the animals. Apart from the odour discrimination tasks described here the setup can readily be expanded to encompass additional sensory cues or even serve as a pre-training phase to screen for high performing animals for use in further studies like awake 2P imaging or electrophysiological recordings.

This work was supported by funding from the Deutsche Forschungsgemeinschaft SPP 1392 “Integrative Analysis of Olfaction”.

Brush cells at the 'gastric groove' sense constituents of ingested food

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At the 'gastric groove' in the murine stomach - the border between the aglandular reservoir compartment and the glandular digestive compartment - brush cells are arranged in a palisade-like manner. These brush cells share the molecular phenotype with gustatory sensory cells and are therefore considered as candidate sensory cells involved in monitoring constituents of the ingested food. Food sensing is also accomplished by enteroendocrine cells, which release hormones in response to stimulation; in contrast, brush cells are not endocrine but supposedly transmit their information to target cells via paracrine messengers, such as prostaglandins, nitric oxide and acetylcholine. In search for food constituents which may activate these brush cells, we recently found that they express the receptor for long chain fatty acids GPR120. Previous studies have shown that activation of GPR120 leads to a phosphorylation of the kinase ERK1/2 and to an expression of cyclooxygenase 2 (Cox-2), the key enzyme for the synthesis of prostaglandins. We have used these two features to monitor the response of brush cells to long chain fatty acids. The results indicate that an application of oleic acid - a ligand for GPR120 - elicited an increased phosphorylation of ERK1/2 and induced the expression of Cox-2; expression of Cox-2 is considered as indicative for an enhanced generation of prostaglandins. So, the data support the notion that in response to long chain fatty acids brush cells may release prostaglandin; this paracrine messenger may affect adjacent smooth muscle cells which express prostanoid receptors. Thus, it is conceivable that the sensing of food constituent by brush cells may contribute to control the gastric motility and the transfer of the luminal content.

Calcium-imaging in the olfactory epithelium of *Danio rerio* reveals cell type-specific responses to different odorant classes

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The olfactory epithelium harbors a variety of different cell types including olfactory sensory neurons (OSNs), supporting cells and basal cells (stem cells). In the past, efforts have been made to characterize these cell types morphologically as well as genetically, especially with respect to genes involved in the processing of olfactory stimuli. However, many of their functional properties during olfactory processing remain elusive.

Here we present a simple technique to record cellular activity in response to different odorant classes in the olfactory epithelium of the zebrafish, *Danio rerio*. To the best of our knowledge this is the first time that odorant responses have been recorded in the zebrafish nose in the live tissue. We identify the cell types, which carry these responses by determining morphological parameters and by co-localization studies with cell type-specific markers. Further, we show that distinct regions in the olfactory bulb process these odors by using the immediate-early gene pERK as a marker for neuronal activity.

The Calcium imaging technique described here constitutes a straightforward approach to characterize odorant responses in the zebrafish olfactory epithelium and thus to understand neuronal odor representation at the cellular level within the sensory surface.

CD36 is involved in fatty acid detection by the murine olfactory system.

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Olfactory signals influence food intake in a variety of species. To maximize the chances of finding a source of calories, an animal's preference for fatty foods and triglycerides already becomes apparent during olfactory food search behavior. However, the molecular identity of both receptors and ligands mediating olfactory-dependent fatty acid recognition are, so far, undescribed. We here describe that a subset of olfactory sensory neurons expresses the fatty acid receptor CD36 and demonstrate a receptor-like localization of CD36 in olfactory cilia by STED microscopy. CD36-positive olfactory neurons share olfaction-specific transduction elements and project to numerous glomeruli in the ventral olfactory bulb. In accordance with the described roles of CD36 as fatty acid receptor or co-receptor in other sensory systems, number of olfactory neurons responding to oleic acid, a major milk component, in Ca²⁺ imaging experiments is drastically reduced in young CD36 knock-out mice. Strikingly, we also observe marked age-dependent changes in CD36 localization, which is prominently present in the ciliary compartment only during the suckling period. Our results support the involvement of CD36 in fatty acid detection by the mammalian olfactory system.

Chemo- and thermosensory signaling in the Grueneberg ganglion

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The Grueneberg ganglion in the anterior nasal region of mammals is considered as a dual sensory organ since its neurons respond to cool temperatures as well as to distinct odorants, including the heterocyclic compound 2,3-dimethylpyrazine (2,3-DMP). The ganglionic cells extend axonal processes to the olfactory bulb of the brain. Investigations using transgenic mice with specifically labeled Grueneberg neurons allowed us to determine more precisely the axonal circuitry. It was observed that the axons terminated in nine distinct, round or oval-shaped glomerular structures (termed Grueneberg glomeruli) which were distributed in a characteristic topographical pattern in dorsal, lateral, ventral, and medial regions of the bulb. To assess whether these glomeruli were activated upon stimulation of Grueneberg ganglion neurons, the expression of the activity-dependent marker c-Fos in juxtglomerular cells of Grueneberg glomeruli was monitored. In these approaches, following an exposure of mice to the odorant 2,3-DMP, all of these glomeruli were found to be activated, irrespective of their position in the bulb. Experiments with mice lacking the cyclic nucleotide-gated channel CNGA3, which is critical for chemo- and thermosensory signal transduction in Grueneberg ganglion neurons, confirmed that the activation of Grueneberg glomeruli was indeed based on a stimulation of Grueneberg ganglion neurons. Since the Grueneberg ganglion neurons do not only respond to chemical stimuli but also to cool temperatures, the question arose whether thermosensory information is also processed by Grueneberg glomeruli in the olfactory bulb. In this context, exposure of young mice to coolness also led to an activation of the Grueneberg glomeruli. In mice deficient for CNGA3, the activation of these glomeruli by cool temperatures was significantly attenuated. These findings demonstrate a shared processing of chemo- and thermosensory information in Grueneberg glomeruli of the olfactory bulb.

This work was supported by the Deutsche Forschungsgemeinschaft.

Default glomerular activity maps in the olfactory bulb of awake mice

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In the mammalian olfactory bulb (OB), glomeruli represent functional units formed by afferent fibers of the olfactory receptor neurons (OSNs), apical dendrites of principal neurons (mitral/tufted cells) and dendrites from local glutamatergic and GABAergic interneurons, collectively referred to as juxtglomerular cells (JGCs). The functional properties of the glomerular units were mostly studied under anesthesia (Kikuta et al. 2013, Homma et al. 2013) and therefore there is little data describing the basal (spontaneous) activity of the units as well as their odor response properties in the awake state.

Using a novel genetically-encoded ratiometric Ca^{2+} -indicator Twitch-2B, we monitored basal glomerular activity in awake, head restrained mice by means of in vivo two-photon Ca^{2+} -imaging. The bright fluorescence of Twitch-2B enabled us not only to measure basal activity and odor-evoked responses of different glomeruli but also to identify the associated JGCs by tracking their dendrites into the parent glomerulus. In awake mice, cellular (cpVenus^{CD}/mCerulean3) ratios, which are proportional to the intracellular free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), ranged from 1.60 to 8.55. Strikingly, the basal activity patterns of glomerular units were not uniform. Instead, color-coded ratio maps of the imaged area displayed a heterogeneous landscape of basal glomerular activity. Within these color maps individual glomeruli could clearly be identified based on their basal activity level. Moreover, the activity levels of individual glomeruli remained stable over a prolonged time period (up to 8 days). Cells projecting into a given glomerulus shared similar ratio levels with this glomerulus, whereby cells had the tendency to have somewhat higher basal Ca^{2+} -levels than their parent glomerulus. Anesthesia reduced significantly glomerular basal activity and thus diminishing the heterogeneity of activity maps.

To understand the mechanisms underlying the striking heterogeneity of the default glomerular activity maps we applied tetrodotoxin (TTX) into the nose and observed a slight reduction of basal glomerular $[\text{Ca}^{2+}]_i$. Chemical ablation of OSNs by i.p. injection of dichlobenil (2,6 dichlorobenzonitrile, a potent olfactory toxicant that specifically induces lesions both in the Bowman's glands and in the nasal epithelium (Brandt et al. 1990)), led to a much stronger reduction of the basal glomerular $[\text{Ca}^{2+}]_i$, wiping out the heterogeneous appearance of the glomerular landscape. Thus, OSNs do contribute to the heterogeneity of the default glomerular activity maps under physiological conditions.

In conclusion, our data identify a new feature of the signal-processing network in the glomerular layer of the olfactory bulb: a strikingly non-uniform default glomerular activity map. This map can only be observed in the awake state and the underlying activity is largely driven by the OSNs. The functional role of this activity remains to be established, but its loss might underlie the differences in sensory processing between the awake state and anesthesia.

References:

- Kikuta et al. 2013, Neuron, 77(6):1122-35.
- Homma et al. 2013, Front Neural Circuits. 7:23.
- Brandt et al. 1990, Toxicol. Appl. Pharmacol., 103, 491-501.

Glomeruli of the OR37 subsystem possess a more stable interneuronal network than others

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The olfactory subsystem OR37 is targeted to the paraventricular nucleus (PVN) of the hypothalamus and seems to be involved in social buffering effects under moderately stressful conditions. Due to the pheromone-like character of the system we hypothesized that its neuronal network in the olfactory bulb may be more stable than those for general odorants. Therefore we have analyzed adult-generated interneurons around transgenically labeled glomeruli of mOR37 members in comparison to glomeruli of receptors responding to general odors.

Results of BrdU labelling experiments indicated that at glomeruli of the OR37 subfamily the proportion of adult born interneurons was significantly lower than at glomeruli of mOR256-17 or mOR18-2. Surprisingly, the number of arriving immature neurons, labeled by the neuroblast marker Doublecortin, differs around individual glomeruli indicating an inconstant arrival rate and also an inconstant integration rate when compared to BrdU-labeled, mature adult-born neurons.

Thus, the proposed role of OR37 odorant receptors in social communication coincides with a more persistent, "hard-wired" functional glomerular domain, though these differences are probably not due to a lower proportion of arriving neuroblasts in the regarding area of the olfactory bulb.

This work was supported by the Deutsche Forschungsgemeinschaft.

Illuminating the function of inhibitory microcircuits in the zebrafish homolog of olfactory cortex

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The brain creates dynamic representations of the sensory environment by extracting stimulus features at early processing stages and synthesizing more abstract object representations in higher brain areas. We dissect the function of neuronal microcircuits in a higher olfactory brain area to identify elementary computations of basic cortical circuits and to analyze the underlying cellular mechanisms. We use a combination of genetic, electrophysiological and optical approaches to visualize and manipulate different types of interneurons (INs) in the posterior zone of the dorsal telencephalon (Dp) of adult zebrafish. This brain area is homologous to olfactory cortex in mammals and assumed to be involved in olfactory object representations and associative memory. We identified two types of inhibitory INs that have similar electrophysiological properties but are differently connected to other neurons in Dp. Both IN types provide divisive inhibition, a particularly important form of inhibition in auto-associative memory networks, which has so far not been observed in olfactory cortex. In addition, we observe that Dp interneurons are involved in other functions, including separation of similar odor representations, representation of odor objects, and in regulating network activity after plasticity.

Impact of basal forebrain stimulation on olfactory bulb output in awake mice

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Odors are probably the most complex and diverse sensory input for an organism. Processing and filtering of olfactory information starts as early as the olfactory bulb (OB), where information from olfactory sensory neurons is transmitted to higher olfactory areas. However, activity within the OB is not only determined by sensory input but also strongly influenced by cortical as well as neuromodulatory feedback projections. Here, we aim to shed light on the specific role of neuromodulatory inputs in early sensory processing, using the mouse olfactory system as a model.

The OB receives neuromodulatory input from diverse areas, such as serotonergic and noradrenergic inputs from the raphe nuclei and the locus coeruleus, respectively. The horizontal band of Broca (HDB) is the main source of cholinergic input to the OB (Zaborszky et al. 1986). The HDB is known to be modulated in general behavioral states like attention and arousal. Cholinergic input to the OB has been associated with odor learning and discrimination (Hasselmo 1999). Slice experiments revealed that muscarinic acetylcholine receptor activation can inhibit granule and short axon cells in OB (Pignatelli and Belluzzi 2008). In agreement with these findings, we recently demonstrated that specific activation of cholinergic HDB terminals in the OB had an excitatory influence on OB output activity in anesthetized animals (Rothermel et al. 2014).

However, recent studies showed that wakefulness greatly alters OB network and output activity (Kato et al. 2012). In anesthetized animals inhibitory granule cells are largely inactive, whereas their stronger inhibitory tone in awake animals likely causes mitral tufted cells (MTC) activity to become sparser and temporally more dynamic.

Therefore, we decided to investigate the effects of HDB stimulation on MTC activity also in awake animals. We selectively expressed GCaMP6 in MTC using an AAV mediated retrograde labelling approach in transgenic animals (Rothermel et al. 2013). MTC activity was visualized using widefield and two-photon microscopy (2PM). Widefield imaging data showed long-lasting excitatory effects of odor responses of MTCs following electrical stimulation. This excitatory effect was still visible immediately after odor offset as well as during odor presentation in the following trial. 2PM also showed increased excitation of odor responses evoked by electrical stimulation in interleaved trials. Surprisingly, the long-lasting electrical stimulation effects observed in 2PM consisted of a general reduction of odor response amplitudes. In summary, our preliminary results show that electrical HDB stimulation in the awake animal is causing relatively long-lasting effects that, in agreement with data from anesthetized animals, result in an enhancement of odor-evoked responses.

Our group also investigates the specific cell types affected by HDB feedback projections. Our current experiments focus on electrical and optical HDB stimulation experiments in anesthetized mice, while simultaneously recording spontaneous as well as odor evoked responses from defined cell types in the OB. We predict that electrical stimulation of the HDB will have excitatory effects on MTCs and inhibitory

effects on granule cells (Kunze et al. 1991). In future studies we will perform behavioral experiments in order to investigate the effects of HDB stimulation on odor-discrimination learning.

Innate fear responses induced by pyrazine odors originated from wolf urine in deer and rats

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Urine excreted from common grey wolf (*Canis lupus*) has been used as a repellent for various kinds of mammals, because their urine odors act as kiromones, which induce fear-related behaviors. Various fear-inducing substances activate neurons at the main and/or accessory olfactory bulb, medial and central amygdala, and ventromedial hypothalamus. Our previous study showed that pyrazine analogues (P-mix) contained in wolf urine induced avoidance and fear-related behaviours in laboratory mice. Exposure to wolf urine and P-mix induces Fos-expression, a marker of excitation in neurons, in the accessory olfactory bulb (AOB) of mice. In the present study, we explored effects of P-mix on rats and domestic deer in Japan. Exposure to P-mix induced avoidance and fear-related behaviours in rats and deer. P-mix but not the mixture of i-amyl acetate, linalool and d-carvone (O-mix), which have flower and fruit flavour, increased Fos-immunoreactivity in the AOB, several regions of the amygdala and the VMH of adult rats. Exposure to P-mix also induced avoidance, immobilization and Fos-expression at the AOB, amygdala and hypothalamus in juvenile rats. The present results suggest that P-mix odour induces innate fear-related behaviours.

Network Formation and Regeneration in the Olfactory System of *Xenopus laevis*

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Comprehending the mechanisms that make lifelong neurogenesis possible has a clear interest for the better understanding of basic principles that govern cellular and molecular interactions in the nervous system, as well as a relevant clinical interest. The general inability of the nervous system to generate new neurons in order to replace those that have been lost is a formidable obstacle to recovery from neuronal damage caused by injury or neurodegenerative disease. 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. For this to be possible, neuronal stem cells must go through several stages of maturation, including proliferation, migration, differentiation, and integration, to become fully embedded in an existing neural circuitry.

Here we investigated the timing of degeneration and subsequent regeneration of the receptor neuron population after transection of the olfactory nerve of larval *Xenopus laevis*. Results obtained using immunohistochemistry, as well as neuronal labeling and functional calcium imaging, indicate that neuronal cell death peaks 48 hours after nerve transection. Proliferating epithelial stem cells are quickly upregulated after lesion. Supporting cells maintain both morphological and functional integrity. The olfactory epithelium recovers its original morphology 1 week after transection, at which time the first axons reach the olfactory bulb. Only spontaneous activity of mitral/tufted cells is observed in the olfactory bulb during the first weeks after transection. After 3-4 weeks first glomerular responses were observed upon epithelial odor stimulus application, but the response and glomerular morphology were still clearly altered as compared to control. 3 weeks later olfactory bulb morphology and glomerular responses seem to have fully recovered, indicating that the olfactory system of larval *Xenopus* recovers morphologically and functionally 6-7 weeks after nerve transection.

P2Y1 receptor-mediated modulation of neuronal activity in the mouse olfactory bulb

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Purine nucleotides such as ATP and ADP modulate the communication between cells throughout the nervous system. In the mouse olfactory bulb (OB), the first olfactory relay station, ATP is released from sensory axons together with glutamate as a neurotransmitter and stimulates calcium signaling in glia cells.

It has recently been shown that ATP also evokes neuronal network activity in the OB (Fischer et al., *Purinergic Signal.*, 2012), though the origin of this effect could not be identified so far. We used spatiotemporal defined photolysis of caged ATP or caged ADP in acute mouse OB slices to further dissect purinergic modulation in the olfactory bulb. Therefore, we mimicked incoming odor signals by releasing ATP or ADP locally restricted to a glomerulus, a specific processing unit in the OB and recorded the response in output neurons (mitral cells and external tufted cells) conveying to this glomerulus by whole-cell patch clamp.

The release of ATP led to a P2Y1 receptor-dependent increase in synaptic activity in mitral/tufted cells (MC/TC) and a prominent depolarization of MC/TCs independent of glutamatergic and GABAergic neurotransmission. This depolarization was strongly reduced in TTX, suggesting that the major ATP response of MC/TCs is indirect and mediated via a different population of neurons by a yet unknown mechanism. By means of confocal calcium imaging we identified a population of juxtglomerular neurons selectively activated by ATP via P2Y1 receptors, possibly being the trigger of the ATP-evoked increase of neuronal activity in the OB.

However, in the presence of TTX, ATP as well as ADP still caused a small P2Y1-mediated direct inward current in MCs. Given the increase in membrane conductivity underlying the induced inward current and the negative holding potential, we conclude that purine nucleotides also seem to modulate unspecific cation channels in mitral cells.

Our results show, that purinergic signaling is present in one of the main processing units in the OB and presumably is able to modulate the processing of odor information.

Supported by the DFG (HI1288/3-1)

Passive perception of odors modulates functional activity of human brain

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Background: The perception of smell surroundings is a complex system process that initiates activation of cognitive-emotional mechanisms of psychical activity and simultaneously modulates their development due to the morpho-functional features of cerebral olfactory system cortex. This process depends on such cognitive functions as emotions, memory, semantic processes and other internal mental activities, and in turn involves virtually all rhythmogenic neural networks (theta-, alpha-, beta-bands). Based on the three-factor model of perception (attention, hedonic value, gender) we conducted a comprehensive comparative neurophysiological study of neocortical mechanisms for the effects of olfactory sensation on functional activity of human brain (using EEG).

Methods: 687 healthy volunteers (347 male and 340 female students aged 18 to 24 with no documented manifestations of rhinal pathologies) participated in this study. EEG was registered during the rest state (5 min) and under odor stimulation (5 min) with essential oils (Lemon, Melissa, Ilang-ilang, Lavender, Rosemary, Bergamot, Pine, Mint, Anise, Artemisia, Valeriana, Rose essential oils). We estimated the spectral power density (SPD) and the levels of coherence of all the frequencies from 0.2 to 35 Hz.

Results: We demonstrated that the passive perception of odors, as well as the analytical odor detection, is accompanied by the activation of cortical neural networks. It stimulates the mechanisms of cortical-hippocampal loop (theta-waves), which leads to the expansion of cortical-cortical and cortical-subcortical interactions (coherence) and modulates mental, emotional and verbal activity (theta1,2-; alpha3-; beta1,2-; gamma-bands). We believe that such effects appear due to the ability of smell perception to change excitability of brain networks (experiments with the use of photic stimulation effects). In addition, we revealed that prolonged resting states under background odoration by essential oils are accompanied by the development of a specific combination of substantial (almost generalized) increase of coherence in alpha1- and alpha-, beta1- bands. According to modern neurophysiology, this condition is described as a tonic alertness - perception of new information and readiness to react while relaxing. At the same time, increase of interfrontal coherence is described in the rest state right after the completion of external tasks. It appears only on a background the olfactory activating, unconnected with environment and anymore expressed in the case of positive attitude toward a concrete odor. Positive evaluation of smell is associated with strengthening of functional brain activity, whereas negative or aversive evaluation - with its inhibition. We also examined gender differences of brain activity under olfactory stimulation. Based on the comparative analysis of neurodynamics, we suggest that olfactory perception in humans is caused by the mental-psychological strategies of formation of introspective processes and behavior fulfillment that differ in men and women (insightful and intellectual, respectively).

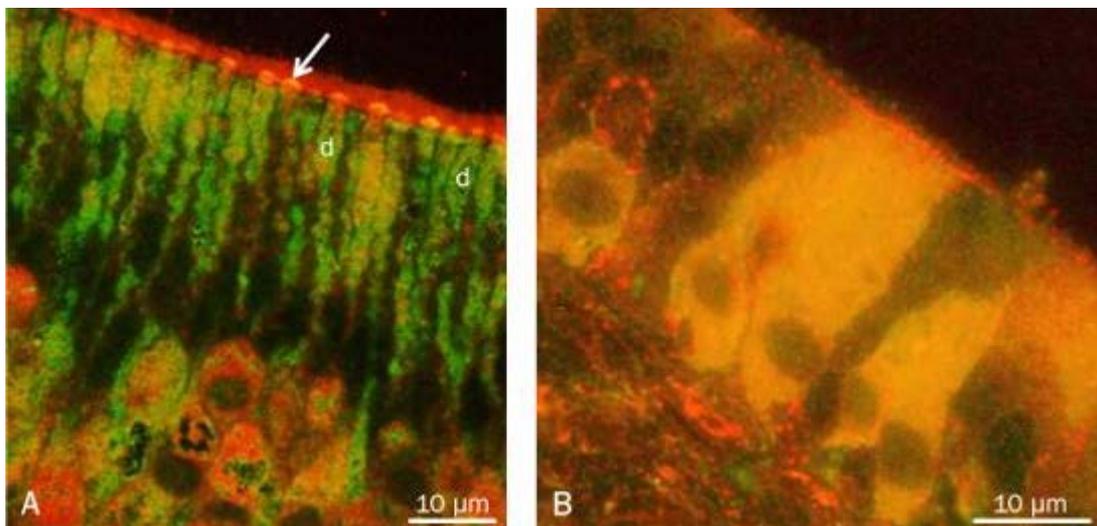
Conclusions: An important point in the structure of olfactory perception is the analysis of olfactory information in the first moment of detection, accompanied by a strong activation of cognitive memory circuits, emotions, semantic analysis, etc. At the same time, the mechanisms of "familiarity" evaluation and biological significance of the incoming sensory information turn on.

The rat vomeronasal organ is a vitamin D target

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Vitamin D3 analogues seem to play a role in olfactory communication in certain reptiles (Martin and Lopez 2006). Here we studied the expression of vitamin D receptor (VDR) and of vitamin D binding protein (DBP) in the rat vomeronasal organ (VNO). Both proteins are expressed in the sensory and non-sensory epithelium of the VNO. A portion of the sensory cells in the VNO contained nuclear and cytoplasmic immunoreactivity for VDR (Fig. A). VDR occurred also in many of the sensory microvilli. DBP was observed in the cytoplasm of sensory neurons, mostly confined to apical dendrites (Fig. A, d). Both proteins were in part colocalized. This colocalization was most pronounced in knob like protrusions of sensory dendrites (Fig. A, arrow). Electron microscopy (EM) revealed that this double staining was associated with tight junctions between sensory dendrites and supporting cells. VDR and DBP were also observed in cytoplasm of single ciliated cells within the non-sensory portion of the VNO (Fig. B). In EM images we could show that these cells had neuronal contacts similar to gustatory cells. DBP was found in some of the vomeronasal glands (VNG), indicating secretion of the binding globulin. In situ hybridization with synthetic oligonucleotide probes complementary to DBP encoding transcripts revealed specific hybridization signal in some of the sensory cells, in single cells of the non-sensory epithelium and in VNG. With RT-PCR of RNA extracts from rat VNO we were able to amplify measurable amounts of both VDR and DBP encoding transcripts. It is likely that the rat VNO is a vitamin D target. Vitamin D3 and related metabolites may have pheromone like properties in rodents.



Readout of electrical activity from calcium signals in vomeronasal sensory neurons of mice

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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 peripheral vomeronasal organ (VNO), vomeronasal sensory neurons (VSNs) translate chemosensory information into electrical activity. However, electrophysiological analysis of this activity depends on single neuron 'low-throughput' readout methods. By contrast, vomeronasal population activity can be analyzed using bulk loading of synthetic calcium indicators high-throughput imaging approaches that allow simultaneous investigation of multiple cells. While useful as a proxy for electrical VSN activity, calcium signal shape has so far not been related to specific discharge patterns in the VNO. Therefore, we asked how intracellular calcium transients in VSNs are correlated with electrical activity. To address this question, we combined single cell calcium imaging with electrophysiological recordings in acute coronal slices of the mouse VNO and performed an event coherence analysis. Targeted VSNs were filled with the calcium-sensitive ratiometric reporter fura-2 via the patch pipette. Both current-clamp and voltage-clamp protocols were performed during simultaneous electrophysiological and imaging recordings. We investigated the influence of different inter-stimulus intervals, depolarization durations and discharge frequencies on cytosolic calcium levels. Based on these data, we aim to infer electrical activity from population calcium imaging experiments to develop a tool that allows large-scale analysis of VNO electrical activity.

The v1r-related Ora receptors are expressed in a specific spatial distribution in the major olfactory Organ of *Danio rerio*

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Olfaction or the sense of smell constitutes an essential sense in most species. Animals use it to search for food, prey and mating partners, and to avoid predators. *Danio rerio* (zebrafish) emerged as an excellent model system for studying vertebrate olfaction due to several technical advantages compared to higher vertebrates. Furthermore, olfaction in zebrafish shows the same fundamental properties as mammalian olfaction, such as axonal convergence and monogenic expression. Moreover, zebrafish possess orthologs of most mammalian olfactory receptor families, to wit ORs, ORAs/V1Rs, OlfCs/V2Rs and TAARs. However, in contrast to mammals, teleost fish exhibit a single olfactory epithelium, in which all four olfactory receptor families intermingle. We have previously reported that zebrafish ORs are expressed in so-called expression domains or zones, distinctly different for different genes, albeit broadly overlapping ¹. Here we have examined how expression of the small ORA family fits into the expression patterns generated by the much larger OR family. In teleost fish the ORA family consists of six rather conserved genes, in stark contrast to the dynamically evolving mammalian V1R families ^{2,3}. The spatial distribution of ORA-expressing cells within the olfactory epithelium was analysed by quantitative in situ hybridisation. The position of each labeled cell was quantified in three dimensions, radial distance from the center of the lamella, laminar height within the lamella, and height within the olfactory epithelia. We could show that different ora genes have distinctly different expression zones inside olfactory epithelia that overlap both with each other and with those reported for or genes ¹. The results also suggest that laminar height may be regulated independently from radial position, because preferred positions in these two dimensions do not co-vary.

¹ Weth F, Nadler W, Korsching S. Nested expression domains for odorant receptors in zebrafish olfactory epithelium. *Proc Natl Acad Sci U S A*. 1996 Nov 12;93(23):13321-6.

² Saraiva LR, Korsching SI. A novel olfactory receptor gene family in teleost fish. *Genome Res*. 2007 Oct;17(10):1448-57.

³ Zapilko V, Korsching SI. Tetrapod V1R-like ora genes in an early-diverging ray-finned fish species: the canonical six ora gene repertoire of teleost fish resulted from gene loss in a larger ancestral repertoire. *BMC Genomics*. 2016 Jan 27;17:83.

Wiring and information processing in an amphibian olfactory network

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The glomerular layer of the main olfactory bulb is characterized by an array of ovoid neuropil agglomerates, the olfactory glomeruli. They represent the first relay station of olfactory information processing where the axon terminals of olfactory receptor neurons (ORNs) synapse onto the apical dendrites of mitral/tufted cells. In rodents, each glomerulus is innervated by the single, unbranched axons of a specific ORN population. Remarkably in larval and adult *Xenopus laevis* the axons of ORNs bifurcate and terminate in one or multiple anatomically distinct glomeruli. To determine the underlying wiring logic, we focus on an amino-acid sensitive olfactory subsystem in larval *Xenopus laevis*. Via fast two photon imaging and neuronal tracing, we characterized the position and chemosensory map of the amino acid responsive glomerular array as well as its innervating ORN population in the main olfactory epithelium. Single cell electroporation of those ORNs in combination with postsynaptic calcium imaging revealed that both axon terminals of a bifurcating ORN functionally innervate their respective glomeruli. In addition we found the vast majority of mitral/tufted cells to exhibit multiple apical dendrites projecting their tufts into either one or multiple anatomically distinct glomeruli. The targeting of multiple glomeruli by single neurons from both, the pre- and postsynaptic side, rules in the possibility of a yet unprecedented olfactory wiring logic. We are currently using transsynaptic tracing or a combination of single cell electroporation and two photon calcium imaging in order to understand the significance of this unique circuitry for vertebrate olfaction in general.

A challenge for a male noctuid moth? Discerning the female sex pheromone against the background of plant volatiles.

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Like many insects, males of the noctuid moth *Heliothis virescens* have to find their mating partners in order to ensure reproduction. They use olfactory cues, a species-specific pheromone blend, being released by their conspecific females. While tracking these blends, male moths are continuously confronted with a wide range of other volatiles from the environment, mostly plant compounds. Therefore, we analyzed the effect of a plant odor background on pheromone-guided flight behavior in male *H. virescens*. In order to create a more natural stimulus, we used the headspace of two host plants as background odor in our wind tunnel and tracked the male behavior while being attracted to the conspecific pheromone blend. Since the volatile emission of a plant is also dependent on the internal state we furthermore compared the pheromone attraction when presenting the headspace of both damaged and intact plants. Surprisingly, our results show that a plant odor background did not affect pheromone-guided flight behavior at all as it has been shown in previous investigations at the sensory level. As odor background we also tested single plant-emitted compounds, which were shown to be neurophysiological relevant. Adding those compounds led to a dose dependent reduction in pheromone attraction of male *H. virescens*. Using GC-MS analysis we show, that those concentrations, which had an impact on pheromone-guided flight behavior, were in a supra-natural range. We could not find those quantities in the headspace of our host plants. Hence, our results lead to the conclusion that pheromone-plant interaction in *H. virescens* might be an effect of stimulation with supra-natural plant odor concentrations, whereas under more natural conditions the olfactory system of the male moth appears to be well adapted to follow the female pheromone plume without interference from plant-emitted odors.

A second insect olfactory center

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The olfactory pathway in insects starts with the olfactory sensory neurons, mainly located on the antenna, they project into the glomeruli of the paired antennal lobe, where the signals are processed by local interneurons. From there, projection neurons send their axons in higher olfactory integration centers – the mushroom body and the lateral horn. The gustatory sensory neurons located on the antenna project to the primary gustatory center, located in the gnathal ganglion.

In *Tribolium castaneum* we selectively labeled a subset of olfactory sensory neurons in the antenna and mouthparts using a partial *Orco*-Gal4 line and analyzed their projections. This analysis, combined with RNA-sequencing, revealed that the antenna and palps contain both, large numbers of olfactory and gustatory sensory neurons. Furthermore the olfactory sensory neurons from the mouthpart project not into the antennal lobe, but in an unpaired glomerular organized area in the anterior part of the gnathal ganglion – the gnathal olfactory center. Some of the neurons pass through the gnathal olfactory center, ascend via the neck connectives and terminate ipsilaterally in an area medioventral to the AL, resembling the lobus glomerulatus. In summary, the olfactory system of *T. castaneum* seems to follow a different logic than hitherto thought for insects, including olfaction and taste dispersed to a high degree on antenna and mouthparts and a selective processing of olfactory stimuli depending on the location of the olfactory sensory neurons.

Currently we use immunohistochemistry against various neuromediators in combination with transgenic lines, antennal and mouthpart backfills, confocal laser scanning microscopy and 3D-reconstruction, to further characterize the gnathal olfactory center.

cAMP modulates response sensitivity of Olfactory Receptor Neurons in *Drosophila* larvae.

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Drosophila Odorant Receptors (ORs) constitute together with an Odorant Co-receptor (ORCO) ionotropic cation channels that, upon odorant binding, induce neuronal excitation. Additional intracellular signaling cascades have been shown to contribute to the neuronal odor response. cAMP has been proposed as a second messenger in Olfactory Receptor Neurons (ORNs), but it remains elusive how it affects the neural odor-induced response. We use *Drosophila* larva as a model system, and combine optogenetic manipulations with electrophysiological and optical recordings of neural activity and with an analysis of the animals' behavioral response. We investigate how cAMP modulates the neuronal ORN excitation, and whether this modulation is directly or indirectly dependent on the presence of ORCO.

We show that optogenetic induction of cAMP synthesis using a photosensitive adenylyl cyclase increases both the ORN firing rate and the intracellular Ca^{2+} concentration. At high light intensity the optogenetically induced increase in firing frequency is sufficient to drive a behavioral response. Optogenetic induction of cAMP synthesis in ORCO mutant larvae also causes an increase in Ca^{2+} and in the firing rate, though to a lower degree than in control animals. This lower activation is insufficient to drive a behavioral response in our experimental setup. We conclude that ORCO is not necessary for the cAMP mediated activation of ORNs, although it contributes to it, either indirectly by increasing the ORN basal activity or directly through modulation by cAMP.

Upon sustained optogenetic induction of cAMP synthesis, firing rates return to baseline within a minute, but the neuronal response to odor stimuli remains drastically reduced. We show that this reduction can be similarly induced by an adaptation to a background odor. So cAMP can modulate ORN response sensitivity to odors.

Finally, *in situ* hybridization experiments show that 5 adenylyl cyclases (ACs) are expressed in the larva dorsal organ. However downregulation of each single one of these ACs has no consequence on the odor dose-response curve. Overall our results suggest a dominant role of cAMP in modulating ORN response sensitivity in a context dependent manner, rather than mediating odor response to short and isolated odor stimulations.

Categorization of olfactory, visual and olfactory-visual compound stimuli in mushroom body output neurons

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Brains receive a tangle of multi-modal input and hold the capacity to simultaneously select adequate stimuli while ignoring others to extract the behaviorally relevant information. The resulting percept reflects a multi modal construct rather than the neural representation of single (context less) modalities. How the brain connects the environmental indentations to guide an organism's performance, at which neural level, and how the modalities interact with each other, in many cases, remained an open question.

To study the neuronal representation of the single modalities odor and light and their olfactory visual compound we use honeybees as model organisms. To receive the nectar or pollen reward from a flower honeybees, in some cases, have to dive into flowers. In that moment olfactory receptor neurons at the antennae are flooded with the odor plume. Simultaneously the ommatidia of the compound eyes are doused with visual information. The food source, therefore, is associated with an olfactory-visual compound rather than a single modality.

Multi-modal integration involves convergence of different sensory pathways at a higher brain level where individual neurons display stimulus-evoked activity to both of two modalities which may converge to be integrated. The honeybee's mushroom body (MB) represents such a higher-order integration center. Its ~170.000 Kenyon Cells (KC) are organized in layers each receiving input from a different modality. MB output is conveyed to ~400 MB extrinsic neurons (MBON). Using extra cellular multi-unit recording techniques we record from MBONs on a reliable basis. We established an experimental design allowing characterization of olfactory, visual, as well as olfactory-visual induced activity in the same MBONs. Our results show that the layered input of the MB is represented in a subpopulation of MBONs responding to either only odor (~6% of recorded MBONs) or light stimulation (~36% of recorded MBONs), whereas a substantial proportion of MBONs was sensitive to both modalities (~50% of recorded MBONs) and thus integrated olfactory-visual information across MB input layers at this level. A subpopulation of MBONs (~6%) did not respond to any of the presented stimuli. Although the majority of MBONs responded to both modalities, olfactory as well a visual induced ensemble activity clearly separates both categories (vision and light). However, the population activity did not reveal any obvious intra modal quality separation. Meaning, neither monochromatic light of different wavelength nor different single odorants were clearly separated in distinct groups at the population level. Taken together our results suggest that at the MB output categories are coded rather than single stimulus properties like identity and quality.

Supported by a grant of the German Excellence Initiative to the Graduate School of Life Sciences, University of Würzburg to MSB and the DFG Priority Program SPP 1392 (Ro1177/5-2) to WR.

***Drosophila* Kenyon Cell responses to asynchronous odorant mixtures**

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Turbulent air movements distribute natural odor plumes in complex spatiotemporal patterns. These patterns contain information about the location and the composition of the odorant's source. For example, the time courses of mixture components' concentration changes carry information about the number of different odor sources, as odorants from spatially separated sources disperse in a way that they arrive asynchronously at the nose, whereas odorants from the same source arrive simultaneously. Behavioral experiments showed that insects can use such temporal cues for odor-background segregation.

To investigate how temporally structured odorant mixtures are processed in the brain, we developed an olfactory stimulator for delivering olfactory binary mixtures with millisecond precise, independent control of the components. Employing 2-photon calcium imaging with single-cell resolution, we measured neuronal responses to synchronous and asynchronous odorant mixtures in the *Drosophila* mushroom body, a brain region for odor learning and recognition.

We found that Kenyon cells are sensitive to the relative timing between two odorants. An asynchrony of 33 ms in odorant arrival evoked both inhibitory and excitatory mixture interactions in Kenyon cell responses. These mixture interactions could not be predicted from Kenyon cells' responses to the single components and synchronous mixtures. We conclude that the *Drosophila* olfactory system transforms the relative timing between odor-evoked activity patterns in the periphery into a spatial representation in the mushroom body. The time-sensitive mixture effects on Kenyon cell activity patterns could serve as a neural basis for the analytic extraction of individual odors from background odors.

Functional Analysis of Interneurons and Projection Neurons in the Antennal Lobe Network of the American Cockroach.

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In the insect olfactory system peripheral input from olfactory sensory neurons is strongly processed in the antennal lobe, the analogue to the olfactory bulb in vertebrates. Processing in the antennal lobe is accomplished by a highly interconnected recurrent network of interneurons. This interaction in the antennal lobe shape olfactory representations, e.g. regulating the tuning profile of projection neurons. In the antennal lobe of *Periplaneta americana* we distinguish two broad classes of spiking (Type I) and non-spiking (Type II) local interneurons (Husch et al. *J Neurosci* 29: 716–726, 2009; Fusca et al. *J Neurosci* 29: 716–726, 2009). A comparably small number (125, in *P. americana*) of projection neurons represent the antennal lobe output and project to higher brain centers. This small projection neuron population encodes olfactory information with a dense spatiotemporal odor code (Krofczik et al. *Front Comput Neurosci* 2: 9, 2008). The processing in the antennal lobe network not only reduces the spatial dimension - from a high dimensional input of many thousands of input neurons to a small population of output neurons - but also processes fine temporal structure of the olfactory input within few milliseconds (Szyszka et al. *Chem Senses* 41: 379–435, 2016) to compute and construct the spatiotemporal population code of projection neurons (Krofczik et al. *Front Comput Neurosci* 2: 9, 2008).

Characterizing the functional properties of antennal lobe neurons is crucial for understanding odor response processing in this network. Here we investigate the intrinsic cell properties of projection neurons, type I and type II local interneurons using current clamp recordings. To this end we employ two complementary approaches. The traditional approach derives active and passive cell parameters from responses to constant current injections, through a wide array of hyperpolarizing and depolarizing protocols. In a model-driven approach we perform noise current injections in order to fit a generalized integrate-and-fire neuron model (Pozzorini et al. *PLOS Comput Biol* 11: e1004275, 2015) and add on to the characterization of antennal lobe neurons. Such inputs allow us to take into account the fluctuating nature of synaptic inputs. Our results indicate functionally relevant mechanistic differences for the antennal lobe neuron types - such as different spike frequency adaptation behaviors - and we anticipate this work to ground realistic models of AL neurons in the cockroach *P. Americana*.

In search for candidate pheromone receptors in the desert locust *Schistocerca gregaria*

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The desert locust *Schistocerca gregaria* is an agriculture pest insect in northern Afrika, the Middle East and parts of Asia. Locusts are unique by their behavioral plasticity to switch between a solitary and a gregarious phase. There is ample evidence indicating that locust body volatiles may play an important role in this process acting as aggregation pheromones, in particular in shaping and maintaining the gregarious phase. Previously we have identified the “sensory neurone membrane protein 1” (SNMP1) from *S. gregaria* and characterized its topographic expression in antennae. SNMP1 is considered to be a marker for pheromone-sensitive olfactory sensory neurons in several holometabolic insect species. In the present study we have screened an antennal transcriptome from the desert locust for odorant receptors and performed double fluorescent *in situ* hybridization experiments to identify receptors co-expressed with SNMP1. A co-expression with SNMP1 could be indicative for candidate pheromone receptors in *S. gregaria*. This approach led to the identification of a group of nine odorant receptors which are co-expressed with SNMP1. Scrutinizing the topographic expression in antennae, we have found by means of *in situ* hybridization, that two receptors, OR2 and OR8, are expressed in a significantly higher number of cells compared to the other members, such as OR1, OR3, OR5, OR6 and OR9. Double *in situ* hybridization experiments utilizing probes for the OR-members and the olfactory co-receptor Orco revealed that most of the receptors were expressed in olfactory sensory neurons residing in sensilla basiconica with the exception of OR3, which we found to be expressed in sensilla trichodea. The notion that in *Schistocerca gregaria* both sensilla types could be involved in pheromone detection would be in contrast to most holometabolic insects, where pheromone detection seems to be restricted mainly to sensilla trichodea. These findings indicate the presence of candidate pheromone receptors in a locust species and may help to eventually identify pheromone components involved in locust behavior, which is considered as an important step towards an efficient locust control.

This work was supported by the Deutsche Forschungsgemeinschaft.

Microcircuits of a specialized olfactory glomerulus in *Drosophila melanogaster*

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The brain is a network of neurons, generating specific behavior. Knowing the former is crucial to understand the latter. Therefore, a comprehensive account, of local circuits and their pattern of synaptic connectivity, is indispensable.

We are exploring these aspects in the first relay station of olfactory processing, the antennal lobe (AL), in *Drosophila melanogaster*. The AL consists of morphologically conserved spherical compartments, the olfactory glomeruli. Each of these compartments is innervated by incoming olfactory sensory neurons (OSNs) expressing the same receptor. The OSNs synapse onto projection neurons (PNs), modulated by local interneurons (LNs). Odor-induced activity patterns are further conveyed to higher brain centers via PNs, where the olfactory information is integrated with other sensory modalities.

Different glomeruli show distinct levels of specification, i.e. some glomeruli are innervated by narrowly tuned OSNs, while others are activated by multiple odorants. Furthermore, glomeruli are distinguishable by anatomical position, volume and their unique numerical neuronal composition in correlation with its functional specificity. Due to these facts, it can be assumed that neuronal architecture of the AL is not homogenous.

We now want to address, if a specialized glomerulus, the DA2, which is exclusively activated by the aversive odor, Geosmin, shows a unique and specific identity on the level of neuronal ultrastructure, i.e. synaptic inventory and connectivity. These results will be compared with glomeruli, showing opposing odor-activation.

To accomplish this we are developing a correlative approach, comprised of a genetically-encoded fluorescence label, user-defined fiducial two-photon laser marks and subsequent volume targeting electron microscopy (vEM), employing focus ion beam (FIB). With this approach we can specifically target the DA2 glomerulus. Currently, our focus is on AL output neurons, the PNs. To highlight this neuronal subtype, we will implement an EM-compatible mark, by inducing photoconversion of the genetically-encoded fluorescence label.

With this project we address the unique identity of glomeruli on the synaptic level in the context of the antennal network and contribute to a better understanding of the specific glomerular networks in correlation of their respective functionality.

Modification of sex pheromone responses by plant volatiles in a male moth

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Moth reproduction highly depends on communication with sex pheromones. The male's olfactory system is well tuned to the species-specific ratio of several pheromone components emitted by conspecific females, and males respond with a characteristic upwind flight behaviour. Highly specific olfactory receptor neurons (ORNs) on the male antenna detect individual pheromone components, and the primary olfactory centre in the brain, the antennal lobe, contains a specialized area treating sex pheromone information, the macroglomerular complex (MGC). Neurons within the MGC code in different ways for the different parameters of the intraspecific signal: component- and blend-specific neurons exist, and dose-dependence and temporal resolution of the responses vary.

In a natural environment, male moths encounter sex pheromone signals within a background of abundant plant volatiles. Such plant volatiles can influence male behaviour in response to sex pheromones and interfere with pheromone detection and central processing. In the male moth, *Agrotis ipsilon*, we have investigated the effects of the flower volatile heptanal on oriented flight behaviour in a wind tunnel, antennal detection by single sensillum recordings and antennal lobe processing (using intracellular recordings) of the sex pheromone blend and its components. A 1% heptanal solution masked the detection of the major pheromone component, but not of a minor component in antennal ORNs. This masking effect was also observed for pheromone blend responses in MGC neurons, in addition to increased response latency, corresponding to increased behavioural pheromone response latency in the presence of heptanal.

We show here that a plant volatile can indeed interfere with sex pheromone information and modify olfactory responses at the behavioural and neural level. Future studies will have to show now, how more complex plant signals might contribute to adaptive mechanisms of species recognition in a complex plant environment.

Odor evoked calcium signals in functional compartments of olfactory local interneurons

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In insects, olfactory information is detected from antennal olfactory sensory neurons and first relayed and processed in the antennal lobe. Here a complex network of inhibitory and excitatory local interneurons (LNs) mediates interactions between the glomerular pathways, which ultimately determines the tuning profiles of the (output) projection neurons [1]. In the antennal lobe of the cockroach *Periplaneta Americana*, we can discriminate two main LN types with distinctive physiological properties and morphological features: 1) Type I LNs, which generate sodium driven action potentials upon odor stimulation and exhibit GABA-like immunoreactivity. This type is characterized by multiglomerular heterogeneous innervations. 2) Type II LNs, in which odor stimulation evokes depolarizations but no sodium driven action potentials, since they do not express voltage dependent transient sodium currents. Accordingly, these neurons cannot trigger transmitter release by sodium driven action potentials. These LNs are omniglomerular and a defined subpopulation of them exhibit ChAT-like immunoreactivity. The distinctive morphological and physiological characteristics of different LN types imply important consequences for their computational properties and the olfactory processing that they perform. To better understand their role in processing of olfactory information we used whole-cell patch-clamp recordings combined with calcium imaging to analyze the odor evoked calcium dynamics in the different LN types. Type I LNs express different branching patterns in different glomeruli suggesting a polar organization with defined input and output regions. Accordingly, the synaptic input from a defined 'receptive field' (e.g., one or a few glomeruli) would be integrated into action potential firing. These action potentials would spread to other innervated glomeruli and provide a defined array of glomeruli with synaptic input. This is reflected in identical glomerular tuning curves of every imaged glomerulus of a type I LN. In this model, glomeruli could interact independently of their distance: not only nearest-neighbor glomeruli could interact, but also glomeruli that are distributed throughout the entire antennal lobe. Type II LNs have very similar branching patterns in all glomeruli, suggesting that they can receive synaptic input from all innervated glomeruli. However, during odor stimulation synaptic input will be typically restricted to a few glomeruli, in which graded postsynaptic potentials will be generated. These potentials will spread only within the same glomerulus or to glomeruli that are electrotonically close to the stimulated glomerulus. Accordingly, glomeruli of a type II LN show individual odor specific tuning curves. Olfactory neurons including LNs, uPNs, and sensory neurons are known to express GABA receptors. To investigate the contribution of GABAergic input to the glomerular odor representation, the effect of a GABA_A- and GABA_B-receptor blocker was analyzed.

[1] Hindennach S, Fusca D, Nörling D, Kloppenburg P, Nawrot MP (this volume) Spatial and Temporal Aspects of Olfactory Computation in the Cockroach Antennal Lobe Network

Olfactory sensory neurons use temporal dynamics to encode odor identity

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Olfactory sensory systems are able to encode enormous numbers of olfactory stimuli with relatively few olfactory sensory neurons (OSN). The high coding capacity originates from the combinatorial nature of the olfactory code where odor identity is encoded in patterns of distributed activity across OSNs. Here we show that additional information about odorant identity resides in the complex temporal time-courses of OSN responses.

We conducted a comprehensive analysis of the temporal structure of responses that a set of 99 odorants elicits from eight classes of *Drosophila* OSNs. We find that response dynamics are diverse, exhibiting excitatory, inhibitory and biphasic responses. The type of response depends on the combination of odorant and OSN tested. We show that a classifying algorithm improves in performance when trained on dynamical response information instead of response strength alone, demonstrating that odorant identity information resides in the temporal structure of OSN responses. Furthermore we analyzed responses to odorant mixtures on the single OSN level and across the ensemble of OSNs and find that the response dynamics of a mixture response can be different from the individual components responses.

In summary our data shows that information about odorant identity resides in the temporal structure of OSN responses and thus is potentially able to contribute to the coding capacity of olfactory sensory systems.

Postmetamorphic plasticity of the mushroom bodies

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With its fully annotated genome, the susceptibility for reverse genetics based upon RNA interference (RNAi) and relative longevity (up to 2-3 years), *Tribolium castaneum* is best suited to study the development and plasticity of the nervous system. While plasticity can be provided by various mechanisms, we focus on ongoing cell proliferation in the adult brain. It is well established that neurogenesis persists in the mushroom bodies (MB) of adult insects, including the beetle *T. castaneum* where neuroblasts giving birth to MB Kenyon-cells remain active after adult eclosion. To label cell proliferation in adult *T. castaneum* we successfully combined the 5-ethyl-2'-deoxyuridine (EdU) with immunohistochemistry against the glia-cell marker reversed-polarity and the use of transgenic lines expressing neuronspecific markers. We reliably labeled the progenies of the adult persisting mushroom body neuroblasts, determined their identity and counted the newborn Kenyon cells within the first week after adult eclosion to determine the proliferation rate.

In several studies it was proposed that newborn neurons of MBs may play a role during olfactory processing and learning. To address the question whether adult proliferation of Kenyon cells depends on olfactory input, we used two approaches. First, we enriched the environment of group-reared beetles during their adult life with the food related odor cis-3-hexenol (leaf alcohol). Secondly, we repeated those stimulation experiments, but knocked down the common odorant co-receptor (Orco) by systemic RNAi, resulting in beetles anosmic to the leaf alcohol.

Our data suggest at least two proliferation phases in the early adult. A first phase, direct after adult eclosion that lasts for 3 to 4 days, seems to be independent from olfactory stimulation. In contrast, a second phase that directly follows the first phase seems to depend on olfactory stimulation.

Post-stimulus activity in the olfactory pathway of *Drosophila*

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Animals are able to link immediate positive or negative experiences with stimuli that lie in the past. This time-bridging associative ability requires a stimulus trace, i.e. information about the stimulus that persists after its offset. Stimulus traces have 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 an odor trace in insect olfactory systems which can last for several seconds. However, the neural substrate of this odor trace 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*. Using *in vivo* calcium imaging we measured response patterns in consecutive processing stages: in olfactory receptor neurons (using the Orco driver line) and projection neurons (GH146 line) in the glomeruli of the antennal lobe, in projection neuron somata, and in Kenyon cell (OK107 line) dendrites and somata (in mushroom body calyces). In the antennal lobe, receptor neurons and projection neurons responded to odors with combinatorial response patterns of activated and inhibited glomeruli, as previously described (see also <http://neuro.uni.kn/DoOR>). After odor offset, the activity patterns turned into prolonged post-odor activity patterns, which were dissimilar to the initial odor response, but still odor specific. These post-odor activity patterns were invariant to changes in stimulus length. The somatic responses of projection neurons and Kenyon cells showed odor specific activation patterns during the odor stimulus and prolonged calcium activity after odor offset. In particular, a distributed subpopulation of Kenyon cell somata kept high similarity of post-odor activity with the preceding odor response. These results suggest that neurons of the olfactory pathway exhibit ongoing odor specific calcium activity, which could serve as neural substrate for an odor trace.

Putative odorant receptors in the desert locust *Schistocerca gregaria*

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The desert locust (*Schistocerca gregaria*) can exist in two phases, in a solitary or in a gregarious phase. The latter forms swarms of billions of animals under given environmental conditions leading to massive destruction of crop yields. In both phases, the behavior of desert locusts is largely directed by olfactory cues. However, little is known about the molecular processes underlying odorant detection in this insect species. In locusts, similar to other insects, odorants are detected by specialized olfactory sensory neurons (OSNs) which project their apical dendrites into hair-like structures (sensilla) that are located on their main olfactory appendages, the antennae and the maxillary palps. In general, the responsiveness of insect OSNs is determined by specific odorant receptors (ORs) residing in the dendritic membranes of OSNs. Due to the key role of ORs in the initial step of odor recognition and their proposed potential as possible targets for novel agents to control insects, we have set out to investigate the OR repertoire of the desert locust in the gregarious phase. By sequencing the antennal transcriptome and subsequent bioinformatical analyses, a larger number of nucleotide sequences encoding 119 putative ORs from *Schistocerca gregaria* (SgreORs) were identified. A phylogenetic analysis of SgreOR proteins and the recently identified ORs from the related locust species *Locusta migratoria* revealed several distinct OR groups of different sizes comprising sequences from both species with paralogous and orthologous relationships. In situ-hybridization approaches with selected SgreORs demonstrated that they are indeed expressed in distinct subsets of OSNs. Investigating the tissue specificity by PCR experiments showed for most of the SgreORs tested a selective expression in the antennae. For some OR types, however, an additional expression in the maxillary and labial palps was found.

Taken together, these data indicate that desert locusts express a large repertoire of OR types in distinct populations of OSNs that would allow for the recognition of numerous behaviorally relevant volatiles originating from the environment.

Role of sensory neuron membrane protein 1 (SNMP1) in pheromone detection of *Heliothis virescens*

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Female moths release species-specific sex pheromone blends to attract potential mating partners over long distances. On the male antennae, specialized hair-like structures (sensilla trichodea) house olfactory receptor neurons (ORNs) and glial-like support cells expressing the key proteins that enable an accurate as well as sensitive detection of distinct pheromone components. Pheromone molecules enter the sensillum through pores in the cuticle and are thought to be transported towards specific pheromone receptors (PRs) in the ORN dendrite by pheromone binding proteins (PBPs) which are secreted by support cells into the sensillum lymph.

Data we have collected for the tobacco budworm, *Heliothis virescens*, and other moth species indicate that the sensitive and specific recognition of pheromone molecules is based on a cooperative interaction of PRs and PBPs. In this process, “sensory neuron membrane protein 1” (SNMP1) may also play a crucial role by operating as a co-receptor for docking PBP/pheromone complexes and/or by contributing to the pheromone transfer to PRs. Findings from studies addressing the specific function of SNMP1 in the detection of sex pheromone components in *Heliothis virescens* will be presented.

This work was supported by the Deutsche Forschungsgemeinschaft.

Sensilla specific and cell type specific expression of odorant binding proteins

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Olfactory sensory neurons in the sensilla of insect antennae are enveloped by auxiliary cells, which produce and secrete odorant binding proteins (OBP). OBPs are supposed to facilitate the transfer of hydrophobic odorant molecules through the sensillum lymph and to contribute to the specific and sensitive reaction of the chemosensory neurons to odor stimulation. Whereas most of our knowledge is based on studies on moths and flies, very little is known about the cellular architecture of olfactory sensory neurons and their auxiliary cells in other insect species. Therefore, we have begun to characterize the OBP-expressing auxiliary cells in the sensilla of the desert locust *Schistocerca gregaria*, a representative of the Orthoptera species. As a first step, we identified 10 classical OBPs based on the 6 conserved cysteine residues as a characteristic hallmark. By means of *in situ* hybridization we have assessed the expression pattern for each of the OBPs. The picture emerging from the results indicates that based on characteristic morphological features, two types of OBP-expressing cells can be distinguished. The type-A cells extend large cytoplasmic processes which engulf and envelop the entire OSNs cluster of the sensillum. The type-B cells are arranged in groups of up to four cells without visible extensions. Furthermore, these two types of OBP-expressing cells were found to belong to different sensilla types. Type-A cells were found to be specifically affiliated with sensilla basiconica and sensilla trichoidea, whereas type-B cells were associated with sensilla ceoloconica and occasionally found beneath sensilla chaetica. So far, we did not observe an overlapped expression of OBPs between the two types of cells, but within the same type different OBPs are found to be either partially or not co-expressed. Our findings provide the first insight in the diversity and selective expression of locust OBPs as well as in the organization of OBP expressing cells in the complex cellular architecture in the sensilla of locust antennae.

This work was supported by the Deutsche Forschungsgemeinschaft.

Separation of different pollen types by chemotactile sensing in *Bombus terrestris* – A new method for measuring chemotactile electroantennograms

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The taste of something merges predominantly purely gustatory but to a certain extent also tactile information. When tasting food, animals rely on these gustatory cues to determine whether or not to eat this food. As food nutritional composition has enormous consequences for the survival of animals, food items should generally be tasted before they are eaten or collected for later consumption. Even though recent studies confirmed the importance of gustatory cues, only little is known about the representation of gustatory stimuli at the receptor level, let alone higher brain centers, in animals other than vertebrates. To measure the gustatory activity at the receptor level in invertebrates, we used bumblebees as a model species and combined electroantennography (EAG) recordings with a technique for chemotactile antennal stimulation in bees adapted from a method for close-range stimulation (Brandstaetter et al., 2010, Dummies versus air puffs: efficient stimulus delivery for low-volatile odors, *Chemical Senses*). The recorded EAG responses to chemotactile stimulation clearly separated volatile compounds by both compound identity and concentration, as it is found in conventional airborne EAGs. It also could be successfully applied to test the receptor activity evoked by different types of pollen. We found that two different pollen types (apple and almond) (which were readily distinguished by bumblebees in a classical conditioning task (Ruedenauer et al., 2015, How to know which food is good for you: bumblebees use taste to discriminate between different concentrations of food differing in nutrient content, *Journal of Experimental Biology*)) evoked significantly distinct neural activity already at the antennal receptor level. Our novel stimulation technique therefore enables investigation of chemotactile sensing which is highly important for assessing food nutritional quality while foraging. It can further be applied to test other chemosensory behaviors, such as mate or nest mate recognition, or to investigate whether toxic substances, e.g. in pollen, affects neuronal separation of different food types.

Spatial and Temporal Aspects of Olfactory Computation in the Cockroach Antennal Lobe Network

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Insects provide ideal model systems for studying sensory processing. The insect sensory networks consist of relatively small numbers of neurons, yet they provide efficient coding strategies that underly complex behaviors. The first stage of insect olfactory processing is the antennal lobe (AL). In this confined neuronal network high-dimensional peripheral input from olfactory sensory neurons (OSN) is processed in order to establish a spatio-temporal code in a comparably small number of output neurons (projection neurons, PN) that convey the olfactory information to central brain areas [1]. The AL network of the American cockroach (*Periplaneta americana*) has two major groups of spiking (type I) and non-spiking (type II) local interneurons (LN, see also [2-5]) that interconnect individual glomeruli, spherical structures of dense synaptic contacts.

Using whole-cell patch clamp recordings we investigate the contributions of individual neurons of different types to the olfactory computation in the AL. Two topographical regions in the AL have been identified [6]. Glomeruli in the antero-dorsal (AD) region receive input from sensilla with alcohol and terpene responsive OSNs, whereas the postero-ventral (PV) region is targeted by OSNs responsive to aldehydes, acids and amines. The rate codes of PNs arborizing within single glomeruli reflect this mapping between groups of odors and AL subregions. Pronounced inhibitory responses in PNs coincide with the responses of inhibitory type I LNs that typically consist of only a few spikes. The inhibitory odor responses in uPNs also differ in magnitude across the odor spectrum in correspondence with identity-specific responses in LNs. To analyze the dense population code individually recorded neurons were aggregated in pseudo-populations. The spatio-temporal activity patterns allow to study the temporal evolution of the odor identity code [7]. We found that odor representations in the AL rapidly stabilize shortly after odor onset, which is of behavioral relevance for the animal.

The accessibility and high quality of intracellular whole-cell patch clamp recordings from the AL network in the American cockroach support a detailed understanding of the emergence of dense information codes. Especially interneurons and their specific contribution to shaping the spatiotemporal activity patterns complement our present knowledge on sensory processing in confined microcircuits.

[1] Boeck J, Ernst KD, Sass H, Waldow U. 1984. Anatomical and physiological characteristics of individual neurones in the central antennal pathway of insects. *Journal of Insect Physiology*

[2] Fuscà D, Schachtner J, Kloppenburg P. 2015. Colocalization of allatotropin and tachykinin-related peptides with classical transmitters in physiologically distinct subtypes of olfactory local interneurons in the cockroach (*Periplaneta americana*) *Journal of Comparative Neurology*

[3] Husch A, Paehler M, Fusca D. 2009. Distinct electrophysiological properties in subtypes of nonspiking

olfactory local interneurons correlate with their cell type-specific Ca²⁺ current profiles. *Journal of Neurophysiology*

[4] Bardos V, Betkiewicz R, Deger M, Klußmann J, Radermacher J, Nawrot MP, Kloppenburg P. Functional analysis of interneurons and projection neurons in the antennal lobe network of the American cockroach. This volume

[5] Fuscà D, Pippow A, Kloppenburg P. Odor evoked calcium signals in functional compartments of olfactory local interneurons. This volume

[6] Watanabe H, Haupt SS, Nishino H, Nishikawa M, Yokohari F. 2012. Sensillum-specific, topographic projection patterns of olfactory receptor neurons in the antennal lobe of the cockroach *Periplaneta americana*. *Journal of Comparative Neurology*

[7] Krofczik S, Menzel R, and Nawrot MP 2009. Rapid odor processing in the honeybee antennal lobe network. *Frontiers in Computational Neuroscience*

Spatio-temporal activity patterns in response to colony odors in the antennal lobes of the ant *Camponotus floridanus*

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For ants, the ability to discriminate between members from their own colony (nestmates) and members from foreign colonies (non-nestmates) is important for colony cohesion and for the protection of their own colony's resources. The discrimination is based on complex mixtures of cuticular hydrocarbons (CHCs) on the body surface of each individual ant. Colonies of different species have different CHCs in their colony odors (species-specificity). The colony odors of conspecific colonies usually consist of the same CHCs, only the ratios of CHCs differ (colony-specificity). During allogrooming and trophallaxis, nestmates frequently exchange CHCs, which results in an almost uniform colony odor, still there are small differences between individuals from the same colony. Additionally, colony odors are influenced by the environment and can change over time. Accordingly, ants have to learn their own nestmate odor throughout their life. Although pattern recognition that includes complex and similar mixtures is a difficult task, ants are amazingly fast and precise in discriminating nestmates from non-nestmates.

So far, the neuronal mechanisms of nestmate recognition are not known. Similar to other odors, both nestmate and non-nestmate colony odors are represented in the antennal lobe as spatio-temporal activity patterns of glomeruli. However, based only on the spatial activity patterns, it is not possible to infer, which colony odor was presented because the nestmate odors cause high variability in activation patterns.

Here, we investigate the neuronal activity patterns elicited by different nestmate and non-nestmate colony odors within single individuals using confocal laser scanning microscopy with high temporal resolution. This allows us analyzing a possible temporal coding of colony odors and assessing the variability of activity patterns for various nestmate and non-nestmate colony odors. Highly variable activity patterns elicited by nestmate colony odors may reflect the plasticity of the olfactory system during learning to cope with changes in the colony odor over time.

Taste Reception in *Drosophila* Larvae: Cellular Architecture of the Terminal Organ

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Finding and evaluating food sources is a critical behavior all animals have to succeed in. Therefore, the sense of taste is essential because environmental chemicals have to be detected, processed and responded to. The major external taste organ of *Drosophila* larvae is the terminal organ (TO). Here, we provide a detailed analysis of TO sensilla and sensory neurons. Using volume electron microscopy, a precise three dimensional analysis of the sensory region of all 14 external TO sensilla was obtained. Though ultrastructural organization is diverse, five TO sensillar types can be distinguished: papillum, pit, spot, knob and modified papillum. Further, we investigated the expression of members of the gustatory (Gr), DEG/ENaC (Ppk) and ionotropic (Ir) receptor gene families in the sensilla's sensory neurons using the GAL4/UAS system. We were able to map these neurons to their corresponding sensilla. Function of sensilla can be inferred from the presence of relevant structural and molecular properties. Hence, gustatory function is assigned to specific sensillar types: the papilla and pits. In contrast, modified papillum, spot and knob sensilla receive different environmental stimuli like touch, CO₂ or temperature.

Temporal resolution of olfactory receptor neuron responses in *Drosophila*

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Flying insects encounter fine-scale temporal patterns of olfactory stimuli, which contain information about the distance and number of odor sources. Behavioral experiments show that insects can use temporal stimulus cues for odor detection. This implies fast and precise olfactory transduction in olfactory receptor neurons. However, previous studies suggest that insect olfactory receptor neurons have comparably slow response kinetics with minimum response latencies between 10 to 30 ms and a maximum temporal resolution between 5 to 50 Hz. This discrepancy between fast odor perception and reports on slower odor transduction motivated us to probe the temporal resolution of insect olfactory receptor neurons using single sensillum recordings in *Drosophila melanogaster*. We show that olfactory receptor neurons can respond to an odorant pulse with an action potential within 2.5 ms, and that they can follow pulsed odorant stimuli at frequencies of up to 100 Hz. These fast response kinetics enable olfactory receptor neurons to transmit olfactory information at high rates that occur in nature. Moreover, the fast odor transduction and high temporal resolution of olfactory receptor neurons have implications for current models of odor transduction and neural coding of odor information.

The molecular basis of olfaction in the leaf-cutting ant *Atta vollenweideri*

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Leaf-cutting ants form large societies that have huge ecological impact. By harvesting leaves to cultivate fungus in their enormous underground nests they compete with other herbivores in the neotropics and contribute to landscape structuring. Their biological success is based on their complex social organization, involving millions of workers of different size (polymorphism) and size-related division of labor with workers performing different tasks (alloethism). Leaf-cutting ants of the genus *Atta* have the most elaborate and derived division of labor among social insects. The maintenance of this highly elaborate social organization inside the colony and on the foraging trails relies mainly on olfactory communication using different pheromones. Remarkably, leaf-cutting ants show neuroanatomically distinct phenotypes in olfactory centers that relate to distinct sizes and behavior. This makes them excellent organisms to study the molecular basis underlying the differences in neuroanatomical organization and subsequently, behavioral variation.

Despite the increasing information available about social insect genomes, the genetic basis underlying olfactory-guided behavior in ants remains poorly understood. How the olfactory system of ants, with exquisite sensitivity and specificity, evolved and functions is still an open question.

Here, we will present the results of an analysis of differential gene expression by RNAseq for caste- and subcaste-specific olfactory-related genes that might be directly related with pheromone detection. We currently analyze the cellular expression pattern in the main olfactory organ, the antennae, of the different candidate genes putatively involved in pheromone reception, using Fluorescence In Situ Hybridization (FISH).

Our study aims to reveal the molecular basis of the extreme sensitivity and specificity of a highly evolved chemosensory system. Furthermore, comparative analysis of genomic data will provide information on the evolution of pheromone communication system in social insects.

The Ol₁mpiad, or: Behavioural faculties of stage 1 *Drosophila* larvae

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Mapping brain function to brain structure is a fundamental task for neuroscience. For such an endeavour, the *Drosophila* larva is simple enough to be tractable, yet complex enough to be interesting. It features about 10,000 neurons and is capable of various taxes, kineses, and Pavlovian conditioning. All its neurons are currently being mapped into a light-microscopical atlas, and Gal4 strains are being generated to experimentally access them one at a time. In addition, an electron microscopic reconstruction of its nervous system seems within reach. Notably, this EM-based connectome is drafted for stage 1 larvae - because they are 10 times smaller than stage 3. However, most behaviour analyses were performed for stage 3 larvae – as their larger size makes it easier to handle them. It is therefore warranted to either redo the EM reconstruction for stage 3 larvae, or to survey the behavioural faculties of stage 1 larvae. We opted for the latter.

We probe stage 1 *Drosophila* larvae for 'free' locomotion, feeding, responsiveness to substrate vibration, gentle and nociceptive touch, burrowing, olfactory and thermotaxis, gustatory choice, and odour-taste as well as light-electric shock associative learning. Expectedly stage 1 larvae show lower scores in most tasks, arguably because of their smaller size and lower speed. Qualitatively, however, stage 1 larvae perform strikingly similar to stage 3 in almost all of these tasks, excepting salt-related behaviour and light-electric shock learning. Collectively, our results bolster confidence in mapping brain structure and behaviour across larval stages.

The role of additional chemosensory organs in the terrestrial hermit crab *Coenobita clypeatus*

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Crustaceans are successful inhabitants of very diverse environments. Originating from the sea they also colonized fresh- and brackish water. At least five crustacean lineages succeeded in the transition from aquatic to terrestrial lifestyles, among them the Coenobitidae. The transition has exacted adaptations in the chemosensory system, for instance to prevent desiccation and to detect volatile chemicals.

The olfactory input is received by specialized sensilla on the first antenna (antennules), called the aesthetascs. The information is forwarded to the primary olfactory center by axons of the olfactory sensory neurons (OSNs). This neuropil, the olfactory lobe (OL) is highly increased in size in terrestrial hermit crabs as compared to their aquatic relatives and consists of more than 1000 glomeruli-like structures. This enlargement might be one of the reasons why most of the research on terrestrial hermit crab olfaction has been focused on the OL and the antennules.

Coenobita clypeatus (Anomura, Coenobitidae) that lost their antennules are still able to find odor sources. This response has to be mediated by other chemosensory sensilla on other body parts. Multimodal sensilla on the crab's second antenna (Antenna), for example, are able to perceive volatile chemical stimuli. RNA-seq results and PCR-based methods showed that members of an olfactory receptor family, the variant ionotropic receptor family, are indeed expressed outside the antennules.

In our study we combine morphological, electrophysiological and molecular techniques with behavioral studies conducted in a crab wind tunnel to evaluate the impact of different appendages of the crab and to investigate the strategies the crab uses to locate an odor source.

This study is supported by the Max Planck Society and the DFG (HA2540/13-1, HA5871/5-1).

There is no evidence for an Orco-based ionotropic pheromone transduction mechanism in the hawkmoth *Manduca sexta*

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Insect odorant receptors (ORs) are 7-transmembrane proteins with inverted membrane topology. They heteromerize with Orco (olfactory receptor coreceptor) *in vitro* and *in vivo*. Orco serves as a "chaperon" that locates and maintains ORs in the ciliary membranes of olfactory receptor neurons (ORNs). Since deletion of Orco depletes ORs from the cilia of fruitfly ORNs Orco mutants cannot smell any OR-dependent odorants. In addition, in Orco mutants ORNs loose spontaneous action potential activity. Thus, Orco, which forms a spontaneously opening, unspecific cation channel in heterologous expression systems, serves as a pacemaker channel in *Drosophila* ORNs, controlling their spontaneous activity. It still is not resolved whether Orco plays also a role as OR-Orco-receptor ion channel complex for pheromone transduction *in vivo*, as suggested by heterologous expression studies.

Here, we examined the role of Orco in pheromone transduction in the hawkmoth *Manduca sexta*. Hawkmoth Orco expressed in HEK cells promotes Ca²⁺ influx which is increased dose-dependently via Orco agonist VUAA1. In addition, in Ca²⁺ imaging experiments of primary cell cultures of hawkmoth pupal antennae, containing mature ORNs that are able to respond to pheromone, VUAA1 dose-dependently promoted Ca²⁺ influx in ORNs. Furthermore, Western blots demonstrated that Orco is present in pupal antennae already before ORNs start to respond to pheromones. Thus, an OR-independent function of Orco during development is likely. *In vivo* tip-recordings of pheromone-sensitive sensilla of adult males Orco antagonist OLC15 blocked spontaneous activity as well as VUAA1 induced activity dose-dependently. Thus, Orco controls spontaneous activity as a pacemaker channel also in hawkmoth ORNs, is activated via VUAA1, and blocked via OLC15, also *in vivo*. To determine, whether Orco also plays a role for ionotropic OR-Orco-based pheromone transduction we examined whether Orco agonists and antagonists affect the first 100 ms of the phasic pheromone response. Since neither VUAA1 nor OLC15 interfered with phasic pheromone responses Orco does not open within the first 100 ms of the pheromone response, in contrast to amiloride derivatives tested. Instead, both Orco agonist and antagonists affected the late-long-lasting pheromone response that occurs a few seconds after pheromone application. Since Orco is opened via depolarizing inward currents in ORNs *in vivo* Orco is a voltage-gated spontaneously opening pacemaker channel that is further activated via pheromone-dependent depolarizations. We conclude that *M. sexta* Orco is not involved in an ionotropic pheromone transduction mechanisms *in vivo*.

[Supported by DFG grants STE531/ 20-1,2 and DFG SPP 1392 to MS.]

Towards the deorphanization of candidate pheromone receptors in the desert locust *Schistocerca gregaria*

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The desert locust (*Schistocerca gregaria*) can change progressively from a solitary to a gregarious phase. Animals in the gregarious phase are capable of forming huge swarms which tremendously threaten agricultural crops in Africa and Asia. Swarming is based on massive reproduction as well as aggregation. In desert locusts, both reproductive and aggregation behavior are supposed to involve pheromones detected by the olfactory system. So far, the molecular basis of pheromone detection in the antenna of *Schistocerca gregaria* is unknown. By RNA sequencing and bioinformatical analyses of an antennal transcriptome from *Schistocerca gregaria*, we have recently identified a larger number of nucleotide sequences which encode candidate odorant receptors (ORs). Among these receptors, a small group of nine members was found to be co-expressed with the “sensory neuron membrane protein 1” (SNMP1) which is indicative of pheromone-responsive olfactory sensory neurons (OSNs) in insects. Therefore, these receptors are considered as putative pheromone receptors.

To scrutinize whether these nine ORs are indeed pheromone receptors, the present project aims at the identification of ligands which stimulate the subsets of OSNs expressing these ORs. Towards this goal, desert locusts will be exposed to pheromones and other behaviorally relevant odorants of this species and the activity pattern(s) of OSNs expressing the putative pheromone receptors will be monitored. To visualize stimulation of the respective OR-expressing OSNs by chemical compounds, expression of immediate early genes (IEGs) will be used as a marker of neuronal activity. Currently, IEG-encoding sequences from antennal tissues of *Schistocerca gregaria* are identified and tested for their potential to serve as activity markers in OSNs expressing putative pheromone receptors in the antenna of *Schistocerca gregaria*.

Revealing the valence of single olfactory sensory channels in *Drosophila melanogaster*

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Like all animals flies experience in their environment thousands of odors. While some of them have a distinct ecological meaning (e.g. pheromones or food odors) and are detected by olfactory sensory neurons (OSNs) expressing highly specific olfactory receptors, most odors are detected by several types of OSNs. At the same time OSN types expressing widely tuned receptors can detect many different odors. By using this so-called olfactory code the fly *Drosophila melanogaster* with its about 60 different olfactory receptors can detect and probably discriminate a huge number of odorants. Apart from some dedicated lines detecting either signals of danger or potential mates the impact of most olfactory receptors on the fly's behaviour is unknown. In this study we investigate this issue using artificial activation of single OSN-channels by red light. To do so, we express the red-shifted channelrhodopsin *Chrimson* under control of single olfactory receptors in OSNs of *Drosophila melanogaster*. We analyse the flies' behaviour using the Flywalk, i.e. a behavioural paradigm in which flies that freely walk within an airstream are exposed to pulsed light. Artificial activation of OSNs that carry a positive valence leads to upwind movement (as the fly upon artificial stimulation tries to reach the odor source), while activation of OSNs carrying negative valence leads to downwind movement or freezing.

Poster Topic

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- [T20-1C](#) Neuronal Correlates of Social Representations in Freely Interacting Rats
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- [T20-3C](#) Optogenetic neuromodulation of cortical circuits underlying nociception.
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Corinna Martin
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Gaetan Lepreux, Stephan Suichi Haupt, Volker Dürr
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Johannes Strauß, Reinhard Lakes-Harlan, Nataša Stritih

An fMRI study of central effects of peripheral nerve injury-induced neuropathic pain in mice

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Introduction:

Neuropathic pain has been described as the “most terrible of all tortures which nerve wound may inflict” [1]. A peripheral nerve injury often leads to the development of persistent neuropathic pain, which is characterized by spontaneous especially burning pain, allodynia (pain responses to non-noxious stimuli) and hyperalgesia (exaggerated pain responses to noxious stimuli) [2].

Methods:

The effects of the chronic constriction injury (CCI) model on central nociceptive processing in mice were imaged using BOLD fMRI (4.7T Bruker Biospec, 200mT gradient system, quadrature head coil, GE EPI sequence [TE_{eff} 25.035ms; TR 2000ms; FOV 15x15mm; matrix 64x64; slice thickness 0.5mm; 22 slices axial], scan duration 75min). Experiments were performed on male C57BL/6-wildtype mice weighing 25±4g. Under anesthesia, an approximately 1cm long incision was made into the skin, located in the area between the gluteus and biceps femoris muscles of the left hind paw. While exposing the sciatic nerve, 3 ligatures were placed proximal to the sciatic trifurcation around approximately 1/3 to 1/2 the diameter of the sciatic nerve. In sham-operated mice the nerve was exposed, but not ligated. The dorsal sides of both hind paws (right and left) were stimulated with 3 repetitions of increasing temperatures (non-noxious 40°C and 45°C and noxious 50°C and 55°C). Following heat stimulation, a second fMRI measurement was performed. The plantar sides of both hind paws were stimulated with 6 sets of pneumatically operating vonFrey filaments (40g). The experiment lasted 56 days in total, with fMRI measurements on day 0 (1 day before the surgery), and day 4, 6, 8, 14, 21, 28 and 56 post op. Before every fMRI measurement, the behavioral reaction to heat stimulation (Hargreaves) and mechanical stimulation with an electronic pressure-meter test (“plantar test”) was investigated.

Results:

The partial ligation of the sciatic nerve in the mouse produced a persistent decrease of thermal (hyperalgesia) and mechanical (allodynia) behavioral thresholds over the whole 56 days. On the other hand, in sham-operated animals the threshold decreased after surgery and returned after day 14 to the baseline. These behavioral measurements demonstrate that partial ligation of the sciatic nerve in the mouse produces profound behavioral changes characteristic of a neuropathic pain state. The preliminary data of fMRI results showed a significantly decreased response to nociceptive stimuli for operated animals compared to the sham group. Reduction of both activated brain volume and BOLD amplitude could be found noxious thermal and mechanical stimuli primarily in medulla oblongata, thalamus, sensory cortex and hypothalamus. The activity in motor areas remained comparable between both groups. Therefore, we conclude that signal suppression seems to be specific for the nociceptive system.

Conclusion:

Conclusively, chronic, here neuropathic pain changes profoundly the processing of nociceptive signals in the brain. Using BOLD fMRI studying nociception in mice can lead to a better understanding of the transformation of the central processing of neuropathic pain.

References:

- [1] Mitchell S.W. Injuries of nerves and their consequences, JB Lippincott, Philadelphia, 1872.
- [2] Woolf C.J., Mannion R.J. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet* (1999) 353 1959–1964.

Cell-type and connectivity specific sub- and supra-threshold correlations of spontaneous activity in mouse layer 2/3 in vivo

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A central theory of cortical function posits that monosynaptically connected cortical neurons form functional ensembles that process and distribute more similar information than unconnected neurons (Hebb, 1949). Some evidence exists for functional specificity of connectivity, with brain slice work (Yoshimura et al., 2005) showing that groups of layer 2/3 pyramidal neurons are more likely to be connected if linked by common layer 4 input. Moreover neurons tuned to more similar visual input in vivo have been found more strongly connected in vitro (Ko et al., 2011). However whether connectivity is related to the input-induced membrane potential correlations in vivo is a fundamental but unanswered question. To address this open question we used two-photon targeted multiple (2 to 4) whole-cell patch-clamp recordings in anesthetized mice (Jouhanneau et al., 2015) and studied the correlations of sub- and supra-threshold neuronal activity of connected and unconnected neuron pairs during spontaneous activity. We recorded from both excitatory, glutamatergic pyramidal neurons (PYRs) as well as different subtypes of local GABA-ergic inhibitory interneurons (parvalbumin (PV), somatostatin (SST), and vasoactive intestinal polypeptide (VIP) expressing interneurons). Connectivity was identified by evoking single or multiple spikes in a pre-synaptic neuron via current injections and measuring the evoked excitatory or inhibitory post-synaptic potentials in the post-synaptic neurons. This approach allows us to study the correlations of sub- and supra-threshold spontaneous activity of connected and unconnected neuron pairs in a cell-type specific manner. Here we will present our preliminary analysis of how connectivity relates to correlations during spontaneous activity in vivo.

References:

- Hebb DO (1949) The organization of behavior. Oxford University Press.
- Jouhanneau J-S, Kremkow J, Dorn AL, Poulet JFA (2015) In Vivo Monosynaptic Excitatory Transmission between Layer 2 Cortical Pyramidal Neurons. *Cell Rep* 13:2098–2106.
- Ko H, Hofer SB, Pichler B, Buchanan KA, Sjöström PJ, Mrcic-Flogel TD (2011) Functional specificity of local synaptic connections in neocortical networks. *Nature* 473:87–91.
- Yoshimura Y, Dantzker JLM, Callaway EM (2005) Excitatory cortical neurons form fine-scale functional networks. *Nature* 433:868–873.

Cellular basis of motor-sensoric modulation

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An organism's capability to react to incoming stimuli from its environment is one of the fundamental qualities that define life. When stepping on a pin, for example, the inflicted tissue damage causes sensory afferents to excite central motor neurons, which in turn elicit muscular contraction and eventually removal of the harmful stimulus.

However, far from purely eliciting an adaptive response, activation of cortical motor areas can also directly bring about a change in sensory perception.

Although this motor-sensoric modulation has found clinical applications in pain therapy, the underlying circuits and mechanisms of this phenomenon are still not completely understood. In this study, we therefore aim to elucidate underlying connections first anatomically by viral-mediated bidirectional tracing experiments in transgenic mice followed by detailed immunohistochemical analysis to address neuronal subtypes.

The intricate interconnections between the sensory cortex and its immediate rostral neighbour -the motor strip- could be hypothesized to largely mediate this modulation of sensory inputs by the motor cortex. However, several additional pathways appear to play a role in fine-tuning of sensory experience.

Arising also directly from the motor cortex, prominent projections modulate the sensory thalamus, leading to both inhibition and disinhibition of its gatekeeper functionality.

Located even deeper within the brain, we observed that the descending sensory modulatory centres (e.g. the periaqueductal grey) are also modulated by the motor cortex, which thereby provides the capacity to modulate pain at the entrance-level of the central nervous system, i.e. the spinal dorsal horn.

We are currently addressing the impact of modulating motor cortex activity on pain via behavioural assays and elucidating the nature of circuits that are activated or inhibited during this process. Our experiments promise mechanistic insights into a promising clinical therapy for pain irresponsive to conventional treatment and could help refine the procedure for maximising efficacy.

Cortical oscillatory patterns during acute and chronic pain in rodents

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It remains a key challenge to find a specific signature of pain in the brain. Oscillatory patterns in the human somatosensory cortex are closely correlated to the perceived pain sensitivity. How oscillatory patterns change during ongoing pain remains however unclear.

For a longitudinal assessment of oscillatory changes during chronic pain the use of animal models is required. We used a multichannel recording approach in freely moving mice. We chronically implanted microelectrodes over the hindlimb representation area of the primary somatosensory cortex. We evaluated changes in oscillatory patterns during acute evoked as well as long lasting chronic inflammatory pain. For the induction of inflammatory pain we injected complete Freund's adjuvant (CFA) in the contralateral hindpaw. Cortical activity was recorded four days after CFA injection.

In line with the results from human studies we could observe an increase in power in the gamma range under evoked acute pain stimulation. Interestingly, preliminary results indicate that animals that underwent intraplantar CFA injection showed an increase in gamma-band oscillatory power in comparison to naïve animals in the absence of additional stimulation.

Our results suggest that pain leads to specific changes in oscillatory patterns that persist during the time of pain chronicity. We are further planning to record cortical network activity in models of neuropathic pain.

Effects of optical activation of groups of sensory neurons in the femoral chordotonal organ of *D. melanogaster*

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Terrestrial animals rely on robust and rapidly adaptable locomotion for survival. To achieve this, the neuronal networks controlling locomotion must be flexible enough to adapt to general variability in motor output and to external perturbations, such as variations in the walking surface or unforeseen obstacles. In order to produce an appropriate motor output, this process relies on the integration of both internally and externally generated sensory signals. During walking, the rhythmic motor output observed in a single leg generally arises from interplay between central pattern generators in the central nervous system and input from peripheral mechanoreceptors in the legs. In insects, much is known about the broad organization of the neural circuits controlling locomotion; however, our knowledge on the role sensory organs play in the generation of leg movements *in vivo* is still very limited.

In insects, the angle and movement of the femorotibial joint are encoded by the femoral chordotonal organ (fCO), an internal proprioceptor in the proximal femur. Functional studies during walking are limited. In order to elucidate the functions of primary sensory neurons within this organ, we identified eight transgenic Gal4 drivers in *D. melanogaster* that target specific neuronal subsets localized within the fCO. We first analyzed the distribution of the sensory neurons in the fCO and their projection patterns in the VNC. We then tested for potential influences of fCO sensory neurons on tibial muscle activity by optogenetic activation of these subsets of neurons in the quiescent fruit fly while monitoring tibia position. Importantly, this fictive stretching or relaxation of the fCO in different Gal4 lines had different influences on reflexive tibia muscle activity (e.g., generating flexion or extension of the tibia). Further experiments will be performed to investigate how their activity affects motor neuron activity and, ultimately, inter-leg coordination during locomotion. This will eventually lead to a more detailed understanding of the roles of subsets of neurons within peripheral mechanosensors as well as of sensorimotor integration in locomotion.

This work is supported by DFG-RTG 1960.

Endoscopic in vivo imaging of thalamic neuronal ensembles mediating cortico-thalamo-cortical communication

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The coherent activity of distinct neuronal ensembles likely forms the basis for sensorimotor integration. We hypothesize that this integration exists in the network linking primary and secondary cortices through the thalamus. To test this, we devised a protocol capable of activating the primary somatosensory cortex (S1) through a physiological stimulus or the creation of an artificial neuronal ensemble in layer 5B (L5B). The corresponding activity patterns of the relaying ensemble in the thalamic posteromedial nucleus (POm) will be recorded in anesthetized or awake mice. To achieve this, we established the implantation of GRIN lenses at the POm nucleus in conjunction with viral expression of genetically encoded calcium indicators (GCaMP6 variants). We use epifluorescence (Inscopix nVistaHD) and 2-photon imaging to determine the activity patterns of thalamic neuronal ensembles. These activity patterns are likely to be defined by L5B to relay cell connectivity, because the synapse connecting L5B neurons with thalamic relay neurons in POm acts as a driver synapse able to generate action potentials in POm relay neurons. The unusual delayer and amplifier properties of this synapse could be instrumental in recruiting single neurons into thalamic ensembles. Selective genetic perturbations of this synapse will help to delineate the function of cortico-thalamo-cortical (CTC) loops/ensembles. As a perspective, this approach will allow addressing the behavioral function of the higher order thalamus conferred by neuronal ensembles of the CTC network.

Epileptiform activity in the CNS of decapod Crustaceans following treatment with electrical current (electric stunning)

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Arthropods have often been investigated using electrophysiological methods. In our approach we implanted electrodes into the CNS of decapod crustaceans to facilitate measurements of the arousal status of experimental animals as well as the response to external stimuli like e.g. a touch. As extracellular hook electrodes we used teflon coated steel wires (75 μ M), which are stable and allowed measurements with a good signal to noise ratio. The implanted electrodes allowed the animals to move freely in the aquarium and therefore an investigation of the nervous system activity during different treatments. The summation potentials were analyzed using the fast fourier transformation (FFT), which allowed to compare the resulting power spectra, regarding frequency content and amplitudes of signals during behavior. One treatment was electrical stunning between two electrodes, which lead to a paralysis (of the musculature) and to a very strong epileptiform-like activity within the CNS, which overlies incoming signals from external stimuli. The activity of the nervous system declined gradually during this rhythmic activity of the CNS. After a nearly complete decline, the nervous system activity started to recover until after 18h or more the response to external stimuli and the behavior (fully?) returned back to normal behavior as well as CNS activity. This method allows the measurement of the arousal status of the animals by comparing the FFTs during specific time intervals during treatments.



Evaluation of the effects of three kynurenic acid analogues on the neuronal nitrogen oxide synthase levels in the nitroglycerin model of migraine

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Migraine is a common neurological disorder with high prevalence and an unknown pathomechanism. The systemic administration of nitroglycerin (NTG) is a widely used model of migraine headache both in human subjects and in animals. The nitrogen monoxide released from NTG is able to induce an immediate headache in healthy subjects and in patients, but in migraineurs a delayed migraine-like headache attack also occurs.

In rats, NTG administration activates the trigeminal system, which is demonstrated by increased c-Fos protein expression in the caudal trigeminal nucleus. Furthermore, the level of neuronal nitrogen oxide synthase (nNOS) is also increased in the same area after NTG administration, suggesting the occurrence of a self-amplifying process, which could be related to central sensitization. In this activation and sensitization process of the trigeminal system glutamate neurotransmission plays a key role. Kynurenic acid is an endogenous ionotropic glutamate receptor antagonist, with potential to decrease the activation and sensitization of the trigeminal system. Previously two analogues of kynurenic acid proven to be effective in the NTG model, and based on their promising effects novel molecules were developed. In our study, we examined the effects of these new analogues, SZR104, SZR105 and SZR106 on nNOS expression by Western blotting in the NTG model. Male SPRD rats between 250-300 g were used. The first group received physiological saline (p.s.) intraperitoneally (i.p.) as vehicle for the analogues, the second, third and fourth group received the analogues SZR104, SZR105 and SZR106 dissolved in saline (final volume 1.5 mL, concentrations 1 mmol/kg, 0.5 mmol/kg, 0.5 mmol/kg respectively). One hour later every group was halved, one half receiving p.s. as a vehicle for NTG, and the other NTG i.p. (10 mg/kg, from undiluted 1mg/mL Nitropohl infusion). Four hours later the animals were deeply anesthetised, perfused with ice cold phosphate buffered saline, and the dorsal horns of the medulla and upper cervical spinal cords were removed for Western blot detection of nNOS and β -actin as loading control. nNOS specific bands were detected at 155 kDa, while β -actin at 42 kDa. The analogues were not able to reduce the nNOS protein level elevation induced by NTG administration. However they showed a tendency to even raise the level of nNOS in the absence of NTG.

These analogues have a completely new chemical structure, and were selected based on in vitro electrophysiological findings suggesting their direct neuronal effects. The exact mechanism of their action is not known, but our results suggest that they are not effective in reducing central sensitization of the trigeminal system. However the possible antinociceptive and behavioural effects of these compounds will be the subject of further investigations.

Excitability of dorsal root ganglia neurons in response to oxidized phospholipids

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In inflammation, leukocytes produce reactive oxygen species, which oxidize phospholipids (OxPL). We recently showed that intraplantar injection of the OxPL 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OxPAPC) into rat paws induces persistent hyperalgesia, possibly due to peripheral sensitization of nociceptors for inflammatory mediators. In murine dorsal root ganglion neurons (DRG), OxPAPC elicits calcium influx through the non-selective cation channels transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1).

Here, we asked whether OxPAPC activates intracellular signalling pathways, increases the excitability DRG neurons and hereby induces a higher frequency of spontaneous calcium transients in DRG neurons. Secondly, we raised the question whether OxPAPC sensitizes DRGs for inflammatory mediators.

Murine DRG neurons were cultivated for 24 h in presence of nerve growth factor. Indirect immunofluorescence labelling was performed against TRPV1, filamentous actin, the neuronal marker III-tubulin and the somatodendritic marker Map2. Confocal imaging showed a high abundance of TRPV1 in the somatodendritic area of DRGs, but also localized TRPV1 to axonal growth cones. This suggested that OxPAPC may be able to increase the excitability of DRG neurons by local action on growth cones. To answer this question, we performed calcium imaging with high spatial and temporal (10 Hz) resolution using the high-affinity calcium indicator Oregon Green BAPTA-1. Under control conditions, DRG neurons showed spontaneous, spike-like global calcium transients, but also local calcium signals in soma, axons and growth cones (Figure 1). DRGs pre-treated with OxPAPC (10 μ M) for 1 h showed frequencies of spontaneous calcium transients in growth cones comparable to controls, indicating that OxPAPC does not increase long-term excitability.

Next, DRG neurons were pre-treated with OxPAPC and then stimulated with a mixture of pro-inflammatory mediators (IS, 50 nM bradykinin, 1 μ M histamine, 500 nM prostaglandin E2) or forskolin (10 μ M), a cell-permeable activator of adenylyl cyclase. These agonists induced different patterns of calcium signals. Notably, forskolin-stimulated DRG neurons showed higher responsiveness when pre-treated with OxPAPC, suggesting that OxPAPC sensitizes DRG neurons for other mediators.

In conclusion we found localized, spontaneous calcium transients in growth cones of DRG neurons, indicating that the growth cone structure might serve as a model to investigate local signalling effects in nociceptors. Furthermore, we show that long-term treatment of DRG neurons with OxPAPC sensitizes DRG neurons for forskolin similar to the observed in vivo hyperalgesia.

This work was supported by the Graduate School of Life Science, University of Wuerzburg (GSLs) and the Interdisciplinary Centre for Clinical Research, Wuerzburg (IZKF, project N-261).

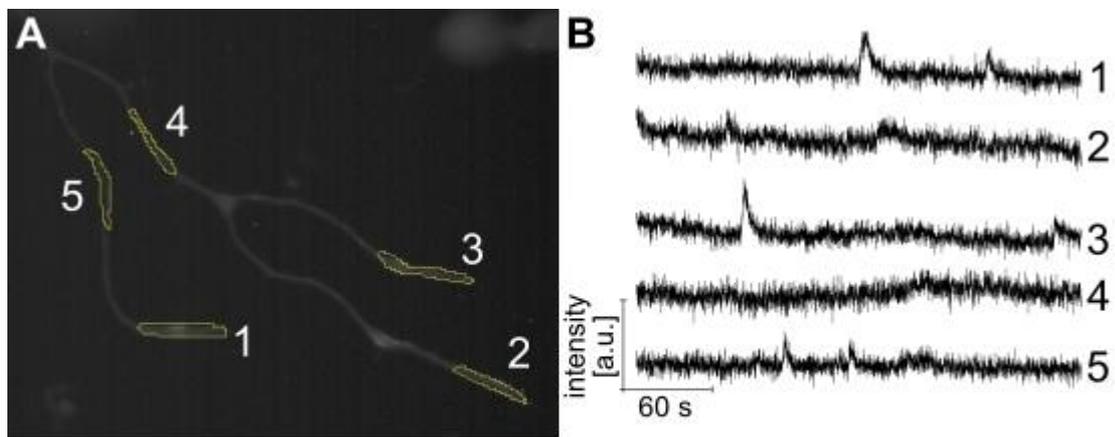


Figure 1. Highly resolved calcium imaging recording showing neurites and axonal growth cones of a cultivated DRG neuron (A). Local calcium signals in neurites and axonal growth cones (B). The graphs labelled 1 to 5 correspond to marked regions of interest in A.

Functional magnet resonance tomography in $\text{Na}_V1.8$ -deficient mice upon cold and heat noxious stimulation: An investigation of activity and connectivity changes in central projection areas driven by the sensory neuron sodium channel $\text{Na}_V1.8$.

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Pain fulfils a protection and warning function for the living organism against noxious stimuli. Evolutionary, protective behaviour requires that nociceptive processing also works not only for heat at high but also at low temperatures. The tetrodotoxin-resistant voltage-gated sodium channel subtype $\text{Na}_V1.8$ is expressed in the peripheral nervous system in the majority of primary afferent nociceptive C-fibers and is essential for the detection of noxious cold signaling. In our recent behavioural and electrophysiological studies on $\text{Na}_V1.8$ deficient mice the tetrodotoxin-resistant voltage-gated sodium channel $\text{Na}_V1.8$ proved to play an essential role for the sustained excitability of peripheral nociceptors in cold states.

In the present study we aimed at analyzing and visualizing genetically driven alterations and the cerebral components of cold versus heat nociceptive signaling in $\text{Na}_V1.8^{-/-}$ in comparison with WT mice by utilizing functional magnetic resonance imaging (fMRI). By using this approach we were able to identify brain structures where noxious cold and/or heat signals are decoded. We applied cold (0-20°C) or heat stimuli (40-55°C) to the dorsal surface of the right hindpaw of these mice via Peltier elements inside a 4.7T Bruker BIOSPEC MRI scanner acting with an optimized data acquisition and analysis scheme.

Using BOLD functional magnetic resonance imaging pronounced differences were observed between both genotypes and stimulation conditions (cold and heat). Differences between $\text{Na}_V1.8^{-/-}$ and WT mice were found for cold sensation especially at 5 °C and 10 °C manifesting in significantly altered functional activity patterns (determined from the functional data set by a second order group statistics) as well as significantly reduced activated volumes and BOLD signal amplitudes in central brain structures of $\text{Na}_V1.8^{-/-}$ mice. These structures included for example parts of the thalamus as well as limbic output structures, sensory input structures, parts of the sensory and association cortex including the cingulate cortex. In contrast, differences of noxious heat processing were less pronounced.

Functional connectivity analysis also showed that dramatic alterations in the sensation of noxious cold stimulation can be detected and clearly reduced interactions between certain brain structures can be found in $\text{Na}_V1.8^{-/-}$ mice compared with the WT: I.e. brain structures belonging to the thalamus, the limbic system (Hippocampus, amygdala), the somatosensory and association cortex as well as structures belonging to the sensory input (e.g. colliculi, PTA). Concerning heat stimulation $\text{Na}_V1.8^{-/-}$ mice showed qualitatively quite the same functional connectivity pattern and consequently less prominent differences in connectivity. These facts show that $\text{Na}_V1.8$ is not only relevant for cold but also for heat pain

processing, albeit to a quite lower extent. We conclude, that the altered phenotype of $\text{Na}_V1.8^{-/-}$ mice and the fact that $\text{Na}_V1.8^{-/-}$ mice do not perceive nociceptive aspects of strong cooling in contrast to their WT littermates seems not only to be an expression of a just peripheral phenomenon going along with diminished peripheral transmission, but also as an effect of the upstream processing with reduced afferent input leading to altered subsequent nociceptive processing in the central nervous system and consequently altered connectivity between pain-relevant brain structures combined with a reorganization of the response to cold noxious stimuli.

Infrared motion detection in the brainstem of rattlesnakes (*Crotalus atrox*)

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Pitvipers (*Crotalinae*) have a specialized sensory system to detect infrared radiation (IR). The sensory periphery consists of bilateral pit-organs located at the upper jaw of the animals' head. Each pit-organ is innervated by trigeminal nerve branches, which ramify further into smaller bundles that terminate in a topographic manner within the pit. Following transduction of IR into spike discharge, the IR-information is transmitted to the nucleus of the lateral descending trigeminal tract (LTTD) in the dorsal hindbrain. Previous studies have found evidence for a spatial contrast enhancement for IR-information in this nucleus, comparable to the lateral inhibition in the retina; the underlying cellular mechanism is however unknown so far. Here, we used intracellular in vitro recordings from second-order neurons in the LTTD in an isolated rattlesnake brain preparation. This approach allowed electrical stimulation of single or multiple, spatially adjacent trigeminal fiber bundles within the pit organ. Neighboring fiber bundles were suctioned into micropipettes and activated by either single electrical pulses or pulse trains. Intracellularly recorded responses in LTTD neurons were classified as mono- or disynaptic based on the known latency of the synaptic transmission, obtained from the delay between the presynaptic afferent and the postsynaptic second-order response components in field-potential recordings. Short-latency, monosynaptic EPSPs were present in virtually all LTTD neurons ($n = 17$), while responses that only consisted of IPSPs were less frequent ($n = 7$) and always delayed with respect to the EPSPs, indicating a disynaptic onset. However, disynaptic IPSPs, likely mediated by local inhibitory interneurons, were often superimposed on monosynaptic EPSPs ($n = 14$), thereby causing truncation of the latter. In order to simulate IR motion, up to three neighboring fiber bundles were stimulated sequentially with pulse trains applied in different temporal order. This stimulus caused activity patterns in primary afferent fibers that mimicked those expected from a moving natural IR stimulus. The spike activity in LTTD neurons revealed a range of different patterns that consisted of mixed excitatory and inhibitory response components with a gradual transition between two extremes that depended on the sequence and timing of the stimulated peripheral fiber bundles. The sequence in which multiple adjacent fiber bundles were stimulated mainly influenced the duration of the inhibitory components, particularly in spontaneously active neurons. In fact, the spontaneous activity recovered more quickly from an inhibition when the stimulus sequence of the fiber bundles mimicked a natural nasal to temporal motion compared to stimulation in the opposite direction. This suggests that during IR motion in the intact animal the activation of neighboring sensory receptors leads to a time-specific summation of the inhibition in second-order LTTD neurons, thereby causing a motion direction-dependent suppression of excitatory activity. Our study provides the first insight into the mechanism by which the LTTD processes IR motion and thus yields further evidence for a retina-like function in the rattlesnake IR system. To further understand the underlying principle of IR motion detection, subsequent approaches will use electrical stimuli that mimic different velocities of moving IR stimuli under natural conditions.

Investigating peripheral nervous system interfaces for somatosensory stimulation

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The ability to apply a graded and spatially located somatosensory feedback is still lacking in modern prosthetic devices. Somatosensation is not only required for dexterous control and the embodiment of an artificial limb, but also plays a role in emotional communication. Until recently, neural interface research for (bidirectional) prosthetic use has focused to a large extent on direct interactions with the brain. An alternative approach is to interface at the peripheral nervous system (PNS) instead. Transverse Intrafascicular Multichannel Electrodes (TIMEs) are thin film, polyimide, electrode arrays that are inserted transversally through the nerve. These allow the stimulation of individual fascicles in the peripheral nerves. To investigate if these PNS interfaces are suitable for somatosensory feedback, we implanted TIME electrodes in the medial and ulnar nerve in the upper limb of a rhesus monkey (*Macaca mulatta*) to assess if we can evoke somatosensation by electrically stimulating the nerve.

Purpose-bred macaque monkeys are trained in a somatosensory discrimination task, which allows them to perform a two-alternative forced choice task, in which they receive either vibrational or electrical stimulation to the median and ulnar side of the hand, by applying tactile vibration of the index or little finger or electrical microstimulation of the median or ulnar nerve, respectively. The animal then has to indicate which side of the hand was stimulated more intensely by pressing a left or a right button. Systematic variation of the tactile and electrical stimulation intensity then allows to obtain psychophysical response curves and to quantitatively compare the perceived strengths of both types of stimuli.

Preliminary results showed that the implantation technique of TIME electrodes in the PNS of rhesus macaques is feasible, including the placement of a subcutaneous cable and a cranial connector, without loss of hand function. Furthermore, two rhesus macaques were trained to differentiate vibrational cues applied to the two regions of the hand with frequencies between 100-200 Hz in steps of 15 Hz. Ongoing experiments also demonstrate the feasibility to obtain psychometric response curves for different tactile (vibrational) and electrical stimulation levels, thus allowing to compare the perceptual strength of both stimuli.

Electrical micro-stimulation of the PNS could therefore play an important role for future neuroprosthetics applications that need the transmission of graded sensory information, like grip force or hand aperture of a prosthetic hand, back to the brain.

Longitudinal analysis of structural and functional changes in peripheral circuits of Streptozotocin (STZ)-induced diabetic mice, mimicking the clinical symptoms of painful diabetic neuropathy

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Diabetic polyneuropathy (DPN) comprises a wide spectrum of painful (tingling, burning pain, heat hypersensitivity) but also non-painful symptoms (loss of pain perception, numbness) related to structural and functional changes of neural circuits within the peripheral and central neuronal system. Polyneuropathies such as DPN are understood primarily as a result of small-fibre dysfunction causing the pain symptoms whereas the negative symptoms with loss of function are believed to be due to loss of terminal nerve fibres. Yet, the lack of a longitudinal study and the lack of methods for non-invasive microstructural imaging of specific peripheral nerve types have limited the knowledge about the precise role of distinct microstructural alterations for the quality, intensity and temporal evolution of definite symptoms. Using an experimental mouse approach, aim of the study is to unravel (i) which precise types of sensory afferents undergo structural remodelling in DPN, (ii) how the functional excitability of distinct types of sensory afferents change over the course of DPN and (iii) what are the causal contributions of diverse types of sensory afferents to nociceptive hypersensitivity. All analyses will be conducted in close conjunction with assessment of pain-related behaviour, e.g. mechanical and thermal sensitivity. As a key method, non-invasive multiphoton-imaging is employed to longitudinally study morphological changes in peripheral nerves as well as functional changes (*in vivo* Ca²⁺ imaging) up to 1 year from onset of diabetes. Therefore, we can rely on a variety of mouse reporter lines labelling A -fibres, nociceptors, C-LTMRs and silent nociceptors. The causal structure-function relationship will be addressed by genetically silencing/ablating specific types of sensory afferents and testing impact on DPN-associated pain. Since neuropathic pain-like symptoms are thought to be mediated by A -fibres in humans and animal models, our initial analyses focus on changes in A -fibres. Of course, longitudinal structural analyses will also be conducted using the other 'fibre-reporter lines' mentioned above. In contrast to a human study, the strength of a mouse study is to provide objective and specific structural as well as functional information (via reporter lines) and to be able to image very distal sensory afferents and terminals *dynamically* at high spatial and temporal resolution. The project is of great interest as the results pave the way for novel therapies specifically addressing painful symptoms in DPN, which for many patients represent the most debilitating manifestation of DPN.

Low back pain model in mice and the impact of stress

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Low back pain (LBP) is a highly prevalent and disabling condition resulting from the complex interplay of anatomical injury and psychosocial factors. Although several injury-based preclinical models of LBP have been described, a “biopsychosocial” model mimicking the human situation is still lacking. In the present study we have established and characterized a LBP model based on the bilateral injection of the nerve growth factor (NGF) in the multifidus muscle of mice, which was recently shown to induce long-lasting hypersensitivity of dorsal horn neurons in rats [1]. We have used a portfolio of behavioural paradigms to measure stimulus-evoked and stimulus-independent pain related responses. Therefore, we have evaluated the nociceptive responses (von Frey filament stimulation, Hargreaves and cold plate tests), light touch sensitivity (adhesive tape test) and the neuromuscular function (grip strength test) at different time points after NGF injection. Our data showed that NGF-treated mice developed a long-lasting hypersensitivity to mechanical, cold and light touch stimuli, and a deficit in the grip strength compared with control PBS-injected mice. We have further characterized behavioural alterations reflecting more changes in the animal wellbeing and spontaneous ongoing pain by using longitudinal home-cage monitoring, voluntary wheel running activity and gait analysis. NGF-injected mice showed a significant decrease in wheel running activity compared to control mice but no significant alterations in gait parameters or home cage activity.

Additionally, since chronic stress is known to be an important risk factor for LBP we are currently investigating the impact of stress on the pain-related alterations, which we found in this model. For this purpose, we have applied a modified chronic unpredictable stress (CUS) procedure [2] up to ten weeks to induce a stable stress condition before the NGF injection. This combinational approach (NGF injection and CUS paradigm) has the ability to mimic the “biopsychosocial” model proposed for human LBP and will provide new insights for the study of the molecular mechanisms underlying this chronic pain condition.

Furthermore, we looked at diverse mediators and markers at the peripheral and central nervous system. Among these, we investigated the expression and activation of microglia and astrocytes and diverse neuronal marker proteins.

References

[1] Hoheisel U, Reuter R, de Freitas MF, Treede RD, Mense S. Injection of nerve growth factor into a low back muscle induces long-lasting latent hypersensitivity in rat dorsal horn neurons. *Pain*. 2013;154:1953-60

[2] Mineur YS, Belzung C, Crusio WE. Effects of unpredictable chronic mild stress on anxiety and depression-like behavior in mice. *Behavioural brain research*. 2006;175(1):43-50

Marker-less motion capture of antennal movement kinematics in honeybees and other Hymenopterans

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Bees and other Hymenopteran insects extensively use their antennae for active tactile exploration, pattern recognition and learning. To date, the honeybee (*Apis mellifera*) is the only insect species that has been investigated systematically in both non-associative motor learning and associative conditioning paradigms. In each of these paradigms, bees actively sample the ambient space with both antennae. For investigating the role of antennal movement sequences in tactile learning, a posture tracking system is needed for resolving antennal kinematics. In our earlier work, we proposed a system for automatic tracking of the antennal tip position [1]. Here, we present a method for automated and reliable marker-less posture-tracking of both antennae in Hymenopterans with geniculate antennae, honeybees in particular. The underlying idea is to use a kinematic template with three degrees of freedom of rotation (DoF) in the head-scape (HS) joint and one DoF in the scape-pedicel (SP joint), i.e., the "knee" of the geniculate structure, and to find the best posture of this template to fit the posture observed.

To this end, we captured stereo videos of spontaneous antennal movements in tethered honeybees and bumblebees (*Bombus terrestris*) under different conditions, varying the colour of light (white or red), degree of blind-folding (sighted, unilateral and bilateral coverage of the eyes) and body orientation (horizontal or vertical). Bees were fixed in metal tubes and recorded by two digital video cameras equipped with macro lenses, for periods of 60 s at 100 fps. For automated posture tracking, videos were processed by subtracting a mean image, thresholding and filtering, yielding pixel clusters that correspond to parts of the antennae. The head coordinate system and the segment lengths and root location of the template were determined by manual labeling of morphological landmarks in a single stereo-pair of frames. The tracking algorithm varied the four DoF per template, determined the resulting posture of the antenna model and calculated the correspondence between the projections of the model into both camera views and the pixel clusters of the processed video. The correspondence was optimized by a particle swarm algorithm with 8 to 64 particles that adjusted the 2x4 DoF.

So far, the precision and robustness of the algorithm was evaluated by comparison with three manually tracked videos. Mean squared errors per frame were frequently below 5 degrees in single frames, with the rms error per DoF and for the entire video ranging between 10 and 15 degrees, except the roll angle of the HS joint. The latter becomes harder to estimate the more the SP joint of the antenna is extended: in the extreme case of a fully out-stretched antenna, this roll angle is undefined. As bumblebee antennae were strongly extended more often than in honeybees, overall tracking performance was better for honeybees than for bumblebees. Moreover, bumblebees often showed prolonged episodes with no movement, in which case the mean image could contain parts of the antenna, thus preventing automated tracking. Further technical problems include posture-dependent shadows, depending on the lighting conditions, and ill-constrained angular ranges of the four DoF of the template. For example, successful tracking of bumble bee antennae required different joint angle constraints than successful honeybee tracking.

Mechanoreceptor arrangement at the antennal base helps crickets to differentiate between active and passive antennal touch

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Crickets use their antennae as tactile sense organs to actively explore the surroundings. The antennal-tactile sense enables them to overcome obstacles, discriminate surface structures, and also react to approaching predators and conspecifics. I used light and electron microscopy to systematically map the distribution of mechanoreceptors in the two antennal base segments (scape and pedicel) and traced sensory fibres in the antennal nerve to reveal the central projection patterns of different antennal sensilla and proprioceptors in the cricket brain. A total of about 600 sensilla were counted on the surface of the scape and about 250 on the pedicel. Based on their distinctive morphologies they can be classified as *sensilla campaniformia*, *basiconica*, *coeloconica* and *chaetica*. Chaetic sensilla are arranged in hair fields at the actively moveable head-scape and scape-pedicel joints to monitor the actual position and active movement of the antenna. Large and small campaniform sensilla are arranged in a band at the distal edge of the pedicel to detect deflection of the flagellum. Retrograde nerve labelling demonstrated a single mechanosensory cell innervating each chaetic and campaniform sensillum. Basiconic and coeloconic sensilla, however, have several sensory cells and appeared to be randomly scattered on both, scape and pedicel. Furthermore, anterograde and retrograde staining revealed about 40 sensory neurons forming a chordotonal organ in the ventral pedicel and 5 somata of pedicellar strand receptors in each side of the brain. The anatomical findings suggest that the specific arrangement of sensilla and internal proprioceptors at the antennal base can be used to differentiate between active and passive antennal touch.

Moderate anesthesia may promote the study of temporal coding in sensory cortices.

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Spiking neurons in sensory cortices encode stimuli by means of latency-modulation, rate coding and/or temporal patterning, such as phase-locking to stimulus periodicities. While neurons at subcortical and peripheral sites generally respond robustly with (sub-)millisecond precision, cortical responses are marked by large variability - even in primary sensory areas. It was shown that this variability is strongly correlated to the pre-stimulus level of ongoing activity that reflects non-sensory influences such as attention or expectancy. Such contextual modulation of sensory responses is crucial to the control of goal-directed behavior, but complicates studies into sensory coding at the cortical level. For rate-coding aspects, this issue may be overcome by subtracting pre-stimulus spike rates on a trial-by-trial basis. This is not an option if temporal patterns of responses matter, e.g., in frequency-coding by locking of spikes to stimulus-phase. In a recent study on encoding of whisker vibration in the rat's barrel-cortex, we obtained evidence that the reduction of ongoing activity by anesthesia reduces components of activity that otherwise blur phase-locking in the awake animal.

Spiking activity was recorded extracellularly from the barrel cortices of awake or anesthetized rats (see companion poster by Vahle-Hinz et al.). For sensory stimulation, single whiskers were deflected sinusoidally (~100 µm, 20-200 Hz, 1 s), mimicking stimulation by rough surface textures during tactile exploration. We applied a moderate, faithfully controlled general anesthesia (isoflurane; 0.9-1.5 end tidal vol%), which should suppress modulations by conscious influences such as attention or fear. On the neuronal level, this anesthesia regime reduced ongoing activity substantially, without inducing non-physiological states such as neuronal bursting activity. As a result, spike rates and the degree of temporal patterning (phase-locking) of responses were no longer correlated with pre-stimulus activity. In the awake state, where response rates were strongly correlated to the rate of pre-stimulus spiking, phase-locking strength showed a negative correlation to both pre-stimulus and response rates. This suggests that additional spikes stemmed from ongoing activity, i.e., they were 'non-sensory', a 'contextual modulation' rather than the result of recruiting more neurons or increasing the individual cell's responsiveness. We assume that the reduction of ongoing activity under anesthesia corresponded to a selective reduction of this contextual, modulating activity that blurs temporal coding in the awake animal. In conclusion, a moderate, carefully controlled level of general anesthesia can ease investigations into sensory coding not only in terms of animal handling and stimulus control, but also by reduction of non-sensory activity that can blur coding in the awake animal.

Supported by: EU (IST-2000-28127) and DFG (SCHW577/10-2; SFB936/A2/Z1; EN 533/13-1)

Neuronal Correlates of Social Representations in Freely Interacting Rats

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Social and sexual relations are key determinants of biological fitness. Knowledge about sex, kinship and sexual status of other individuals are relevant in terms of sexual contacts and other features of a relationship. For instance, the rank of an individual in a hierarchy or the personal relationship to a conspecific might affect daily habits like feeding and social interactions. Despite the importance of socio-sexual information processing, we have only very limited information, how social representations map onto the rodent forebrain. This dearth of information reflects the fact that so far only few neural recordings were obtained in interacting animals, and that it is unknown, which cortical areas carry social information. Here, we overcome those limitations and record neuronal responses in freely interacting rats. To cope with the complexity of free interactions we utilize wireless recording and multi-modal behavior surveillance of these interactions. Our recordings target the representation of genitals and the trunk in somatosensory cortex. Genital somatosensory cortex was chosen as a recording target, because receptive field properties, its topography (a phallic penis representation), microstimulation-evoked sexual behaviors and comparative data all point to a sexual function of this region. Trunk somatosensory cortex was chosen as a recording target, because of its role in social behaviors such as tickling, huddling and grooming. Preliminary data of neuronal responses to a variety of social interactions suggest that these areas are indeed activated in various social contexts. Currently we compare the neuronal responses between interactions with different sexes.

Non-visual Functions of Opsins in *Drosophila* Larval Mechanosensors

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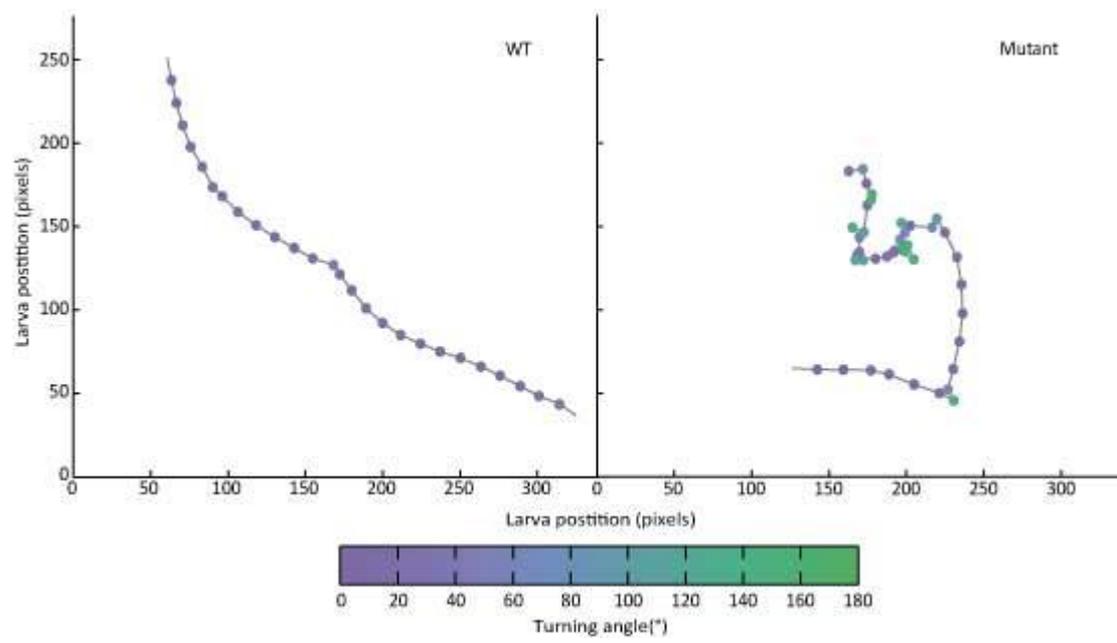
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Evidence is accumulating that opsins can sense more than light. In *Drosophila*, opsins were recently implicated in larval temperature preference behaviour and in hearing in adult flies [1,2]. Here, we report that *Drosophila* larvae also require visual opsins for locomotion, and show that the proprioceptors that control locomotion do express opsins. Opsin mutant larvae had reduced muscle contraction amplitude, reduced locomotion speeds and increased turning behaviour. When we genetically rescued the function of the respective opsin gene, normal locomotion was restored. In addition calcium responses of proprioceptors to mechanical stimuli were analysed for wild-type and mutant larvae.

Opsin-dependent locomotion defects associated with altered temperature preference behaviours and closely resembled the locomotion deficits of mutants whose chordotonal neurons are impaired. Promoter-fusions revealed that opsins are expressed in the serially arranged, proprioceptive chordotonal neurons in the larval body wall. Opsin expression was confirmed with antibodies, and chordotonal neurons seemed to be the only neurons that express opsins outside the larval eye. This suggests that larval locomotion and temperature preferences might converge on chordotonal neurons. It also strongly supports the idea that light-independent opsin functions evolutionarily predated their use as photoreceptor proteins [1].

1. Shen, W.L., Kwon, Y., Adegbola, A.A., Luo, J., Chess, A., and Montell, C., 2011. Function of Rhodopsin in Temperature Discrimination in *Drosophila*. *Science* 331, 1333–1336.
2. Senthilan, P., Piepenbrock, D., Ovezmyradov, G., Nadrowski, B., Bechstedt, S., Pauls, S., Winkler, M., Möbius, W., Howard, J., and Göpfert, M., 2012. *Drosophila* Auditory Organ Genes and Genetic Hearing Defects. *Cell* 150, 1042–1054.



Optogenetic neuromodulation of cortical circuits underlying nociception.

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Sensory cortical centres in the brain are ultimately responsible for processing and integrating nociceptive inputs from the body. Although many brain regions including the amygdala, somatosensory, anterior cingulate and insula cortices have been previously shown to be functionally active during pain perception, the crucial region(s) and types of neurons (e.g. excitatory, inhibitory) involved in modulating the nociceptive processing of specific sensory modalities (e.g. mechanical, thermal, etc.) in the brain remains unclear. For example, the anterior cingulate cortex (ACC) is consistently activated during pain, but the functional specificity of particular cingulate divisions, their roles at distinct temporal phases of central plasticity, and the underlying projection pathways involved remain unknown. Additionally, it is also unclear if distinct cortical regions (e.g. cingulate vs somatosensory cortices) play different roles in modulating nociceptive processing.

In this study, optogenetic and behavioural approaches are used to investigate the role(s) of specific cortical neurons in the processing of acute peripheral hypersensitivity in various subdomains of the cingulate cortex previously implicated in pain processing. Expression of blue light-driven channelrhodopsin 2 (ChR2; cationic channel) and/or archaerhodopsin (ArchT; proton pump) in neurons of the brains of adult C57BL6 mice was achieved via adeno-associated viruses. Light delivery into the brain regions of interest was carried out with an optic fibre inserted via a chronically implanted cannula or ferrule.

Mechanical sensitivity (von Frey test) of the hindpaws were assessed at either basal conditions or at various time periods after a peripheral capsaicin injection in the absence or presence of photo-illumination with either yellow (589nm) or blue (473nm) light. Inhibition of cortical excitation resulted in significant decreases in capsaicin-induced hypersensitivity compared to controls and that this effect was reversible upon cortical activation via ChR2 stimulation, providing direct evidence that activity in a specific population of cortical cells is critical for driving hypersensitivity in the periphery.

Order under the guise of chaos: functional neuroanatomy of the somatosensory cortex of the reeler mouse

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Layer IV (LIV) of the rodent somatosensory cortex contains the somatotopic barrel field. Barrels receive much of the sensory input to the cortex through innervation by thalamocortical axons from the ventral posteromedial nucleus. In the reeler mouse, the absence of cortical layers results in the formation of mispositioned barrel-equivalent clusters of LIV fated neurons. Although functional imaging suggests that sensory input activates the cortex, little is known about the cellular and synaptic properties of identified excitatory neurons of the reeler cortex. We examined the properties of thalamic input to spiny stellate (SpS) neurons in the reeler cortex with in vitro electrophysiology, optogenetics, and subcellular channelrhodopsin-2-assisted circuit mapping. Our results indicate that reeler SpS neurons receive direct but weakened input from the thalamus, with a dispersed spatial distribution along the somatodendritic arbor. These results further document subtle alterations in functional connectivity concomitant of absent layering in the reeler mutant. We suggest that intracortical amplification mechanisms compensate for this weakening in order to allow reliable sensory transmission to the mutant neocortex.

Organization of the isthmic system in the western-diamondback Rattlesnake (*Crotalus atrox*)

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Across the animal kingdom, diverse types of thermoreceptors are found. In reptiles, a specialized infrared (IR) sensor is present in three groups of snakes (boids, pythons and pit vipers). These snakes can perceive electromagnetic radiation in the IR-spectrum with so called pit organs. From a thin pit membrane, where sensory transduction takes place, IR signals are conveyed via branches of the trigeminal nerve to the LTTD (nucleus of the lateral descending trigeminal tract) and subsequently to the RC (nucleus reticularis caloridis) in the hindbrain. The RC projects to the contralateral optic tectum, where IR information forms a spatiotopic multimodal map with inputs from the visual system.

In vertebrates the propagation of retinal inputs from the optic tectum to higher visual areas such as the nucleus rotundus is selectively influenced by feedback signals from the isthmic system. Although the underlying neuronal network is well understood in birds and mammals, less is known about the isthmic system of reptiles. Since in IR sensitive snakes the infrared system is integrated into the visual system at the level of the optic tectum, it is hypothesized that the isthmic system also modulates IR signals conveyed to the nucleus rotundus.

We therefore used immunohistochemistry and performed neuroanatomical tracings to first investigate the general organization of the isthmic system in snakes. Injections of neurobiotin into the optic tectum revealed ipsilateral projections to an area of the midbrain, where the nucleus isthmi is located in other reptiles such as turtles and geckos. Besides axon terminals, also cell bodies were stained, hinting for a reciprocal connection of this area with the optic tectum. Additionally, cell bodies in the identical contralateral area were stained, indicating a unidirectional projection from the isthmic nuclei to the contralateral optic tectum, which has already been described in the gecko.

Injections into the area where the isthmic nuclei are suspected led to labeled projections that spread across the entire optic tectum.

To test whether subnuclei of the isthmic system can be differentiated we used immunohistochemistry (anti-ChaT, anti-Calbindin and anti-Calretinin). Anti-ChaT-stainings showed two distinct nuclei where the isthmic system was suspected, one of which corresponded to the location of cell bodies and terminal fields labeled by tectal injections. To further distinguish between the parvocellular and magnocellular nuclei of the isthmic system, anti-Calretinin and anti-Calbinding stainings were performed. While anti-Calretinin did not yet yield any results, anti-Calbinding also labeled cells in two areas similar to those labels by anti-ChaT.

These results clearly identified the subnuclei of the isthmic system in rattlesnakes. To verify if the isthmic network is also involved in processing of IR-information an electrophysiological approach will be done in a future study.

Funded by the DFG (KO4835-1-1)

ORTHODROMIC AND ANTIDROMIC SPIKE PROROGATION AND DISSIMILAR EXPRESSION OF ATP-GATED AND CAPSAICIN-SENSITIVE CHANNELS IN TRIGEMINAL SENSORY FIBERS IN MENINGES

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Migraine is a common neurological disorder characterized by a strong headache which mechanisms remains unclear. The peripheral axons of the trigeminal nerve in dura mater play an important role in the development of migraine pain. These axons are primarily designed to generate and propagate action potentials (AP) from the periphery to the brainstem. However, a number of studies have shown the peripheral release of CGRP, a key migraine mediator, in response to stimulation of the trigeminal ganglion. These data indicate an important role of both orthodromic and antidromic propagation of excitation in these fibers. To study these issues we studied generation of AP in the peripheral versus central branches of the trigeminal nerve in hemiskull preparation isolated from P35 rats. The aim was to characterize the nociceptive traffic and expression of ATP-gated P2X and capsaicin-activated TRPV1 receptors in different parts of the sensory axons. We found that the baseline frequency of APs in the proximal nerve was $0.23 \pm 0.05 \text{ s}^{-1}$ ($n=10$), whereas, in distal part, it was significantly higher ($0.79 \pm 0.22 \text{ s}^{-1}$, $n=7$, $P<0.05$) consistent the main function of the former to propagate nociceptive signals to the brainstem. Application of ATP ($100 \mu\text{M}$) significantly increased the frequency of APs in the distal axon to $1.98 \pm 0.79 \text{ s}^{-1}$ ($n=7$, $P>0.05$). In contrast, there was no significant action of ATP on the proximal part ($0.79 \pm 0.26 \text{ s}^{-1}$, $n=10$, $P>0.05$). Capsaicin was effective in both parts of the nerve (increase in distal part to $6.41 \pm 1.08 \text{ s}^{-1}$, $n=10$, $P<0.05$ and to $2.21 \pm 0.47 \text{ s}^{-1}$ in proximal part, $n=9$, $P<0.05$). These results suggest a bidirectional generation and propagation of APs and the asymmetric distribution of P2X3 and TRPV1 channels along the axons. These data advance our knowledge on information processing in these structures important for understanding the neurochemical mechanisms of migraine related trigeminal pain.

Oxidized phospholipids acutely increase the firing rate of dorsal root ganglia neurons and induce pain behavior

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Medications such as non-steroidal anti-inflammatory drugs are the most widely used painkillers worldwide. Despite their unequivocal effectiveness serious side effects restrict non-steroidal anti-inflammatory drug usage, indicating the need for alternative treatment options. In order to develop new therapeutic approaches, it is necessary to define the molecular mechanism of nociception after inflammation. In a recent study we found experimental evidence that oxidized phospholipids (OxPL) are generated in inflamed paw tissue and are potent novel targets to treat acute and chronic inflammatory pain (Oehler et al., 2016). Furthermore, the transient receptor potential channels TRPA1 and TRPV1 were identified as molecular targets of OxPLs. Here we analyzed OxPL-mediated activation of TRPA1 and TRPV1 in vitro (HEK293) and in cultured sensory neurons with calcium imaging and whole cell patch clamp recording. After analyzing the stimulation effect of OxPL on receptor potential channels on nociceptors, we investigated the influence of OxPL on the excitability of small-diameter DRG neurons. Here, we show that local 500 ms application of 30 μ M OxPAPC, an OxPL compound mixture, is sufficient to trigger and increase the action potential firing rate in DRGs for almost 10 min (Fig. 1 A-E). Furthermore, the excitation threshold was significantly decreased from $-25.47 \text{ mV} \pm 1.81$ to $-32.22 \text{ mV} \pm 1.47$ after OxPL stimulation (Fig. 1 F). This effect could also be detected when the OxPAPC subcompound PGPC was used for DRG stimulation. As OxPL-induced receptor potentials are sufficient to increase DRG firing, we were questioning whether Nav1.9, a voltage-gated sodium channel involved in DRG excitability (Östmann et al., 2008) and nociception (Leipold et al., 2013), is needed to mediate OxPL-induced pain behavior. In wild-type littermates, OxPAPC injection into paws induced pain behavior, an effect which is most pronounced at one to three hours after OxPAPC injection. In Nav1.9 k.o. mice, this OxPAPC effect is not disturbed, indicating that Nav1.9 is not affecting OxPL-induced pain behavior on this timescale. First electrophysiological experiments indicate that Nav1.9 deletion does not affect OxPL-induced action potential firing in DRGs, and is probably not involved in the fast sensory perception of OxPL compounds. In conclusion, TRPA1 channels are molecular targets of OxPL-induced activation and OxPL induce hyperexcitability in DRGs neurons. Nav1.9 is not affecting OxPL-induced pain behavior on long term and seems to have no role in OxPL induced hyperexcitability.

#equal contribution

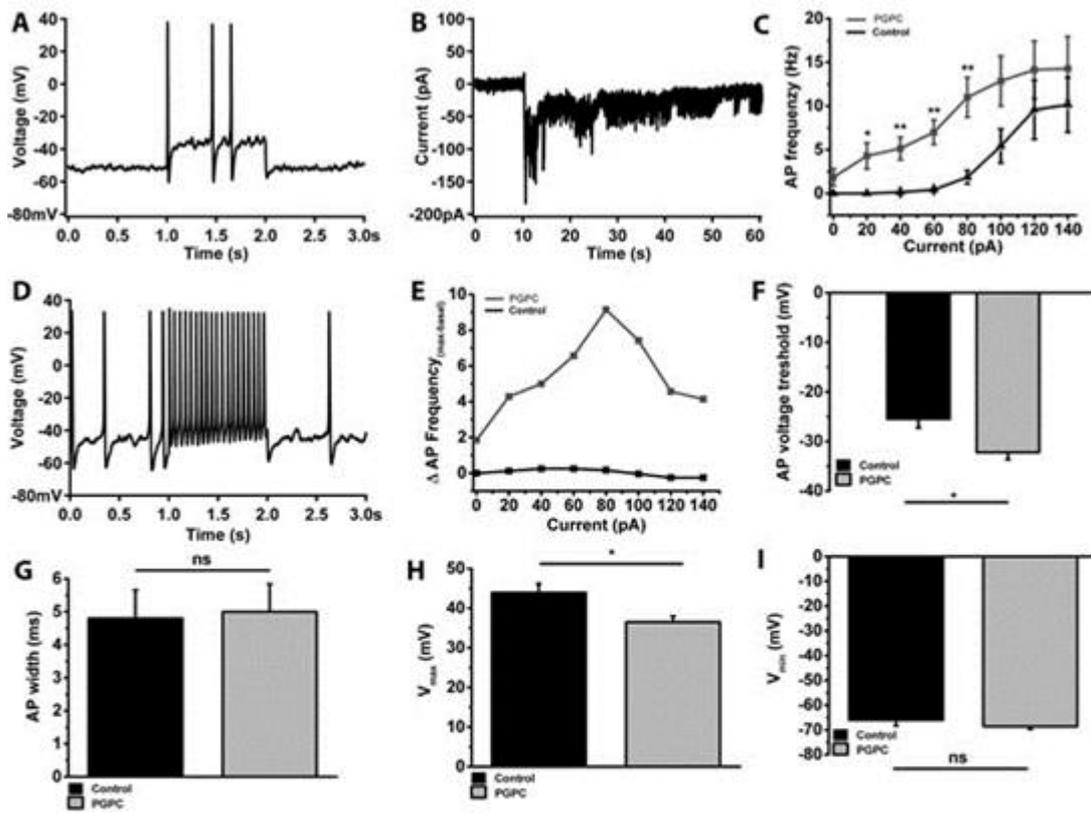


Figure 1: PGPC induces hyperexcitability in DRG neurons. Representative trains of action potentials, recorded from murine DRG neurons in response to 1 s current injections of 80 pA. AP firing rate frequency after PGPC addition is increased (D, C, E; n=7) compared to control measurements (A, C; n=10). PGPC induces only small currents in DRG (B) and decreases the excitation threshold (F) and V_{max} (H) while APwidth (G) and V_{min} (I) are not affected (n=7). Data points represent mean values and significance between pairs of data was tested with a one-way ANOVA (*p<0.05; **p<0.001; Holm-Sidak).

Passive versus active sensing: a giant descending interneuron in a stick insect conveying information about antennal movement.

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Stick insects use their antennae to continuously search and sample the environment ahead during walking. In *Carausius morosus*, the antennae and front legs are equally long, so that any obstacle encountered by the antennae is reachable by the legs. Indeed, stick insects respond to antennal contact with an obstacle with aimed front leg movements. The information required for this behaviour could be conveyed by previously identified descending interneurons that connect the brain to the thoracic ganglia, and convey short-latency information about antennal movement (Ache et al., 2015). For example, the contralateral On-type velocity-sensitive neuron (cONv) encodes contralateral antennal joint angle velocity but also responds to substrate vibration. Since cONv receives multimodal input and has highly fluctuating spontaneous activity, the objectives of this project were to test (i) how cONv can reliably encode single-trial joint movement in the presence of its strongly fluctuating spontaneous activity and (ii) how cONv responds depending on the behavioural state of the animal and stimulus modality? These questions were studied by intra- and extracellular recordings and antennal motion tracking in otherwise stationary animals. Substrate taps at a rate comparable to stepping during walking were reliably encoded by a single spike per tap, while lowering spontaneous (i.e., irregular) spike activity. Thus, the presence of substrate vibration may improve the encoding of antennal movement cues.

The response of cONv to passive deflection of the antenna is reliable, strong and velocity-dependent. However, spontaneous activity of cONv is unchanged during rhythmic exploratory antennal movements, while its sensitivity to passive deflection persists. Provided that the response to passive deflection is similar to the response to interrupted active movement, cONv could serve as a reliable antennal contact detector under behaviourally relevant conditions.

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.

Rate code and temporal code: complementing mechanisms in signalling rapidly varying stimuli in the rat's barrel cortex.

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During tactile exploration, rough surface textures cause whiskers to vibrate at high frequencies. To decipher relevant coding mechanisms, we studied the responses of cortical neurons to high-frequency vibrations of whiskers and compared recordings obtained under isoflurane anesthesia with those from awake rats.

Mobile multielectrode arrays were either chronically implanted or used acutely for extracellular recording of single- and multi-unit spiking activity in trained awake head-fixed rats or in mechanically ventilated isoflurane-anesthetized rats, respectively. Sinusoidal deflections were applied to single whiskers in rostro-caudal direction at frequencies of 20-620 Hz for 1 s. Precision of phase-locking was addressed by computing angular dispersion around a preferred stimulus phase and vector strength. Fidelity of phase-locking to every stimulus cycle was tested by inter-spike interval (ISI) measures. The results are based on single trial analyses.

Pre-stimulus ongoing activity was 5 times higher in the awake state than under anesthesia, therefore, only response rates above ongoing activity were compared. Under anesthesia, the responses to the 1-s vibration were marked by a strong onset activity of a few milliseconds, followed by oscillations (~15 Hz) for up to 300 ms and a late epoch of sustained firing. The spike rates of onset and late response epochs were unrelated to stimulus frequency. In contrast, in the awake state, the onset response was smaller (0.4 times) and immediately merged with sustained firing which was closely related to vibration frequencies in both rate and pattern. Response pattern reflected the phase-locked occurrence of spikes for the entire 1-s stimulus in the awake state and for the late epoch under anesthesia.

The results show that rapidly varying stimuli may be encoded by spike rate as demonstrated for the early response epochs (the first ~10 ms) in the awake state (but not under anesthesia). In addition, the temporal aspect of prolonged movements is reflected in phase-locked discharges. These may develop also under anesthesia towards late response epochs (>300 ms). Here, periods of faithful reflection of stimulus frequency resulting in discharges with one spike per cycle may occur as is the case for the entire 1-s response in the awake state.

Supported by: EU (IST-2000-28127) and DFG (SCHW577/10-2; SFB936/A2/Z1; EN 533/13-1)

Resiniferatoxin administration reveals two distinct brain networks involved in nociceptive processing of the rat

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Introduction:

Resiniferatoxin (RTX), an extract from the spurge plant *Euphorbia resinifera*, is a potent receptor agonist of the transient receptor potential cation channel subfamily V member 1 (TRPV1). TRPV1 is mainly expressed on small-diameter peripheral nociceptive C-fibers. Besides chemical substances and protons, the channel is opened by temperatures above 43°C, which is close to the pain threshold. In high doses, peripherally administered RTX can be used to ablate TRPV1-expressing neurons via calcium-mediated excitotoxicity, and therefore to diminish the response of the thermo-receptive system to noxious temperatures.

Aim of this study was the characterization of the influence of the missing TRPV1-signaling on CNS processing of innocuous and noxious temperatures.

Methods:

Male Wistar rats (~400g), were separated into two groups, one remained naïve (n=18) while the other one was treated with RTX (n=9). RTX was injected s.c. into the neck skin on 3 consecutive days with increasing doses (30, 70, 100µg/kg).

fMRI BOLD-measurements (4.7T Bruker Biospec, 2 x 2 array head coil, matrix 64 x 64, FOV 25 x 25mm, voxel size 391 x 391µm, slice thickness 1mm, axial, 22 slices, GE single-shot EPI (TR = 4000 ms, TE_{eff} = 24.38 ms)) were conducted under mild isoflurane anesthesia 8 days after the last injection.

A contact heat stimuli sequence between 40°C and 54°C in 2-degree-steps was presented in a pseudo-randomized way at the dorsal side of the left hind paw (duration of each stimulus 20sec with 5sec plateau, 3min and 40sec interval; each temperature repeated three times in total; actively feedback computer-controlled Peltier heating device).

BOLD responses (amplitude and volume) as well as changes in brain functional connectivity were analyzed for each group and each stimulation temperature.

Results:

RTX-mediated ablation of TRPV1-expressing neurons suppresses effectively the BOLD brain response and reduces the activated brain volume evoked by stimuli above 46°C in almost all brain regions except parts of the brain stem. The brain's response to stimuli below 46°C was comparable between both groups.

For temperatures above 48°C, network analyses showed a significant reduction of functional connectivity within two distinct sets of brain structures: one consisting of the lateral and medial thalamus, the hippocampus, the superior colliculi and the brainstem, while the other one contains the primary somatosensory cortex, association cortex and the cingulum. We see a great reduction of intra- and extra-thalamical connectivity in the RTX group which is of course due to the lack of nociceptive input. Furthermore, while thalamo-cortical connections remain unaffected, we see for RTX a decrease of inter- and intra-cortical connectivity. These networks are normally in charge of sensory, cognitive and emotional processing of the nociceptive signals.

Conclusion:

The blockade of TRPV1-signaling via RTX suppresses effectively CNS response to noxious heat stimuli above a nociceptive threshold of 46°C. Via network analyses, we can distinguish two affected brain networks which are involved in filtering and cognitive processing of nociceptive signals, as well as in sensory location and emotional interpretation.

Responses of the femoral chordotonal organ of adult *Drosophila melanogaster* to vibrational stimuli

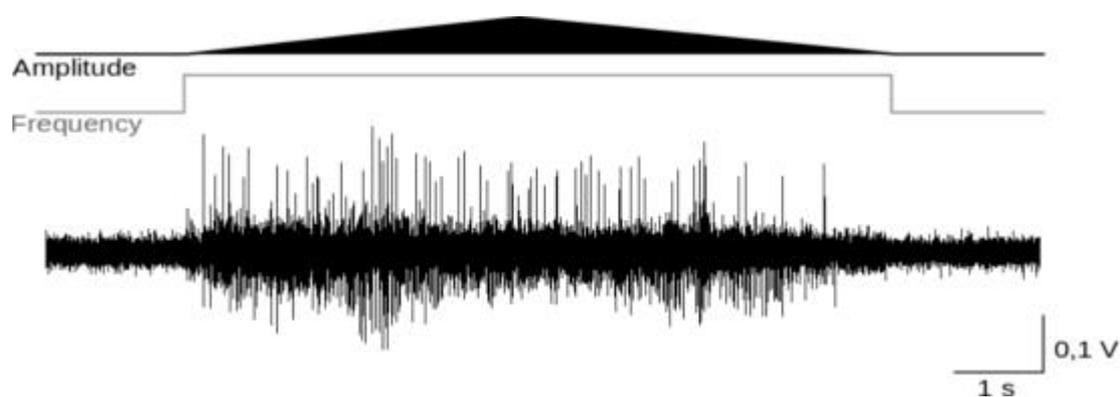
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In the all three leg pairs of adult *Drosophila melanogaster* a femoral chordotonal organ (feCO) can be found. Chordotonal organs are mechanoreceptors composed of scolopidial units and located in and between different joints of insect body to detect position and movement. The feCO of *Drosophila melanogaster* is a large sensory organ consisting of three groups of scolopidia. One large group terminates at the cuticular surface of the distally femur whereas the other two groups are distally associated with the femoral muscle (Shanbhag et al. 1992, Int. J. Insect Morphol. Embryol. 21:311-322). The organ is involved in controlling the leg movement and in proprioception. Here we investigate physiological properties in respect to vibration perception.

Therefore the legs of adult *Drosophila melanogaster* were stimulated and extracellular recordings from the leg nerve were made. As sensory stimuli either frequency-modulated (1-4000 Hz, 8 s) or amplitude-modulated (0-approx. 24m/s², 4 s) ramp-shaped stimuli were used. The ramp-shaped stimuli were designed with a rising phase and a decreasing phase. The responses of different neuronal units were extracellularly recorded with sharpened tungsten electrodes and analysed for threshold and intensity-response curve. To determine functional specializations in due to the body position recordings of the three leg pairs were compared.

The amplitude-modulated stimulation reveals a asymmetric electrophysiological response regarding increasing and decreasing accelerations. The response to the decreasing phase was much lower. The frequency-modulated stimulation elicits a distinct frequency dependent reaction of the leg nerve even in frequency ranges above 1000 Hz. The comparison of the leg pairs showed no major differences regarding the investigated properties.



Thalamocortical innervation of GABAergic interneurons in the mouse barrel cortex

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GABAergic interneurons are thought to play a key role in neocortical processing. Even though they show a striking diversity, they can be subdivided into non-overlapping subgroups according to the expression of certain marker proteins. It is long known that the group of FS/PV cells are strongly innervated by thalamocortical fibers. However, studies investigating other groups of GABAergic interneurons are sparse and rarely extend beyond LIV.

Hence, this project investigates the innervation of SOM and VIP cells of the mouse barrel cortex by their primary relay nucleus, the VPm. Our aim is to probe cells for connections in all layers and to assess the effect of their activation, overall strength and other synaptic properties. Understanding how GABAergic interneurons are recruited by thalamocortical fibers is crucial to understand their overall role in neocortical processing.

As methods we use optogenetics combined with *in vitro* whole cell recordings. Animals of the SOM- and VIP-Cre reporter lines are injected stereotactically with an AAV at age P19-25 leading to expression of ChR2. After 2- 3 weeks animals are used for acute slice preparation. In order to isolate monosynaptic input, the drugs TTX and 4-AP are washed in.

Thalamocortical stimulation leads to a stronger depolarization in VIP cells than in SOM cells. About a third of VIP cells can be driven to spike. Cells which differ in recruitment also show differences in several intrinsic properties. Monosynaptic innervation can be found in almost all VIP cells in all layers. Innervation of SOM cells on the other hand is comparatively weak. SOM cells which are closer to the FS phenotype also show stronger inputs than the remaining cells.

These data give several new insights into the thalamic innervation of GABAergic interneurons. Most prominently, they show a strong and almost ubiquitous thalamic innervation of VIP cells, which are thought to be predominantly driven by corticocortical or neuromodulatory inputs. Furthermore, they show a weaker but consistent innervation of SOM cells, which is dependent on the respective subpopulation.

The role of the leech *Anterior-Pagoda* cell in tactile information processing

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The medicinal leech is an excellent model system in order to analyze the neuronal basis of specific behavioral patterns. It possesses one of the smallest neuronal systems with around 10,000 neurons in total, arranged in a segmented ventral nerve cord with subunits (ganglia) consisting of about 400 neurons. Touching the skin triggers a precise movement – the local bending. During this behavior the leech bends away very locally from the touch. This behavior arises from a three layered network, consisting of about 50 neurons located within one ganglion. In the first level of the network, three types of mechanosensory neurons (touch (T) cells, pressure (P) cells and noxious (N) cells) process the information about the touch. The second and third layers of the network consist of interneurons and motor neurons, respectively. Only few interneurons are known and functionally described, whereas the sensory neurons and motor neurons have already been studied in more detail (Kristan, 1982; Lockery & Kristan, 1990a, 1990b).

Tactile stimuli-experiments triggering the local bend behavior activate a large number of active neurons on the ventral side of the leech ganglion (Fathiazar et al., 2016). The Anterior-Pagoda (AP) cell, a spontaneously active, paired cell type is among these cells. In order to investigate how the AP cells are involved in the processing of pressure stimuli and what role they might have in the local bend response, anatomical and physiological characterizations of the AP cell were performed in the first part of this study.

The morphology of the AP cell, visualized by intracellular dye injections, showed a contralateral stratification of the main process, being typical for a motor neuron. The main focus of the physiological analysis was to characterize of general response features (e.g., spike count, spike latency or interspike intervals) using intracellular single unit recordings. The depolarized membrane potential and a characteristic spontaneous activity support the conclusion that the AP cell could be a motor neuron.

In the second part of the study, the function of the AP cell as a postsynaptic target of the P sensory cells (Sunderland, 1980; Zhang et al., 1990, 1995) was further investigated physiologically. AP cell responses differed between ipsilateral and contralateral stimulation in spike numbers and latency. The results indicated that the AP cell could be involved in the discrimination of sensory stimulus location. Additionally, intracellular injections of neurobiotin that passes gap junctions and intracellular double recordings yielded potential candidates for connection partners, including the well-known interneuron 212 and the contralateral AP cell.

This study provides for the first time a comprehensive overview of the morphological and physiological characteristics of the leech Anterior-Pagoda cell and gives insights into a wide range of functional properties of this cell type in the overall network.

Based on these results, a profound understanding of the function of the AP cell in the processing of sensory information in the neuronal network of the medicinal leech could be obtained by additional studies, for example, the investigation of the involved neurotransmitters.

Regulatory Mechanisms underlying motor neuron functional diversification

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Accurate neural control over the movement apparatus relies on circuits operating through fusimotor neurons to regulate muscle tone and maintain protective reflexes during movements. Fusimotor neurons achieve this by tuning the sensitivity of muscle stretch receptors, but how they acquire properties distinct from the more numerous α -motor neurons controlling the working musculature remains unclear. We aimed to evaluate the role of orphan-nuclear receptors (ONRs) in the functional specification of fusimotor neurons using chick and mouse models. We hypothesized that ONRs regulate the functional diversification of motor neurons into fusimotor and α -motor neurons by regulating fusimotor electrophysiological properties (1) and their incorporation into fusimotor circuits to determine accurate motor outputs in mice (2). To test our hypothesis, we used a transposon-mediated gene transfer method for long-term expression of candidate ONRs. This involved transposon-mediated manipulation of ONR gene expression in late-gestation chick embryo which enabled us to: Analyze electrophysiological properties of motor neurons within the spinal cord (1) and verify candidate genes in RNA-expression profile to explicate a potential mechanism of functional diversification (2). Furthermore, we performed conditional mutagenesis in postnatal mice and analyzed: Electrophysiological properties using Whole-Cell Patch Clamp recordings (3), Gait behavior using DigiGait, Step ladder, and Beam walk behavior assays (4), and muscle-spindle activity using EMG activity assays (5). We report that the generation of mice selectively lacking ONR function in motor neurons demonstrates the importance of these mechanisms for the assembly of functional fusimotor circuits in the context of the behaving animal through real-time gait and posture analysis and motor performance assays.

Untangling VIP neuron diversity: A quantitative analysis of firing patterns and the influence of neuromodulation

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VIP expressing interneurons are an essential component of cortical circuitry. They are commonly described as the most heterogeneous class of inhibitory interneurons. However, it is not known whether VIP neurons form distinct subgroups based on morphology and/or electrophysiology. Here, we focused on the electrophysiological diversity by targeting genetically labeled VIP neurons in acute thalamo-cortical slices of the barrel cortex in mice. Based on whole cell patch-clamp experiments in all cortical layers we characterized the electrophysiological profile of VIP neurons. Firing patterns were quantified using a novel method based on adaptation rates, current dependency, and frequency spectrum to describe VIP neuron diversity in detail. Based on these data, VIP neurons can be subdivided into 5 electrophysiological types. The distribution profile of these types throughout the barrel cortex shows that bursting VIP neurons are located in layer II/III exclusively with a tendency towards upper layer II/III. In contrast, low frequency-continuous adapting (LFCA) VIP neurons are found predominantly in lower layer II/III and layers IV-VI. The remaining three types are found ubiquitously distributed throughout all layers of the barrel cortex. Additionally, we studied the responses of VIP neurons to noradrenalin (NA), acetylcholine (ACh), and serotonin (5HT). In layer II/III, VIP neurons are depolarized by NA, ACh, and 5HT. The depolarization induced by ACh is mediated by nicotinic non- $\alpha 7$ ACh receptors. Despite the current classification as 5HT_{3a} receptor expressing interneurons, only 46% of all VIP neurons show 5HT_{3a} receptor mediated currents. But all of them show 5HT₂ receptor mediated currents. Interestingly, depolarizations induced by ACh and 5HT trigger a change in the firing behavior of bursting VIP neurons. Their firing pattern switches from bursting to tonic whereas the firing pattern of all other electrophysiological types remains unchanged. The firing behavior of bursting VIP neurons suggests an additional specialization to integrate brain states. In conclusion, using a quantitative method to identify electrophysiological types resulted in a detailed description of firing patterns of VIP neurons. These firing patterns were highly diverse in firing rate adaptation and frequency spectrum. Furthermore, some VIP neurons show an intrinsic change of firing patterns which can be induced by neuromodulation.

Vibrosensory organs and vibration transmission over the legs of the cave cricket *Troglophilus neglectus*

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Many orthopteran insects use substrate vibrations for intraspecific signalling or predator detection. Vibrosensory organs are usually chordotonal organs located in the tibia (subgenual organ complex) and femur (femoral chordotonal organ) of all leg pairs. Here we match the characteristics of sensory organs and of vibration transmission over the legs of the cave cricket *Troglophilus neglectus* (Orthoptera: Rhaphidophoridae).

In the subgenual organ complex, three chordotonal organs are present: the subgenual organ, intermediate organ and accessory organ, containing together ca. 50 sensilla (Jeram et al. 1995, J Morphol 223: 109; Strauß et al. 2014, Royal Society Open Science 1:140240; Strauß and Stritih 2016, Acta Zoologica 97: 187). The subgenual organ spans the dorsal hemolymph channel of the tibia. It is the main vibration detector responding with high sensitivity to vibration stimuli (acceleration) with best frequencies between 200 – 1000 Hz. The intermediate organ is located at the anterior tibia and extends distally of the subgenual organ; it responds to somewhat higher frequencies than the SGO. The accessory organ is closely associated to the cuticle in the posterior tibia and has been discussed as a low frequency detector below 200 Hz.

We tested for a possible match of the divergent frequency sensitivity and the asymmetric arrangement in receptor organs with distinct propagation of sinusoidal pulses of different carrier frequencies delivered from a minishaker over the cuticle at different planes and positions. In particular, we studied whether the location of the accessory organ corresponds to differences in mechanical properties of the anterior or dorsal cuticle. Scanning was registered by laser-Doppler vibrometers from two positions of the leg simultaneously.

Vertical leg vibration showed a flat response up to 400 Hz and a sharp peak at 600–800 Hz, which was much more pronounced on the tibia than on metatarsus, and roughly reflected the IO frequency response. Transverse leg vibration showed on the tibia a broader peak at 300–400 Hz of about 10 dB lower intensities than vertical vibration, by which its average values closely matched the SGO frequency response. No difference was found between fore- and midlegs or between the anterior and posterior part of the tibia.

These findings suggest selective resonance of the leg cuticle and hence frequency-specific transmission of vibrations to different proximo-distal parts, but not the anterior and posterior parts of the tibia. Differences in the tuning of the proximal tibia by vibration in different planes suggest that different morphological attachments may allow specific organs to respond differently to similar leg vibrations. In addition, extensive stimuli prolongation was found at frequencies around 100 Hz, apparently resulting from reflections and resonance. These effects were the strongest in the proximal tibia and might have influenced the development of the accessory organ in this part of the leg through the enhanced stimulus detectability. The absence of differences in vibration transmission between the leg pairs is reflected in the highly similar serial organisation of vibratory organs.

Poster Topic

T21: Motor Systems

- [T21-1A](#) A local, load-based mechanism for inter-leg coordination in insects
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- [T21-2A](#) Adaptive Motor Control: Task Specific Processing of Movement Feedback in the Curve Walking Insect.
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A local, load-based mechanism for inter-leg coordination in insects

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Stable walking requires that a leg's step cycle is coordinated with the step cycles of the other leg(s). How does the nervous system coordinate the respective neural locomotor networks? The properties of load sensors in both mammals and insects suggest a particularly elegant, leg-local mechanism: A leg starts its stance-to-swing transition when sensing a reduction of its own load induced by the touchdown of a neighboring leg. That is, the touchdown of a neighboring leg could be signaled mechanically through the ground as opposed to neuronally. To test whether animals can take advantage of this leg-local, load-based mechanism, two critical hypotheses have to be tested. First, the load sensors of a leg must be able to reliably signal the unloading of that leg in natural walking conditions and elicit motor effects that initiate its swing phase. Second, the unloading must be caused mechanically through load transfer to a neighboring leg. We tested both hypotheses in freely walking stick insects (*Carausius morosus*). The primary load sensors of stick insects, campaniform sensilla, are particularly well studied. Most importantly, the comparatively large size and sprawled posture of stick insect legs allow simultaneous recordings of joint kinematics, ground reaction forces and muscle activity in freely walking animals. With respect to the first hypothesis, we show that campaniform sensilla can reliably encode the unloading of the leg at the end of the stance phase. Based on joint torques, we predict that two sensilla groups on the trochanter switch their activity with the unloading onset. This should elicit a motor reflex that initiates the leg's swing phase. Indeed, electromyographic recordings reveal that the activity of the stance muscle (depressor) and the swing muscle (levator) is tightly correlated with the unloading onset. With respect to the second hypothesis, we show that the unloading onset is directly linked to mechanical load transfer between legs. The unloading onset follows the touchdown of the posterior neighboring leg with short latency. In addition, a mechanical simulation reveals that this leg takes over load highly effectively during walking. In linking sensory physiology directly to behavior, our results demonstrate that leg-local load signals can contribute significantly to inter-leg coordination by exploiting the mechanical coupling between legs. We predict that this mechanism is used to similar advantage across insect species, in analogy to load-based mechanisms proposed in mammals. It may be implemented in legged robots to increase robustness and reduce complexity of walking control.

Adaptive Motor Control: Task Specific Processing of Movement Feedback in the Curve Walking Insect.

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Walking animals constantly need to adjust their leg movements to serve the given motor task. Curve walking is generated by specific kinematics changes in each leg on both sides of the animal: the middle outside leg generates large amplitude, longitudinally directed stance movements, with minor movement in the femur-tibia-joint, whereas the inside leg generates only small front to back movements but with marked tibial flexion (Gruhn et al., 2009). Hellekes et al. (2012) showed that the task-specificity in leg stepping kinematics on both sides of the curve walking animal is accompanied by differences in the processing of movement-related feedback. Flexion signals from the Femur-Tibia (FTi-) joint, reported by the femoral chordotonal organ (fCO), induce reinforcement of *Flexor tibiae* activity more often on the inside than on the outside.

Here, we investigated 1), if different parameters of tibial movement are processed differently between inside or outside steps, 2) if the motor output of the respective swing motor neurons in thorax-coxa and the coxa-throchanteral joint is affected differently by fCO feedback during inside and outside turns, and 3) the potential cellular mechanism behind the observed effects.

For this purpose, we stimulated the middle leg fCO with a large range of stimulus velocities, amplitudes of FTi-joint movement, and starting angles while intra- and extracellularly recording mesothoracic motoneuron (MN) and muscle activity in curve walking animals. The likelihood for reinforcement of movement for all three modalities was significantly higher during inside compared to outside steps. Reinforcement of tibial MN activity is increased with increasing starting angles and decreasing stimulus velocities (cf. Bässler, 1988) for the inside and outside leg, but unaffected by FTi-joint excursion amplitude. In addition, we observed a decreased efficacy of flexion and extension signals from the fCO in an outside leg compared to rest. We also tested for task-dependent modifications in inter-joint influences of fCO feedback: fCO stimulation affected activity of *levator trochanteris* MNs independent of stepping direction; in contrast, fCO stimulation affected *protractor coxae* MN exclusively during outside stepping.

Currently, we are investigating the neuronal mechanisms underlying the observed task-dependent alterations in influence from fCO feedback. We recorded intracellularly from mesothoracic tibial MNs during outside stepping of the front legs. Initial results show that the short latency depolarization in extensor MNs upon elongation of the fCO is reduced (cf. Driesang and Büschges, 1996). *This work was supported by DFG grant Bu857/14.*

Anatomical and physiological specializations for high spike time precision in *Drosophila* flight steering motoneurons

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The *Drosophila* flight muscles are segregated into two classes with anatomical and physiological differences. The large asynchronous stretch activated indirect power muscles are specialized to maximize power output. In contrast the smaller steering muscles insert directly onto the sclerites of the wing hinge and therefore allow rapid changes in wing kinematics during steering maneuvers. The steering muscles act as a transmission system to precisely transform the mechanical energy provided by the power muscles into wing motion. This is accomplished through conformational changes in the arrangement of the wing hinges. The steering muscles are activated in the conventional 'one spike – one action potential' fashion to enable a precise control of the timing of activation within each wing beat cycle which totals appr. 5 ms. This study combines genetic and molecular biological methods with anatomical and physiological tools to characterize a specific *Drosophila* steering motoneuron, namely b1, as a model for high spike time precision.

We first tested how precise the b1 flight steering motoneuron fires during behaviour. Simultaneous extracellular recordings, high speed video, and laser based wing beat detection reveal a spike time precision below 0.3 ms. In fact, more than 70 % of all b1 spikes occur within a 0.2 ms window at a precise phase during wing downstroke. Anatomically, the b1 neuron shows two striking features. First, the dendritic branches are short and thick, indicating a very short time constant, which will now directly be measured by in situ current clamp recordings. Second, the axon terminals are three times larger than those of indirect flight motoneurons and contain many more active zones; quantification is underway. We will next probe the ionic current complement that, together with anatomical specializations, allows for high precision firing to then probe for the specific contributions of structure and physiology on precision by genetic manipulation. To target genetic manipulation exclusively to the b1 motoneuron without affecting any other neurons, we currently generate split GAL4 lines from the publically available HHMI Janelia Farm fly stocks. We expect this study to reveal fundamental insight into anatomical and physiological specializations concerning speed and precision by characterizing b1 as a model motoneuron.

Antidromic action potentials alter information encoding in a sensory neuron

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The ability of neurons to properly initiate and conduct action potentials along axons is fundamental for the stable and robust functioning of the nervous system. Axons have been shown to possess receptors for amines and neuropeptides, whose metabotropic actions can influence action potential propagation and initiation. Several modulatory and pathological conditions activate new ectopic spike initiation zones in the axon trunk, whose action potentials propagate both ortho- and antidromically. While axonal modulation by synaptic, paracrine, and endocrine pathways is likely common in all nervous systems, little is known about the consequences of such antidromic action potentials for information coding at the primary spike initiation zone.

To address these issues, we utilize a combination of electrophysiology and computer modeling of the anterior gastric receptor neuron (AGR) axon. AGR is a single-cell muscle tendon organ of the experimentally advantageous crustacean stomatogastric nervous system. Its axon is several centimeters long and spontaneously generates ectopic action potentials in its axon trunk. These spikes are generated in addition to action potentials initiated at AGR's primary spike initiation site near the peripheral dendrites. We found that AGR's ectopic spike frequency is modulated by a pair of chemosensory projection neurons, whose co-transmitters elicited distinct and opposing effects: the biogenic amine, histamine, decreased ectopic spike frequency while conversely the peptide co-transmitter, FLRFamide, increased spike frequency and created new spike initiation sites. Octopamine, which is likely present as a hormone modulator in this system, also excites AGR's ectopic firing. To test the hypothesis that modulation of ectopic spike frequency in the axon trunk determines information encoding at the primary spike initiation zone, we first created an axon model based on Hodgkin-Huxley equations. Our initial results indicate that back-propagating ectopic action potentials invade the primary spike initiation zone. The model predicts three distinct frequency-dependent actions on sensory encoding: 1) spike collisions and excitability changes at the primary spike initiation site reduce the number of action potentials 2) spike timing is altered, and 3) the spike frequency in response to sensory stimuli now depends on the frequency of back-propagating action potentials. Thus, modulation of spike initiation in the axon trunk may alter information coding at the primary spike initiation site. We are currently testing these model predictions in the biological system.

Continuity in inter-leg coordination during walking in *Drosophila*

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During legged locomotion, a terrestrial animal needs to coordinate its legs to generate a movement pattern that can reliably propel it forward. This pattern has to be adaptable according to the animal's needs (e.g., the animal must be able to change its heading or speed). Walking speed, in particular, has a strong influence on the locomotor pattern; horses, for instance, change from walking to trotting and, eventually, to galloping when they speed up. These locomotor modes in horses and other large vertebrates are energetically optimal with regard to the speed at which they are used. More importantly, transitions between them are discontinuous, a critical hallmark of true gaits.

Insects also use different locomotor patterns at different walking speeds. Insects use so-called wave gait coordination at slow speeds, tetrapod gait at intermediate speeds, and tripod gait at high speeds. Gait implies that these coordination patterns are relatively invariant and that there are, in fact, three distinct modes of locomotion in walking insects. The existence of these different, speed-dependent inter-leg coordination patterns is well-established; however, whether they are true gaits (i.e., whether the transition between them is discontinuous or if they are merely striking patterns in a continuum) is much less clear. Indeed, existing data suggest that walking insects use a continuum of coordination patterns instead of distinct gaits.

By investigating walking behavior during acceleration or deceleration episodes spanning the animal's range of walking speeds, we can search for discontinuities between coordination patterns to determine if they are true gaits. To our knowledge, only qualitative data exist in this regard for insects, and, surprisingly, systematic studies explicitly searching for these discontinuities in insects, thereby validating or disproving true gaits in insects, do not exist.

To address this, we investigated walking behavior in *Drosophila* while animals spontaneously accelerated from low to high walking speed, or vice versa. We hypothesized that any discontinuities (or lack thereof) would become apparent in this experimental paradigm. To assess continuity, we calculated several important kinematic parameters, such as duty factor and phase relationships, on a step-by-step basis. Our analyses indicate that flies changed their inter-leg coordination continuously while increasing or decreasing walking speed. Consistent with previous studies flies used tripod-like coordination patterns at high walking speeds and tetrapod-like patterns at lower speeds. Importantly, these patterns are parts of one continuous spectrum and, therefore, must be regarded as different variants of the same underlying mode of locomotion. In this notion, tripod and tetrapod patterns are merely striking special cases among many different possible and intermediate coordination patterns.

These findings have implications for our understanding of the neuronal basis of walking in insects. Previous studies often used very specific walking situations and speeds, in particular, to investigate the insect walking system, and often came to different conclusions regarding the nature of neuronal control. We argue that to characterize and, more importantly, to truly understand the neuronal basis for insect walking, future studies must explicitly take into account the speed dependence of inter-leg coordination. *This study was supported by DFG-RTG 1960.*

Feedback integration on the fly – a numerical model for phase-coded locomotor control in flying insects

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Sensory information processing is key for success of motor control in many animals. A well-studied model system for multimodal sensory processing during locomotion is the flight control circuit of insects. In flies, for example, visual signalling from the compound eyes is continuously fused with proprioceptive feedback from mechanoreceptors on wings and gyroscopic halteres that elicits spikes in flight muscle motoneurons. The precise timing of motoneuron spike initiation is crucial because spike timing determines the biomechanical efficacy of flight muscles and thus wing motion. There is a continuing debate on how graded responses of non-spiking visual interneurons are integrated with wingbeat-synchronous spikes of the mechanosensory system. Based on previously published anatomical, electrophysiological and behavioral data, we here present a numerical simulation that explains spike control at the level of dendritic integration by single motoneurons. A Hodgkin-Huxley model simulates the activity of a motoneuron that is driven by visual interneurons and proprioceptive mechanoreceptors via electrical synapses. We determined simulation parameters by fitting the model's postsynaptic subthreshold response to previously measured experimental data. Our simulation shows that the motoneuron's spiking frequency and spike timing depend on magnitude and temporal structure of both visual and mechanosensory feedback. While mechanosensory feedback provides a temporal set point for spike initiation, graded potentials from the visual system may shift spike timing by up to 12% of the stroke cycle. These results are in good agreement with experimental data on vision-induced changes of flight muscle activation timing during maneuvering flight. In sum, the simulation highlights the significance and computational power of single motoneurons for sensory information processing in fast feedback systems. It also supports the idea of information processing by local neural networks for motor control in insects, similar to those networks found in vertebrates.

Hexapedal Inter-Leg Coordination via Physical Coupling Only

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Legged animals show different patterns of inter-leg coordination, i.e., gaits. Typically, gaits change in response to changes in locomotion speed. Whereas in quadrupeds gait changes are best described by discrete switches from one pattern to the other, a “gait continuum” has been observed in insect locomotion (e.g., stick insects: Graham, 1985, *Adv Insect Physiol*; *Drosophila*: Wosnitza et al. 2013, *J Exp Biol*): That is, walking coordination smoothly fluctuates between stereotypical patterns, loosely influenced by locomotion speed. Generally, three walking gaits are distinguished in insects: tripod, tetrapod and wave gaits, involving three, two or one protracting legs (swing movements) at a time, respectively. Intermediate coordination along this continuum occurs regularly.

With regard to control of locomotion, this suggests that precise timing plays a minor role in insect locomotion. In fact, sparse inter-leg coordination rules and sensory feedback have proven to be sufficient to simulate the flexible walking patterns observed in stick insects realistically (Schilling et al, 2013, *Biol Cybern*). Interestingly, this model and earlier variants rely mostly on proprioception of kinematic variables, whereas distributed load sensing has largely been neglected, despite its strong impact on motor activity.

In order to test the potential of distributed load sensing for adaptive control of naturally flexible inter-leg coordination in insects, here we take a radical approach: Based on the systematic evaluation of a dynamic walking simulation, we test to what extent inter-leg coordination may be governed by mechanical coupling sensed by load sensors, only. In other words, we test whether local load sensing, e.g., by campaniform sensilla, is sufficient to support the adaptive control of different gaits as in natural insect locomotion.

To this end, we expand on the model proposed by Owaki et al. (2013, *J R Soc Interface*) that may generate distinct quadruped inter-leg coordination patterns without any neuronal (or electrical) information exchange among legs. Each leg controller comprises a phase oscillator receiving input from local force sensors. According to this approach, gaits emerge through the mechanical coupling of otherwise independent controllers. To test the applicability of Owaki’s model in hexapod locomotion, we extended it by adding two more legs. By means of a sensitivity analysis, types and stability of the resulting inter-leg coordination patterns were explored. In particular, three parameters were varied: the antero-posterior location of the centre of mass (CoM), the oscillator frequency of the legs, and the strength of the force feedback. Results show that physical coupling is sufficient to elicit different leg coordination patterns: the dominant pattern is the tripod gait. Tetrapod patterns differ from the ones observed in insects and occur for a symmetrical load distribution, only. In no case, a stable wave gait emerged. Our results suggest that the CoM strongly influences the type of gait emerging. With increasing strength of force feedback, the possible coordination patterns become more sharply separated in parameter space. The effect of frequency is more inconsistent, but in general higher frequencies are needed to elicit walking with higher feedback strengths. Future work will address the effect incorporation of insect-specific morphological features that affect load distribution and corresponding load-dependent coordination.

How angular velocity signals update a heading representation in the fly brain

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Many animals maintain an internal representation of their heading as they move in their environment. Such a compass representation was recently discovered in a neural population in the *Drosophila melanogaster* central complex, a brain region implicated in spatial navigation. Here we use electrophysiology and calcium imaging in head-fixed walking flies to identify and characterize a population of neurons that conjunctively encodes heading and angular velocity, a tuning property that arises on the single cell level. We further show how the asymmetric turn responses and the neuron's recurrent loop-with-shift connectivity to the compass cells provide an elegant mechanism for updating the fly's heading representation when the animal turns in darkness. This mechanism, which bears a strong resemblance to the ones proposed in theoretical models for rodent head direction cells, provides a striking example of structure predicting function for a broadly relevant navigational computation.

Motor control of *Drosophila* feeding behavior

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The precise coordination of body parts is essential for survival and behavior of higher organisms. While progress has been made towards the identification of central mechanisms coordinating limb movement, only limited knowledge exists regarding the generation and execution of sequential motor action patterns at the level of individual motoneurons. In *Drosophila*, sweet stimuli evoke a robust and highly stereotypic motor behavior, the extension of the proboscis towards the food source. Here, we use this behavior as a model system to study the generation of a reaching-like behavior.

First, we provide a complete neuroanatomical description of all motoneurons and muscles contributing to proboscis motion. By performing a combined behavioral and morphological screen we were able to identify genetic control elements targeting the individual motoneurons controlling the five major sequential steps of proboscis extension and retraction. Activity-manipulations during naturally evoked proboscis extension show that orchestration of serial motoneuron activation does not rely on feed-forward or sensory-feedback mechanisms. Our data support a model in which central command circuits recruit individual motoneurons to generate task-specific proboscis extension sequences.

Neural Joint Control is Constrained and Assisted by Passive Dynamic Muscle Properties

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Physical simulations integrating available data on neural control and biomechanics of stick insect walking so far fall short in faithfully generating natural joint kinematics. Most obvious is the difficulty to tune the interactions between neural control circuits and strong low-pass filter characteristics of the leg muscles such that stable, yet sufficiently fast joint movements result [1]. One out of several hypotheses tested in simulations led to promising improvements in joint kinematics: passive dynamic muscle force represents not just a velocity-dependent damper. Rather it is also dependent on muscle length and movement amplitude, analogous to and extending beyond the force-length and force-velocity relationships of the neurally driven muscle. To verify this hypothesis, the passive dynamic muscle properties of the stick insect *flexor tibiae* muscle were investigated and preliminary results supported the hypothesis [2].

Acknowledging the importance of the interaction of antagonistically acting muscles in joint space, the experiments were subsequently redesigned to work in joint space. This resulted in a fully automated stimulation paradigm consisting of a sequence of muscle stretches mimicking multiple velocities, starting angles, and movement amplitudes. The passive muscle forces/torques evoked by these stretches show a nonlinear dependence on all three parameters. Maximal passive forces at high velocities using the whole physiological working range of 140° reach up to 90 mN on average, which represents about half the maximally generated active force of the antagonistic *extensor tibiae* muscle. Passive dynamic forces can vary 2.5-fold depending on joint position, 4-fold depending on movement velocity and 1.5 fold depending on movement amplitude. Additional experiments were performed to distinguish the dependencies of torque on joint position, velocity and movement amplitude from other history-dependent effects like muscle run-down. Protocols with inverted muscle stretch sequences (N=6 for regular and inverted experiments respectively) and multiple repetitions (n=4) of the entire stretch sequence within the same experiment revealed that the qualitative dependence of torque on all three parameters is independent from the order of stretches (starting at either short or long muscle lengths), from the number of prior stretches, and from the animal tested.

With the implementation of the obtained passive dynamic muscle data, the muscle/joint model can serve now as a solid basis for morphological simulations of stick insect walking. Preliminary results show that simple neural control signals like constant muscle activations lead to complex physiological velocity profiles which combine moving at high speeds with stability through muscle internal attenuation at the end of swing. At the same time these simulations show sensitivity for the individual tuning of agonistic and antagonistic properties and the possibility to produce more complex velocity profiles by differential neural activation of agonist and antagonist.

[1] v. Twickel A (2011). *Embodied Modular Neural Control of Walking in Stick Insects -- From Biological Models to Evolutionary Robotics*. PhD thesis, Universität zu Köln

[2] v. Twickel A, Guschlbauer C, Mantziaris C, Schwarz A, Büschges A (2013). Embodied joint movement control: interaction of neural networks and passive muscle properties in the stick insect. *Proceedings of the 10th meeting of the German Neuroscience Society 2013*

Pilocarpine evoked membrane potential and calcium oscillations in stick insect leg motoneurons do not depend on voltage-gated sodium currents

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In the stick insect *Carausius morosus*, rhythmic activity of leg motor neurons (MN) during stepping is based on synaptic tonic excitatory drive, synaptic phasic inhibition, and phasic excitation¹⁻⁴. The first two are also the basis for rhythmic activity evoked by the muscarinic acetylcholine receptor agonist pilocarpine¹⁻³. It is known that calcium ions (Ca²⁺) contribute to the generation of the tonic depolarization of leg MNs during walking⁴. In this study, we sought to investigate the dependence of membrane potential and Ca²⁺ oscillations on voltage-gated sodium channels upon activation of the locomotor networks by pilocarpine in situ.

To measure intracellular Ca²⁺ transients in retractor MN neurites after blocking voltage-gated fast and persistent sodium currents, we retrogradely filled the mesothoracic nerve n15 with the Ca²⁺-sensitive dye Oregon Green 488 Bapta-1 dextran (OGB-1) and the lidocaine derivative QX-314^{5,6}. Rhythmic activity in MN pools was induced by bath application of pilocarpine and was extracellularly recorded from lateral nerves of the mesothoracic ganglion while changes in MN Ca²⁺ concentration were monitored. Furthermore, we performed intracellular recordings from retractor and protractor MNs with sharp electrodes containing 100 mM QX-314.

Our experiments showed that application of pilocarpine evoked Ca²⁺ oscillations in retractor MNs that were in-phase with the extracellular recorded spike activity of the backfilled nerve (N = 15). QX-314 backfill successfully abolished extracellularly recorded spike activity, whereas Ca²⁺ oscillations were not affected and showed similar oscillations compared to those measured under normal conditions without QX-314 (N = 9). Intracellular administration of QX-314 into retractor (N = 7) and protractor motoneurons (N = 6) via the electrode blocked spike discharges, but pilocarpine induced slow membrane potential oscillations persisted.

Therefore, we conclude that the tonic depolarization in MNs is not mediated by a QX-314-sensitive persistent sodium current (e.g. I_{Nap}) and Ca²⁺ transients are not based on the opening of voltage-sensitive Ca²⁺ channels by sodium spikes in the MNs. Our results suggest that Ca²⁺ enters the MNs when the cells are tonically depolarized due to pilocarpine application. Ca²⁺ may enter the cell directly through ligand gated channels and/or through low threshold Ca²⁺ channels gated by the depolarization. Ca²⁺ oscillations are the result of inhibitory input that sculpts the tonic depolarization and generates the rhythmic pattern.

This project is supported by DFG grants to Ansgar Büschges and Joachim Schmidt, and the RTG 1960.

1. Büschges, A. Brain Res. **783**, 262–71 (1998). **2.** Büschges, A. et al., Eur. J. Neurosci. **19**, 1856–1862 (2004). **3.** Ludwar, B. C. et al., J. Neurophysiol. **94**, 2772–84 (2005). **4.** Westmark, S. et al., J. Neurophysiol. **102**, 1049–61 (2009). **5.** Connors B.W. and Prince D.A., J Pharmacol. Exp. Ther. **220**, 476-81 (1982). **6.** Hu, G.-Y. et al., J. Neurophysiol. **87**, 2676–83 (2002).

Sensory Basis of Force Direction Sensitivity of Motor Neurons in the Stick Insect Leg

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Load and force sensing plays a pivotal role in insect locomotion as it has been shown to be responsible for switching motoneuronal activity from swing to stance motor neuron pools. Load and force sensing is also responsible for the reinforcement of stance phase motor activity to support propulsion of the animal. In insects such as the stick insect, load is perceived through the campaniform sensilla (CS), often oval shaped and direction sensitive sense organs that can be arranged in groups, typically oriented in the same direction. Load on the stick insect leg is, to a large extent perceived through five major groups of CS on the proximal leg, which report cuticular strain in a particular leg plane: two groups of trochanteral CS (1&2) monitor forces generated in the horizontal plane when the leg is bent anteriorly or posteriorly by the protractor and retractor coxae muscles (Schmitz 1993); two dorsal groups of trochanteral CS (3&4) monitor forces in the vertical plane caused through activation of levator and depressor trochanteris muscles (Zill et al. 2012). Finally, one group of femoral CS (gr.5) is known to affect extensor and flexor tibiae muscles (Akay et al. 2003) by rostrally and caudally directed forces generated horizontally to the femur.

Previously, it has been shown, that signals from CS groups 1 and 2 affect the activity of the protractor and retractor coxae (Schmitz, 1993). Here we investigated in the middle leg whether the other three groups of CS might also affect the activation of the two coxal motor neuron pools, or if the force plane for activation of these two muscles is the horizontal front-to-back movement only. We therefore systematically applied horizontal or perpendicular bending stimuli of 50µm amplitude to the fixed leg stump in which the CTr-joint was fixed by means of a minuten pin clamp, and recorded the nerve activity in the nerve roots innervating the protractor coxae (n12) or retractor coxae (n15) muscles. By systematically ablating different combinations of CS groups, we show that protractor coxae activity is affected by CS signals from groups 1 & 2 only. In contrast, retractor MNs were found to be sensitive to ventral deflection of the leg stump. The sensory signals contributing to this response are provided by CS groups 3, 4 and 5 as only ablation of all five CS groups completely abolished retractor response to bending forces.

Our results support the notion that both coxal motoneuron pools differ in the responsiveness to different directions of force applied to the leg. Supported by DFG grant Bu857/14.

SOG-related influences on sensory-motor interactions in locust walking circuits

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The neural basis of insects walking relies on the cumulative outcome of interactions between motor and sensory pathways in the thoracic circuits and head ganglia. One specific region that had drawn attention as a potential locomotion-related center is the suboesophageal ganglion (SOG). The SOG contains both descending and ascending interneurons and modulatory projections that reach the thoracic ganglia and the brain. Despite some evidences that suggest the SOG plays a role in initiation and modulation of locomotion, much is still unknown about it. In specific, it is unknown whether and how it contributes to sensory-motor thoracic processing. Our current study addresses this question, by monitoring responses to specific walking-related stimulation of thoracic leg nerves in the desert locust, *Schistocerca gregaria*. Towards this goal we combine electrophysiology and calcium imaging techniques and study interactions between descending influences from the SOG and walking-related thoracic reflexes. Extracellular recordings from nerves containing the depressor motoneurons during stimulations of sensory nerves before and after neck lesioning between the SOG and the prothoracic ganglion, suggest a contribution of the SOG to intersegmental transfer of sensory inputs from leg nerves. The exact contribution differ in different experimental conditions and among the different thoracic ganglia. To further understand it and point towards specific SOG areas that respond to such legs activations we are developing a preparation for monitoring calcium levels in the SOG, during such stimulations.

Thorax- and Leg-Segment Specificities in the Motor Output of the Turning Stick Insect *Carausius morosus*

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Locomotion depends on neuronal activity, muscle contractions, and sensory feedback. The generation of straight walking has already been well studied and much is known about the neuronal activity underlying basic locomotor patterns in the single leg (Büschges 2005). However, we only begin to understand the neuronal mechanisms underlying behavioral flexibility, and the knowledge on local segmental activity during adaptive locomotion, and the role of descending influences on it is scarce (Hellekes et al. 2012; Martin et al. 2015; Gruhn et al. 2016).

Here we investigate the neuronal mechanisms underlying curve walking in the stick insect (*Carausius morosus*). Turning of the tethered animals on the slippery surface was induced using an optical stimulus, and walking sequences were monitored by video and by EMG recordings of the flexor tibiae muscle in both front legs. We studied the influence of optomotor induced turning on motor activity in all three major leg joints of the deafferented meso- and metathoracic ganglia, that control the middle and hind legs, respectively, by recording extracellularly from leg nerves *nl2* (containing protractor motoneurons (MNs)), *nl5* (retractor MNs), *C1* (levator MNs), *C2* (depressor MNs), *nl3* (extensor MNs), and branches of the *nervus cruris* (flexor MNs) in a reduced preparation with all but the two front legs cut off. In a second set of experiments, a split-bath preparation was used, in which the meso- and/or the metathoracic central pattern generators (CPG) for walking were pharmacologically activated, to investigate the involvement of local CPGs in thorax-segment and leg-segment specificity of the turning related motor output.

The motor activity of the three joints showed three types of responses: 1) a context-dependent change in activity in the subcoxal joint: meso-(Ms) and metathoracic (Mt) protractor MN activity was increased during inside over outside turns (Ms, N=4; Mt, N=12), while retractor MN activity was increased during outside vs. inside turns (Ms, N=4; Mt, N=8); 2) a context-independent activity: the meso- and metathoracic levator and depressor MNs showed little if any changes activity depending on the turning direction. Levator MN activity increased with the start of front leg stepping in either direction (Ms, N=8; Mt, N=6), whereas depressor MN activity ceased (Ms, N=4; Mt, N=6). 3) a segment specific activity: in half of the experiments, extensor (Ms, N=4 of 8) and flexor MNs (Ms, N= 6 of 11) showed direction dependent modifications in neuronal activity in the meso-, but not in the metathorax. We then tested, if the changes in activity were mediated through changes in local CPG activity, by use of pharmacologically activated ganglia. Initiation of front leg turning always modified the rhythmic activity in the motor neuron pools of the subcoxal and coxa-trochanteral joints and in the same way as seen under control conditions in saline.

Our results indicate that changes in meso- and metathoracic motor activity during curve walking of the front legs is leg-joint specific, and can be direction specific, as well as thorax-segment specific. The changes in motor activity in the subcoxal- and coxa-trochanteral joints in the pharmacologically activated preparation furthermore suggest that these effects are mediated through changes in local CPG activity.

Supported by DFG grant Bu857/14 & Konrad-Adenauer-Stiftung.

A functional gradient in the rodent prefrontal cortex supports behavioral inhibition

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Humans and animals share the ability of planning and holding back their responses until the appropriate moment via a well-orchestrated balance between movement initiation and inhibition. In impulse control disorders like fronto-temporal dementia, this balance is disturbed emphasizing the critical role of the prefrontal cortex (PFC) for appropriately timed actions. As rodents are an essential animal model for neural disorders and basic neuroscience, it is important to define the exact functional roles of their PFC subsections in action control which so far, remain controversial. Here, we employed optogenetic and electrophysiological techniques to systematically analyze the impact of five key subareas of the rat medial PFC (mPFC) and orbitofrontal cortex (OFC) on response preparation. Inactivation of mPFC subareas induced drastic performance changes before an external signal, namely an increase (prelimbic cortex - PL) or decrease (infralimbic cortex - IL) of premature responses. Additionally, electrophysiology revealed a strong decrease in neuronal activity of PL neurons prior to premature responses. In contrast, inhibition of OFC subareas (mainly the ventral section – VO) significantly impaired the ability to respond rapidly after external cues. Consistent with these findings, mPFC activity during response preparation predicted trial outcomes and reaction times significantly better than OFC activity. Interpreted in the framework of proactive and reactive behavior, these data support the concept of opposing roles of IL and PL in directing proactive (internally driven) behavior and argue for an involvement of OFC in predominantly reactive movement control. By attributing defined roles to rodent PFC sections, this study contributes to a deeper understanding of the functional heterogeneity of this brain area and thus may guide medically relevant studies of PFC - associated impulse control disorders in this important animal model.

A neural mechanism underlying swimming termination in lampreys

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Locomotor movements such as swimming, walking, and flying, are controlled by specific centers in the brain. The mesencephalic locomotor region (MLR) is one such center and it is located at the junction between the midbrain and the hindbrain. It controls locomotor activity through an excitatory connection onto reticulospinal (RS) neurons that in turn activate central pattern generators (CPGs) for locomotion in the spinal cord. The MLR is present in all vertebrate species tested, including the lamprey. The supraspinal mechanisms that initiate and maintain locomotor movements are well understood. On the other hand, little is known about neural mechanisms underlying the termination of locomotion and this topic fell into the scope of motor control research only recently. RS cells ('Stop Cells') that are implicated in halting locomotion have been identified in mice and lampreys [Bouvier et al., 2015, *Cell* 163(5):1191-1203; Juvin et al., 2016, *Cell Rep.* 15(11):2377-2386]. Intracellular recordings of lamprey 'Stop Cells' revealed a characteristic burst of activity (termination burst) that is linked with the end of swimming. Membrane properties of 'Stop Cells' are unlikely to be involved in the generation of the termination burst, suggesting that synaptic inputs from other brain regions are responsible.

Here, we investigated the role of the MLR in halting locomotion and providing synaptic inputs that generate the termination burst in 'Stop Cells'. Experiments were carried out in lamprey semi-intact preparations (n=44), in which intracellular activity of RS cells was recorded and correlated to active swimming movements of the body. Electrical stimulation of the MLR initiated swimming that often outlasted the stimulation period (28.02 ± 14.18 s; n=5). We found that a second MLR stimulation of lower intensity (50% of control), delivered during the exceeding swimming episode, halted the locomotor bout (within 6.83 ± 3.09 s; n=5). When the low intensity MLR stimulation was delivered during the ongoing swimming bout, halting occurred at the onset of the electrical stimulus. Stimulation of the MLR at the same intensity as the initial stimulation (100% of control), prolonged an ongoing locomotor bout. Interestingly, MLR stimulation elicited similar effects during sensory-evoked or spontaneous locomotion. Injecting small quantities of D-glutamate locally in the MLR also halted swimming. A termination burst was then triggered in 'Stop Cells' as seen with electrical MLR stimulation. Taken together, our results indicate that the MLR not only controls the initiation and maintenance of swimming, but it also provides a stop signal to halt locomotion.

Funding: Funded by CIHR, NSERC and GLFC. SG received studentships from DAAD and UzK.

A novel rotating beam test for detection of sensorimotor deficits in a knock-in mouse model of primary torsion dystonia

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Background: The pathophysiology of dystonia, characterized by sustained or intermittent muscle contractions causing twisting movements/postures, is poorly understood. Although mutation carriers show alterations in neuronal connectivity and sensorimotor deficits, only 30% develop dystonia. Ex vivo studies in mouse models of DYT1 dystonia have shown an abnormal D2 receptor mediated release of acetylcholine from striatal interneurons. DYT1 knock-in mice were reported to not exhibit a dystonic phenotype in standard behavioral tests, potentially reflecting non-dystonic mutation carriers.

Objective: The aim of present study was to (1) generate more powerful tests to detect sensorimotor behavioral alterations in the DYT1 knock-in (KI) mouse model of dystonia and to (2) examine the effects of optogenetic stimulations and pharmacological inhibition of specific striatal interneurons.

Methods: The DYT1 KI mouse model and corresponding wildtypes were tested on a sequence of challenging cognitive, motor and sensorimotor tests. Subsequently, the acute effects of optogenetic stimulation and pharmacological inhibition of specific striatal interneurons on motor behavior in DYT1 KI will be compared to wildtype mice.

Results: In order to test sensorimotor function we developed the “adaptive rotating beam test (ARB)” which includes changing sensory input. DYT1 KI mice showed robust deficits in this test, especially if the surface of the rotating rods was smooth and the diameter small. The higher sensitivity of the ARB test was further validated in another model of movement disorders which has previously not shown a behavioral phenotype. Additionally, the adhesive removal test that explores sensorimotor connectivity revealed significant impairments in the DYT1 KI mice compared to controls. Preliminary results from optogenetic and pharmacological studies indicate genotype-specific effects on (sensori)motor function.

Conclusions: The novel adaptive rotating beam test for the first time detected a phenotype in the DYT1 KI mice. Sensorimotor impairments in this model were further supported by deficits in the adhesive removal test. As indicated by preliminary data, striatal interneurons are likely to play an important role in DYT1 dystonia. Current studies examine the pathophysiological substrate of sensorimotor deficits in dystonia with focus on striatal interneurons and sensorimotor pathways.

Adaptation of motor activity in monkey motor, premotor and parietal cortices during BCI control of 3D reaches

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Adaptation of reach motor behavior in response to changes in the sensorimotor environment has been studied widely from a behavioral and computational perspective, while little is known about the neural underpinnings in primates. Partly, this is because it is very difficult at the neural level to disentangle the multiple sensory and motor related changes observed at the behavioral level in cortical areas which are known for integrating many of these different signals at the same time, like the posterior parietal or premotor cortices. Here we suggest alleviating this conceptual problem by using Brain Computer Interfaces (BCIs). BCIs have been used as experimental paradigms probing sensorimotor adaptation in absence of haptic feedback by directly controlling the relationship among end-effector and neuronal activation, providing better experimental control over the relevant parameters. Proficient BCI control relies on the adaptation of neural networks, and previous studies have pointed-out the importance of designing decoders that take into account how the brain adapts during on-line control. Yet, it is unclear if such learning process is limited to the control of the movement and limited to neurons that directly drive the motor output via the decoder. Here we test whether BCI adaptation acts only locally or modifies a distributed frontoparietal network that is not directly controlling the decoder (structural specificity), and whether adaptation occurs exclusively during movement control proper, or instead also changes motor planning (functional specificity).

Neuronal activity was recorded from a macaque monkey implanted with 2x32 floating multi-electrode arrays (FMAs), in each of three cortical areas M1, PMd, and PRR. A Kalman-Filter decoder was trained by regressing neuronal firing rates from a subset of M1 and PMd (not PRR) single and multi-unit with hand velocity (50 ms steps) while the monkey manually performed a memory-guided center-out reach task in a 3D virtual-reality environment with 8 targets arranged on the vertices of a 7 cm cube. During BCI-control the animal had to control the cursor directly via the decoder. Once the animal became proficient in controlling the cursor (baseline), we perturbed the decoder by applying a 30 degree visuo-motor rotation of the cursor in the fronto-parallel plane (rotation). We compared shifts in neural tuning among population of neurons in the three cortical areas in the rotation phase relative to baseline.

We found that during motor control neurons in all three areas, including PRR, shifted their tuning, to a different extent, but such that it could reflect a re-aiming strategy of the monkey suited to accommodate for the applied perturbation. We also found that in the planning period of movement in the rotation phase different fractions of units from all areas changed their tuning properties, but these changes were less systematic. In conclusion, during BCI learning not only neurons from the local networks controlling the movement, but also neurons not contributing to the motor output in areas separate from the decoding areas co-adapt, and neural adaptation affects not only the movement period but also the planning period. This suggests that BCI learning includes an explicit learning strategy for motor control and a modified motor-control policy reflected in modified motor planning.

Automated experimenter-free analysis of motoric phenotypes in neurodegenerated knock-out rats

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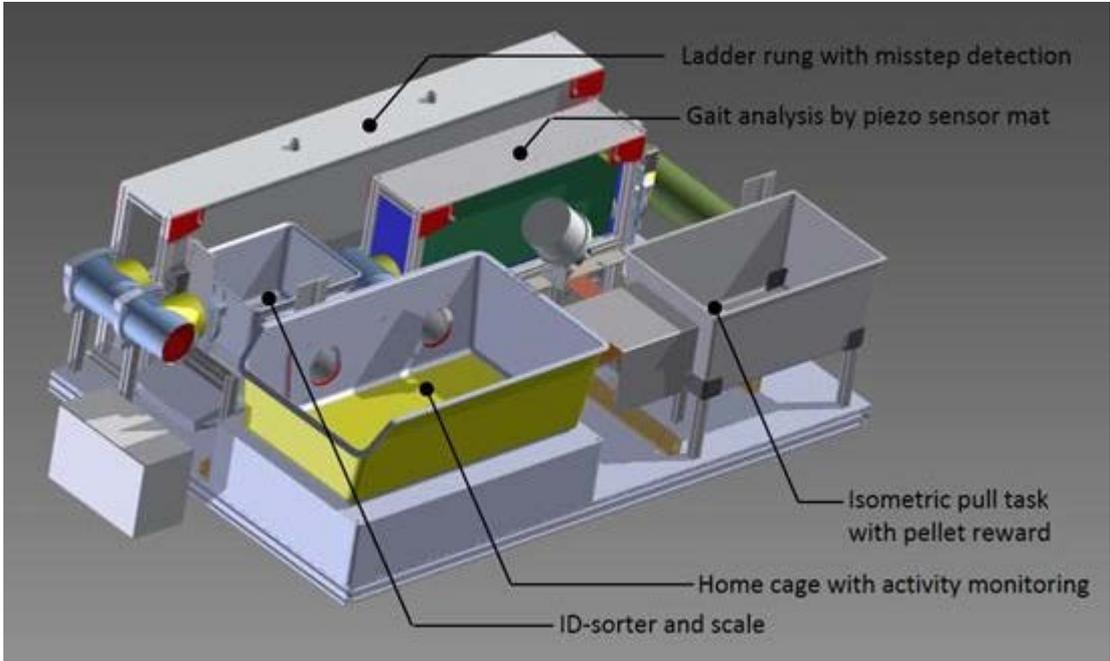
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In order to overcome limitations of behavioral studies with rats, the approach of automated experimenter-free analysis is highly promising: in a system combining the animals' living environment and the experimental set-up, the measured data is automatically acquired and stored. Several significant improvements of the experimental procedure can be achieved by this approach: the confounding factor stress on the rats can be significantly reduced and the behavior displayed by the animals can be seen as self-motivated. As all results are continuously recorded, the influence of observer's bias is immensely reduced, increasing reproducibility while simultaneously reducing time cost and work load for the experimenter.

With the Operator-Independent Motor Analysis System (OptiMan, see Figure 1), we combine the rats' home cage with several behavioral tests for the analysis of motor function: from the home cage, where activity, locomotion distances and spatial preferences can be monitored via Radio-Frequency based identification tags (RFID-tags), the rats can enter a circular arrangement of diagnostic chambers in a self-motivated manner. Unidirectional flaps only permit one-directional runs of single individuals whereby an animal passes a series of behavioral tests assessing motoric abilities: a horizontal ladder task with adjustable rung patterns allows for the quantification of gait parameters such as stride length and the counting of missteps to assess coordination. This first unit is followed by an operant cage with an isometric pull task testing forelimb strength and a gait analysis walkway consisting of a high-density piezo transducer array evaluating kinematics and individual weight bearing.

We aim to explore the potential of an automated, experimenter-free set-up for the analysis of motor function in a rat knock-out model lacking a protein integral to the synaptic active zone. The knock-out results in neurodegeneration presumably accompanied by motoric as well as psycho-social impairments. By means of a comprehensive motor and activity analysis with the OptiMan system, we hope to obtain a detailed description of the phenotype displayed by the knock-out rats and thus elucidate the role of the gene in question.



Electrophysiological Characterization of VTA/SNc Neurons and Their Habenular Inputs in Lamprey

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In vertebrates the basal ganglia is a central brain circuit for action selection, motor control and motor learning and its structure and function is evolutionary well conserved from lampreys to humans [1]. Via efferents to the basal ganglia as well as to motor areas dopaminergic midbrain neurons located in the Ventral Tegmental Area (VTA) and Substantia Nigra pars compacta (SNc) take part in a multitude of motor functions and are indispensable for maintaining normal motor output. Pathological changes in dopamine levels, as e.g. in Parkinson's disease, lead to severe neurological consequences. Whereas their (functional) efferent connectivity is rather well understood the control of the activity of dopamine neurons, especially via the lateral Habenula (IHb), is the subject of current research. The aim of this study is to electrophysiologically characterize immunohistochemically classified dopaminergic (tyrosine hydroxylase positive, TH+) and non-dopaminergic (TH-) neurons and their habenular afferents in the VTA/SNc homologue of the lamprey, the nucleus tuberculum posterior (ntp), with the ultimate goal to understand how dopamine release is controlled.

In a preliminary study [2] patch clamp recordings were performed in 350µm lamprey brain slices containing the VTA/SNc homologue while stimulating major direct and indirect afferent pathways from IHb, the fasciculus retroflexus (fr) and its relay via the Rostral Tegmental Nucleus (RMTg). Both TH+ and TH- neurons displayed spontaneous spike activity but differed with regard to input resistance and action potential half-width. These differences were significant on the population level but could not be used to unambiguously distinguish TH+ and TH- neurons. Upon rf stimulation mainly excitatory, AMPA receptor-mediated responses were observed, in contrast to strong inhibitory inputs via the RMTg pathway predicted from mammalian data.

Here several additions are presented: A) To improve identification of TH+ and TH- cells the effect of bath applications of multiple receptor agonists (Dopamine, Quinpirole, GABA, Baclofen, Met-Enkephalin) on TH+ (n=7) and TH- (n=5) cells was tested. Consistent with mammalian data Met-Enkephalin (30µM) induced a strong hyperpolarization in TH- but not in TH+ cells. In contrast to mammalian data dopamine (30µM) did not lead to hyperpolarizations or reductions in spike frequency in TH+ neurons, but in some TH- neurons. B) Bath application of high-divalent cation ringer (HIDI) demonstrated that at least part of the excitatory rf inputs to TH+ (and TH-) cells are monosynaptic despite their relatively high latencies. C) Patching ntp neurons in thick (1.7mm) transversal brain slices containing ntp as well as IHb, rf and RMTg and stimulating IHb demonstrated that the excitatory, AMPA mediated inputs from IHb to ntp TH+ neurons are not an artifact of the thin slice nor of the rf stimulation. In addition, also TH+ neurons with mainly inhibitory IHb inputs were recorded and bath application of GABAZINE (10µM) reliably resulted in strong and long lasting (up to 20s) depolarizations in TH+ and TH- ntp neurons upon rf or IHb stimulation.

[1] Grillner, S. & Robertson, B.: The basal ganglia downstream control of brainstem motor centres-an evolutionarily conserved strategy. *Curr Opin Neurobiol.*, 2015 , 33 , 47-52.

[2] von Twickel, A.; Walkowiak, W.; Grillner, S.: Habenular Glutamatergic Excitation of VTA/SNc Neurons in Lamprey, *Proc. 11th Göttingen Neuroscience Meeting*, 2015.

Encoding of movement force for decision and action in humans and monkeys

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Goal-directed behavior requires our brain to integrate multiple pieces of information, both for choosing a goal among several, and to perform the action corresponding to the choice. When choosing between two bottles of beverage, the choice will be affected by the type of drink (reward preference), how much is left (reward amount), and how much effort will be needed to get the bottle (effort cost). Performing the action of getting the chosen bottle requires knowing where the bottle is and how heavy it is (so as not to drop it). In this sense, the information on weight and location is relevant and used for both choice and action. Recent neurophysiological evidence (Cisek, 2012; Klaes, Westendorff, Chakrabarti, & Gail, 2011) supports the hypothesis that choice and action form a continuum, where action planning is made in parallel to decision-making, with both processes sharing information and affecting each other (Suriya-Arunroj & Gail, 2015).

This framework has however not been tested for tasks requiring the integration of multiple movement parameters. Here we ask how force information is combined with other movement-related information when choosing and planning arm movements. We performed two complementary experiments in humans and monkeys, where we used a haptic manipulandum to exert forces of varied amplitudes that resisted arm movements.

In humans, we used a psychophysical task to determine how force and other movement parameters affect subjective effort and hence choice behavior in a decision-making context. As a result, subjects estimated effort on the basis of squared force, movement direction and duration, but not movement amplitude.

In monkeys, we address the question with single unit recordings from the frontoparietal sensorimotor cortex. The fronto-parietal reaching network, comprised of the parietal reach region (PRR) and the dorsal premotor cortex (PMd), is a prime candidate for the support of these integration processes. In monkeys, neurons of PRR and PMd represent the direction of upcoming movements, while also showing activity that correlates with upcoming decisions (Klaes et al., 2011). We currently record neural activity from PRR and PMd while the animal is planning and executing arm reaches in different directions and against different forces, both force and direction being cued in advance.

In conclusion, the behaviorally observed dependency of physical effort on squared force during reach choices is reminiscent of a corresponding dependence known from motor control. Therefore, these results strengthen the link between action selection and action execution, as they suggest common minimization principles between effort-based action selection and motor control. Moreover, they suggest integration of force information in neural signals of the sensorimotor cortex of primates during movement planning, not just execution, which we can test with our physiology experiment.

Functional Connectome of the Lateral Habenula-VTA/SNc Circuitry in Anuran Amphibians

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With all vertebrate groups anuran amphibians share basic components of the basal ganglia circuitry which – amongst others – serve to integrate sensory, motor, associative, and limbic information in order to produce context-dependent behavior (Maier et al. 2010). The precise temporal and spatial release of dopamine from the Ventral Tegmental Area (VTA) and Substantia Nigra pars compacta (SNc) within the basal ganglia is essential for motor control and cognitive functions, as demonstrated by severe neurological deficits following pathological changes in dopamine levels (e.g. Parkinson's disease). While the efferent connectivity is well understood, it remains to be established how the release of dopamine is controlled. Over the last decade the lateral habenula (IHb), a phylogenetically old brain structure present in all vertebrates, has shown to take part in regulating the activity of dopaminergic neurons via direct glutamatergic and indirect GABAergic pathways, a feature conserved from lampreys (von Twickel et al. 2017) to mammals (Brown and Shepard, 2016).

The goal of this study is to decipher the functional connectome of the IHb and VTA/SNc circuitry in anuran amphibians by a combination of anatomical and electrophysiological techniques. Preliminary results (Freudenmacher et al. 2015) show that both direct glutamatergic and indirect GABAergic pathway are present. The input to neurons in the anuran SNc/VTA is mainly glutamatergic and AMPA receptor mediated but partly also GABAergic. Evidence indicates that this GABAergic input derives from previously not described GABAergic neurons located adjacent to the dopaminergic nuclei, which, in terms of their connectivity, resemble the mammalian Rostral Tegmental Area (RMTg). Therefore, the main IHb-VTA/SNc circuitry in anurans is evolutionarily conserved.

To shed light on how inhibitory and excitatory inputs to VTA/SNc neurons are generated, extra- and intracellular recordings are performed in the IHb and VTA/SNc of whole isolated brain preparations while extracellularly stimulating varying brain areas including the auditory nerve, amygdala and VTA/SNc homologue. Besides determining the basic electrophysiological properties of the IHb and VTA/SNc neurons and their inputs, the hypothesis, that direct excitatory and indirect inhibitory IHb-VTA/SNc connections originate from topological or at least functionally separate IHb neuron populations, is tested.

Brown, P. L.; Shepard, P. D (2016). Functional evidence for a direct excitatory projection from the lateral habenula to the ventral tegmental area in the rat. *Journal of Neurophysiology* 116, 1161-1174

Freudenmacher, L.; Todorova, V.; Walkowiak, W.; and von Twickel, A.: Habenula Circuitry Controlling the Dopaminergic System in Anuran Amphibians. 11th Göttingen Meeting of the German Neuroscience Society, 2015

Maier, S., Walkowiak, W., Luksch, H., and Endepols, H. (2010). An indirect basal ganglia pathway in anuran amphibians? *J. Chem. Neuroanat.* 40, 21–35.

von Twickel, A; Walkowiak, W; Grillner, S: Electrophysiological Characterization of VTA/SNc Neurons and Their Habenular Inputs in Lamprey, Proceedings of the 12th Göttingen Meeting of the German Neuroscience Society, 2017

Interneuron regulation of motor cortical activity during the execution of a goal-directed forelimb push task in mice.

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The primary motor cortex (M1) is involved in the generation and control of simple and complex motor movements. Pyramidal neurons in M1 show various temporal activation profiles and are sequentially activated during different phases of behaviour such as movement planning, initiation or execution.

Although significant advances have been made in mapping the activity of excitatory microcircuits in M1 during motor behaviour, we still know relatively little about how inhibitory interneurons shape activity changes in M1. Here, we focus on the role of parvalbumin positive (PV) interneurons in shaping motor cortical activity as this group of interneurons provides rapid, powerful perisomatic inhibition that can directly regulate the rate and timing of pyramidal cell output.

To characterize the cellular consequences of M1 PV interneuron activation during behaviour, we developed a goal-directed forelimb object manipulation task – where head restrained mice are trained to execute cued lever push actions for reward – and combined this with resonant scanning 2-photon population calcium imaging of PV interneuron activity. Mice learned to execute the task within days and became ‘expert’ after ~ 10 days of training (i.e. high success rate and reduced reaction times). Expert mice responded to the auditory cue with accurate, reproducible forelimb movements with a latency to movement onset of ~ 1s and performed in excess of 100 successful lever pushes per 30 min training session. This goal-directed forelimb movement paradigm provides a robust model system with which to investigate the cellular and circuit mechanisms underpinning different aspects of a well-defined motor behaviour.

To characterize the timing of PV interneuron recruitment at the single cell and population level we employed resonant scanning 2-photon calcium imaging in layer 2/3 and layer 5 during the execution of our cued forelimb lever push task. To achieve cell-type specific expression of GCaMP6s in M1 PV neurons, we stereotactically injected the adeno-associated virus AAV1.Syn.Flex.GCaMP6s.WPRE.SV40 in the PV-Cre-driver line (B6;129P2-*Pvalb*^{tm1(cre)Arbr/J}). Our data provide a comprehensive description of cell- and layer-specific PV interneuron activity changes across different task-related sequences of mouse behaviour (e.g. successful trial execution, pre-emptive forelimb movements and missed auditory cues), thus providing a detailed spatiotemporal map of PV interneuron activity changes in mouse M1.

Less predominant physical goal encoding and larger dynamical changes during movement control in monkey dorsal premotor cortex compared to parietal reach region

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The dorsal premotor cortex (PMd) and the parietal reach region (PRR) both belong to the frontoparietal reach network in monkeys and are involved in the planning and control of visually guided reach movements. A recent study showed that sustained motor goal encoding in PRR during movement planning predominantly reflects the physical rather than the visual goal of impending reaches conducted under reversed vision (1). Also, preliminary statistics on the numbers of motor-goal encoding neurons in area PMd compared to PRR suggested high similarity in both areas during the planning period of a movement. Yet, pre-selecting and grouping subsets of neurons based on their selectivity as function of spatial task parameters can mask single-neuron heterogeneity. Given both PMd and PRR exhibit highly heterogeneous neural response, excluding neurons that do not show significant selectivity can obscure the differences between PMd and PRR regarding the underlying neuronal computations. Here we ask in how far PMd and PRR differ or match in terms of dynamic neural encoding of movement planning and execution signals in different visuomotor settings.

Two monkeys conducted memory-guided delayed center-out reaches under normal or reversed vision. We applied a neural state space method to construct a high-dimensional state space of all neurons' activity as a function of time, without preselecting 'tuned' neurons. The firing rates across all neurons at a given time correspond to a single point in state space, and trace out a trajectory over time. We tested if, despite the similarity of PMd and PRR during steady-state motor planning, both areas exhibit similar or different spatial encoding properties and neural dynamics during movement execution. First, we assessed the predominance of either physical or visual motor goal encoding in the population dynamics by computing a 'vector of selectivity' (VOS) in the high-dimensional neural state space. The VOS was defined as vector connecting the state space trajectories of trials cueing right- and left-side reaches separately in each viewing context (normal vs. reversed). Opposing VOS between the two viewing contexts, for example, indicate preferential encoding of physical rather than visual goal of movement. During movement planning, PMd exhibited predominant physical goal encoding, but less predominantly than PRR. Second, in both areas physical goal encoding strongly predominated during the early movement period. Finally, the neural dynamics during transition from reach planning to reach execution revealed larger dynamical changes in PMd compared to PRR. These state space dynamics suggest more varied encoding in PMd compared to PRR during motor planning, followed by richer intrinsic dynamics in the transition from planning to movement in PMd. We conclude that PMd during motor planning exhibits more diversity in spatial encoding, i.e., less predominance of a single spatial frame of reference. These results support the view that during on-line motor control, spatial encoding in PRR appears to be more strongly tied to movement-invariant task parameters, while PMd appears to encode movement-variant parameters.

(1) Kuang et al. 2015 Cereb Cortex DOI: 10.1093/cercor/bhu312

Limiting parental feedback disrupts vocal development in marmoset monkeys

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Human vocal development is completely dependent on learning by imitation, through social feedback between infants and caregivers. Based on social feedback, infant vocalization rate declines within the first months and vocal output is pruned towards mature vocalizations. Recent studies revealed similar developmental processes that are influenced by parental feedback in marmoset monkeys. Marmosets produce infant-specific vocalizations that disappear after the first postnatal months. However, it is yet unclear whether parental feedback is an obligate requirement for proper vocal development. Using quantitative measures to compare call parameters and vocal sequence structure we show that, in contrast to normally-raised marmosets, marmosets that were separated from their parents after the third postnatal month still produced infant-specific vocal behavior at subadult stage. Vocal patterns of monkey vocalizations are innate and monkeys seem to be unable to learn new patterns through auditory feedback. However, our findings indicate that direct auditory feedback from a caregiver can shape and prune repertoires of innate call types. This points to a direct modulation of brainstem-based vocal pattern generating networks and might have been one of the critical preadaptations during the evolution of human speech in the primate lineage. Overall, our findings suggest a significant role of social feedback on primate vocal development and further show that marmoset monkeys are a compelling model system for early human vocal development.

Mapping physical and structural MOp connectivity in ALS mouse model: an innovative approach to unmask the rules of neurodegeneration.

Barbara Commisso¹

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Mapping the connectome will let us draw a comprehensive map of neural connections in the brain. MRI data suggest that alteration in connectome may happen in amyotrophic lateral sclerosis (ALS), a fatal degenerative condition characterized by the predominant involvement of primary motor cortex (Mop) in the brain and spinal motoneurons. In particular, the mechanisms responsible for dysfunction and loss of corticospinal motoneurons (CSMN) in motor cortex are not yet resolved and the role of remodeling of the neural network within this process is still unknown. To date, there are no appropriate tools for analysis of the complexity of the motor subnetwork of the cortical connectome in disease models.

We established a new viral tool for high efficiency long-range connectivity mapping by screening different retargeted recombinant adeno-associated viruses of serotype 9 (rAAVs9) derived from selection of an AAV peptide display library. Properties of the viruses were tested by injecting Cre-reporter vectors in striatum and visual cortex of ROSA26-TdTomato reporter mice. We identified an rAAV9-variant (AAV9-SLR) displaying four fold higher retrograde infection and two fold higher local infectivity rate over WT-AAV9.

We have mapped long-range physical MOp input connectivity in the SODG93A-ROSA26-TdTomato mouse, a model for ALS. Recombinant AAV9SLR vectors harboring a Cre reporter gene were injected in MOp, and the whole brain analyzed for the density of RFP-positive cells projecting to MOp. Connectivity was mapped for an early (P30) and advanced (P70) stage of the disease. Decreased spine density in MOp neurons was proved indicating neurodegeneration.

We then analyzed spine density in basal dendrites of pyramidal neurons involved in the motor network to prove the health of their activity. We surprisingly found an increased input connectivity in SOD mice compared to the WT coming from cortical and subcortical regions of the brain. More neurons in somatosensory and contralateral MOp project to MOp displaying an increase of 200%, auditory and contralateral MOs +100%; thalamus and hypothalamus +300% and +100% respectively. The difference was already visible at early stage of the disease (P30). In advanced stage (P70), input connectivity was still higher in some areas but to a lower extent: somatosensory +200%, cMOp +100% and thalamus +100%. In cMOs, auditory cortex and hypothalamus connectivity slips below WT (-50%). In parallel, spine density in basal dendrites is not affected at P30 while decrease of 20% at age P70 in somatosensory, cMO and auditory cortex.

ALS is neurodegenerative disease, therefore we were expecting a gradual decrease of MOp connectivity. Accordingly, our results provide a new insight in the mechanism of neurodegeneration. The network seems to compensate the loss of CSMN population starting from early, asymptomatic, stage of the disease where no other neurodegenerative marks were evident. Progression of the disease implies worsening of the communication within the network, represented by a loss in spine density. Taken together, AAV9-SLR-mediated transduction allows mapping of physical and structural MOp connectivity. This approach may not be limited to the ALS mouse model, but may also be versatile for studying Mop connectivity in further disease models.

Optogenetic tools to study frontoparietal networks in non-human primates – a histological analysis.

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Optogenetics has been successfully applied in several animal models to study neuronal networks. Compared to rodents, implementation in non-human primates has been slow due to the relative scarcity of genetic tools, e.g. specific and robust promoters suitable for viral mediated gene delivery. Remaining challenges are larger tissue volumes, and not well understood long-term effects of opsin over-expression. To study frontoparietal cortical networks in rhesus monkeys (*Macaca mulatta*), we histologically evaluated the effectiveness of viral transduction, neuronal specificity, expression spread, and long-range axonal projections to target areas.

One monkey was injected with an AAV2/5 virus carrying hChR2(H134R)-eYFP and eNpHR3.0-mCherry constructs, both under the CaMKII promoter (UNC Vector Core). NpHR virus was injected in a single penetration into the frontal eye field (FEF) bordering dorsal premotor cortex (PMd). 7 µl were distributed evenly over 7 mm in depth by depositing 1 µl per depth location (spaced 1 mm apart). ChR2 was injected into ventral premotor cortex (PMv) with 3 penetrations at 1.5 mm distance and 1 µl deposits per each of 3 cortical depths. After 10 weeks, immunohistological analyses of 50 µm thick coronal sections of the frontoparietal network were performed.

Both constructs led to a robust transgene expression restricted to cortical neurons, with no signs of inflammation, pathology or tissue damage (apart from the needle tract). The expression appeared predominantly in pyramidal neurons, consistent with CaMKII promoter activity (immunohistological confirmation pending). While ChR2-eYFP was almost exclusively on the cell membrane, NpHR-mCherry also accumulated in intracellular vesicular compartments. Both proteins displayed strong dendritic and axonal expression, including axonal projections terminating in layer 5/6 in respective target areas in the parietal lobe (MIP, LIP, and MT/MST). In injected areas, neurons were infected throughout cortical layers, with strong expression observed within about 1-1.5 mm of each injection site and fairly homogeneous spread through the volume. However, we also observed transduced cell bodies millimeters away from the injection site, even in parietal cortex, only attributable to a retrograde transport of the virus. Concomitant with dense connectivity, the fluorescent signal around the injections sites was spread over 7mm in the rostrocaudal direction. Sparse, retrogradely labeled cells in the parietal cortex were typically found in the external pyramidal layer.

In conclusion, the used virus and injection protocol are well suited for light-driven network manipulations both at the injection site and at distant target areas. While retrograde pick-up creates a potential confounding factor in projection-specific experimental designs, layer specific input and scarcity of retrogradely labeled cells lessens this concern.

Task dependent modulations of the fronto-parietal spike-field coherence network of behaving primates

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Selective communication within and between brain areas leading to behavior is only coarsely known. To better understand how selective communication relevant for behavior is coordinated, we examined the fronto-parietal network for grasping, which is involved in visual processing, decision making, visuo-motor transformation, short term memory, and the generation of movement. To investigate the fine scale neuronal network, two monkeys were implanted with two floating microelectrode arrays per area (32 electrodes each) in the ventral premotor cortex (area F5) of the frontal lobe and in the anterior intraparietal area (AIP) of the parietal lobe. Neuronal activity was recorded in parallel from all electrodes, while monkeys performed a delayed grasping task, in which animals were either free to choose or instructed to grasp a target with one of two possible grip types. 9 recording sessions were used for this study (monkey S: 6; monkey Z: 3). Spike activity of single units and local field potential (LFP) activity of small populations of units was extracted from all recorded electrodes. Network interactions of the single unit population were assessed by calculating pairwise phase consistency (PPC), a metric that evaluates the consistency of the phases of the LFP signal at the time of spike occurrences of each single unit. PPC has the advantage of not being affected by spike-rate variations and it accurately captures oscillatory phase coherence even in the presence of non-Poisson history effects like bursting and refractoriness. Non-parametric cluster based surrogate and permutation testing was applied to test for significant interactions in distinct frequencies at the network level. Beta (18-35Hz) and low frequency (2-8Hz) bands turned out to be the two dominant frequency bands in the fronto-parietal network. In the beta band almost exclusively single units in AIP were synchronized with larger populations in both areas, whereas synchronization with larger populations in the low frequency range was largely confined to single units in F5. The network structure at all conditions was heterogeneous with a small group of single units coordinating the whole network (hubs). When comparing the different conditions with each other, the strongest hubs remained unchanged in their coordinative function for both frequency bands, while less connected hubs turned out to be condition-dependent. The results of this study further strengthen the hypothesis that oscillatory synchrony is of major importance for behaviorally relevant communication in the brain, as demonstrated here in the fronto-parietal grasping network. We found significant condition-dependent reconfigurations of the beta and low frequency network, which could indicate an important role of oscillatory synchrony for network coordination. Surprisingly, the major oscillatory hubs of the network hardly changed for different conditions, reinforcing the notion of a hard-wired backbone of connected single units oscillating at distinct frequencies. In contrast, the group of less connected condition-dependent hub neurons suggests that the backbone neurons recruit the network as required for behavior control. These findings reinforce the notion that oscillatory synchrony is a behaviorally relevant coordination mechanism at the single neuron level, which could also be important for understanding brain diseases associated with disturbed network coordination, such as schizophrenia or autism.

Neural adaptations of the spinal cord evoked by constant motor skill experiences

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Motor behavior enables us to accomplish motor tasks in our daily life. It is modified by musculoskeletal adaptations. On spinal level it enhances particular movements by changing the connectivity between neural networks. In this present study, we specifically focused on the peripheral contribution to skill related neural adaptation.

Former studies in the area of skill related neuromuscular adaptation followed up mainly with changes in cortical input to voluntary control of muscles. Moreover numerous studies have shown that one of the major components of fine muscle control is sensory muscle feedback. Encouraged by those findings we started to investigate the distribution of synaptic input on a large motor neuron pool.

The study was approved by the ethics committee of the University Medical Center Göttingen. We examined a control group of sedentary participants and a group of long-term regular training martial artists, to assess resulting differences of long-term motor skill training. With non-invasive high-density surface electromyography (HDsEMG) we explored the distribution of reflex amplitudes of large populations of motor units (MU). While seated in a chair, the volunteer performed sustained plantar flexion at different percentiles of their maximum voluntary contraction (MVC) force. By stimulating the tibial nerve (TN) with low intensity electrical stimulation, delivered at the popliteal fossa, monosynaptic Hoffmann reflexes (H-reflex) were elicited. Soleus and Gastrocnemius muscle's single MU H-reflexes were recorded. With four different knee angles and adequate resting period between trials, each subject performed four trials.

Statistics (Wilcoxon-Mann-Whitney, $p < 0.05$) show a significant difference between skilled and the control group in respect of reflex amplitudes of single MU. This may indicate higher reflex excitability of the Triceps surae MU pool in the motor-experienced skilled group. Moreover we investigate differences in MU discharge rates.

The afferent system's spinal plasticity is thought to emerge in regards to long-term motor skill training. A longitudinal study should be conducted to exclude the effect of general long-term sport effects. We interpret the result that the lower limb muscle spindle feedback system is altered by changes in neural assemblies. This is thought to cause higher excitability of muscles spindle's IA afferences and results in better fine muscle control and therefor in a better control in space. The investigation of MU discharge rates will provide further insides into skill related neural plasticity.

Poster Topic

T22: Homeostatic and Neuroendocrine Systems, Stress Response

- [T22-1A](#) Anatomy of the neuroendocrine system in *Euscorpium italicus*
Anja Dünnebeil, Nikola Giese, Andrea Wirmer
- [T22-2A](#) Behavioural and physiological functions of the brain-gut allatostatin A peptides in the *Drosophila* larva
Christian Wegener, Jiangtian Chen, Wencke Reiher, Gertrud Gramlich
- T22-3A Retracted
- [T22-1B](#) Impact of PACAP/PAC1 signaling in stress and anxiety: Promising novel targets for the treatment of neuropsychiatric diseases
Veronica Fontebasso, Karl Ebner
- [T22-2B](#) Morphologically different G-cells with neuropod-like processes in the antral region of the stomach
Claudia Frick, Amelie Rettenberger, Malena Lunz, Johanna Bruder, Hanna Martin, Kerstin Lang, Heinz Breer
- [T22-3B](#) Noradrenergic modulation of hypothalamic neurons involved in energy homeostasis
Lars Paeger, Ismene Karakasilioti, Sophie Steculorum, Jens C. Brüning, Peter Kloppenburg
- [T22-4B](#) Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*
Thomas Roeder, Yong Li, Jakob von Frieling, Stella Nolte, Hendrik Beck, Christine Fink
- [T22-1C](#) Oxytocin Neurons Activity in Socially Interacting Rats
Yan Tang, Diego Benusiglio, Valery Grinevich
- [T22-2C](#) Prosencephalic areas associated to the tonic immobility in pigeons (*Columba livia*): a c-Fos study.
José Marino-Neto, Cilene Lino-de-Oliveira, Fernando Falkenburger Melleu
- [T22-3C](#) PVN Neurons in Mice: Identification, Characterisation, Localisation
Andreas Klein, Peter Kloppenburg
- [T22-1D](#) Regulation of hypothalamic neuronal function by glucosylceramide synthase (GCS)-derived gangliosides
Viola Nordström, Silke Herzer, Sascha Meldner, Hermann-Josef Gröne

[T22-2D](#) SIFamide orchestrates orexigenic and anorexigenic peptidergic signals to promote appetitive and feeding behavior in *Drosophila*
Thomas Riemensperger, Ulrike Pech, Simon Kobbenbring, Dennis Pauls, Carlotta Martelli, Britta Bahl, Mirjam Sommer, Atefeh Pooryasin, Jonas Barth, Carmina WarthPerez Arias, Abud Farca Luna, Florian Richter, Christian Wegener, André Fiala

[T22-3D](#) The role of the endothelial cells in leptin transport into the brain
Alessandro Di Spiezio, Helge Müller-Fielitz, Markus Schwaninger

[T22-4D](#) The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia
Jan Siemens, Kun Song, Hong Wang, Gretel Kamm, Jörg Pohle, Fernanda de Castro Reis, Paul Heppenstall, Hagen Wende

Anatomy of the neuroendocrine system in *Euscorpium italicus*

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The neuroendocrine systems of bilaterians fulfill similar regulative functions but are highly variable in structure and position. Within the arthropods, the neuroendocrine system of insects is well studied. Less is known about the system in chelicerates. In scorpions, the most important neurohemal organ is the Tropfenkomplex. Still, it is not clear if the Tropfenkomplex is homologous to either the corpora cardiaca or the corpora allata of insects. We investigated the innervation of the glands in *Euscorpium italicus*. With backfill experiments, we could locate neurosecretory cells in the dorsal protocerebrum and subesophageal ganglion of the scorpion brain. Stainings against various neuroactive substances complete the study. To visualize the results, we used 3D models including material of recent immunocytochemical and former histological staining experiments. Our study gives further insight into the anatomy of the neuroendocrine system of scorpions and allows comparisons to other arthropods.

Behavioural and physiological functions of the brain-gut allatostatin A peptides in the *Drosophila* larva

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Allatostatin A (AstA) neuropeptides are rather widely expressed in the nervous system and the midgut of both larval and adult fruit flies. We could recently show (Chen et al., PLOS Genetics 12:e1006346) that AstA promotes sleep and inhibits feeding in adult flies, and identified a small sleep-promoting subset of AstA neurons in the protocerebrum as a downstream target of PDF, an output factor of the circadian clock. Based on these findings and the results from others, we suggested that pleiotropic AstA signalling by a distinct neuronal and enteroendocrine AstA cell subset adapts the fly to a digestive energy-saving state which can be modulated by the central clock.

In contrast to adult flies, *Drosophila* larvae neither exhibit overt circadian rhythms in behaviour and physiology, nor do they sleep. We are using opto/neurogenetics and different behavioural and physiological assays and will report on the functions of AstA signalling in fruit fly larvae.

Supported by the Deutsche Forschungsgemeinschaft (DFG), grant WE 2652/4-2 to CW.

Impact of PACAP/PAC1 signaling in stress and anxiety: Promising novel targets for the treatment of neuropsychiatric diseases

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Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) is a neuropeptide that was first isolated from ovine hypothalamic extracts in 1989 with neurotransmitter, neurotrophic and neuroprotective properties. PACAP and its preferred receptor PAC1 have been shown to be expressed in brain areas involved in stress and anxiety responses such as the hypothalamic paraventricular nucleus (PVN), lateral septum (LS), amygdala and bed nucleus of stria terminalis (BNST). Intracerebral PACAP administration showed a direct functional interaction with corticotropin-releasing factor (CRF), the main activator of the neuroendocrine stress axis. Moreover, PACAP infusions into the cerebral ventricles lead to behavioral changes that can be observed after stress exposure. However, despite the evidence of an implication of the PACAP/PAC1 receptor system in stress mechanisms, there has been no direct functional evidence for an action of endogenous PACAP in distinct forebrain area on stress responses under ethologically relevant conditions. For instance, the specific role of PACAP/PAC1 receptor system on HPA axis regulation under stress conditions is still unknown. Therefore, aim of the present study was to investigate the role of the PACAP/PAC1 receptor system on neuroendocrine and behavioral stress reactions. We administered a PACAP agonist (PACAP-38) or an antagonist (PACAP 6-38) bilaterally into the PVN, LS or BNST of male Sprague-Dawley rats and tested animals in stress and anxiety-related behavioral tasks such as the forced swim or elevated plus-maze test. In addition, we measured ACTH and corticosterone levels before, during and after stress exposure. So far, we found that intra-PVN and intraseptal administration of the PACAP agonist significantly increased the immobility time and reduced active coping behavior during the forced swim exposure as PACAP-38 treated animals showed enhanced floating and reduced struggling behaviour compared to controls. Thus, our data showed that the PACAP/PAC1 receptor system in the PVN and LS is critically involved in the regulation of behavioral stress function.

Morphologically different G-cells with neuropod-like processes in the antral region of the stomach

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Gastrin-releasing enteroendocrine cells (G-cells) are usually described as flask-shaped cells with a large base and a small apical pole, integrated in the epithelium lining the basal region of the antral invaginations in the stomach. By means of a transgenic mouse line in which the enhanced version of GFP is endogenously expressed under the control of a gastrin promoter, we have analyzed the spatial distribution and morphological and molecular features of G-cells. The results indicate that G-cells are not only located at the basal region of the invagination but to a lesser extent also at the upper region. Visualization of the entire cellular morphology revealed that G-cells show complex morphologies and are not equally distributed along the antrum. While basally located G-cells are roundish-shaped cells which project a prominent apical process towards the lumen and extend basal protrusions containing the hormone gastrin; these processes were frequently found in close vicinity of blood vessels and occasionally close to nerve fibers. Inspection of G-cells in the upper region of antral invaginations disclosed a novel population of G-cells with a spindle-like contour and long apical and basal processes which extend vertically along the antral invagination, parallel to the lumen. Although the functional roles of morphologically distinct G-cells are still elusive, the results of this study are in line with the multiple effects of gastrin in regulating gastrointestinal processes.

This work was supported by the Deutsche Forschungsgemeinschaft.

Noradrenergic modulation of hypothalamic neurons involved in energy homeostasis

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Obesity is a condition that is associated with excessive weight gain and fat mass storage whose prevalence is increasing within western populations. A variety of co-morbidities are linked to obesity such as type 2 diabetes mellitus, cardiovascular diseases and neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease. Together, this contributes to substantial costs of healthcare programs. In non-obese individuals, energy intake and energy expenditure is precisely matched over a long time period in order to maintain energy resources and fat mass. This mechanism, termed energy homeostasis is accomplished by regulatory neuronal networks in the central nervous system (CNS).

To better understand and counteract obesity and its co-morbidities, increasing efforts are being made to define the control mechanisms in the CNS, that regulate body weight and energy homeostasis. Here we focused on the noradrenergic (noradrenaline; NA) modulation of energy homeostasis. Anti-obesity drugs, for example amphetamines, can exert strong anorexigenic effects on eating behavior in humans. However, these drugs generally affect multiple transmitter and neuromodulator pathways, such as the dopaminergic, serotonergic and noradrenergic system, leading to undesired side effects. Pharmacological studies indicate that the anorexigenic effect of amphetamine and related drugs are caused in part by modulation of the NA system. Besides the established role of the paraventricular nucleus of the hypothalamus in NA-mediated modulation of food intake, studies indicate that NA input on the homeostatic system in the arcuate nucleus of the hypothalamus (ARH) might also modulate eating behavior. In the ARH, two key neuronal populations, proopiomelanocortin (POMC) and agouti-related peptide (AgRP) expressing neurons sense and integrate peripheral and nutritional signals. Once activated, POMC neurons promote satiety and activation of AgRP neurons leads to food intake and decreased energy expenditure. Mechanisms that mediate the possible NA action in the ARH are unknown. Therefore, we investigated the effect of NA on POMC and AgRP expressing neurons. Application of NA inhibits POMC neurons, while AgRP neurons are strongly excited. To test which adrenergic receptor (AR) subtypes are expressed within the hypothalamus we performed fluorescence-assisted cell sorting (FACS) of NPY-GFP and POMC-GFP neurons of the ARH. Surprisingly, both cell types express a variety of excitatory and inhibitory ARs, which is also confirmed by specific in-situ-hybridization. To unravel the underlying receptor subtypes mediating the antagonistic effects on both neuron populations, we used pharmacological tools that have high affinities for the different ARs. By specifically antagonizing α_2 -ARs, we reveal that NPY neurons are excited by the activation of α_{1A} – and α_1 -ARs, respectively. Blocking these excitatory receptors unmasked the effect of inhibitory α_2 -ARs. Further pharmacological experiments identified α_{1A} -ARs mediating the strong excitatory effect. In contrast, blocking α_1 -ARs revealed that POMC neurons were inhibited by α_2 -ARs. Again, blocking this effect unmasked the excitatory effect of α_{1A} -ARs. Specific antagonists for α_2 -ARs led to the identification

of α_2 -ARs mediating the strong net inhibitory effect on POMC neurons by NA. Together, this reveals that excitatory and inhibitory ARs are expressed in NPY and POMC neurons but that net effects are excitatory in NPY and inhibitory in POMC neurons, respectively. The strong and differential modulation of the ARH by NA raises the question under which physiological conditions NA is released into the hypothalamus. A combination of behavioral experiments together with cell-type specific activation of NA projections from NA cell groups in the brainstem to the ARH by channel-rhodopsin-2 (ChR2) is performed to answer these important questions.

Taken together, pharmacological experiments revealed that α_2 -, α_1 -ARs and β -ARs contribute to the differential effect of the antagonistic cell populations NPY and POMC in the ARH. The strong modulation by NA indicates a critical role of the catecholamine in the control of energy homeostasis. With respect to these effects, afferent projections from NA nuclei and the physiological conditions under which NA is released into the ARH are of greatest interest. Importantly, further knowledge about the catecholaminergic modulation of energy homeostasis may help to develop new strategies and drugs with minimized side effects in the treatment of obesity.

Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*

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The monoamines octopamine (OA) and tyramine (TA) modulate numerous behaviours and physiological processes in invertebrates. Nevertheless, it is not clear whether these invertebrate counterparts of norepinephrine are important regulators of metabolic and life history traits. We show that flies (*Drosophila melanogaster*) lacking OA are more resistant to starvation, while their overall life span is substantially reduced compared with control flies. In addition, these animals have increased body fat deposits, reduced physical activity and a reduced metabolic resting rate. Increasing the release of OA from internal stores induced the opposite effects. Flies devoid of both OA and TA had normal body weights and metabolic rates, suggesting that OA and TA act antagonistically. Moreover, OA-deficient flies show increased insulin release rates. We inferred that the OA-mediated control of insulin release accounts for a substantial proportion of the alterations observed in these flies. Apparently, OA levels control the balance between thrifty and expenditure metabolic modes. Thus, changes in OA levels in response to external and internal signals orchestrate behaviour and metabolic processes to meet physiological needs. Moreover, chronic deregulation of the corresponding signalling systems in humans may be associated with metabolic disorders, such as obesity or diabetes.

Oxytocin Neurons Activity in Socially Interacting Rats

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The hypothalamic neuropeptide oxytocin (OT) exerts prominent pro-social effects (Grinevich et al, 2016) and hence considered as potential drug for treatment of psychosocial diseases in human patients (Meyer-Lindenberg et al., 2011 Nature Neurosci Rev.). Despite numerous publications focused on pro-social effects of OT, it is still unknown how social interaction affects electrical activity of OT neurons. Recent development of cell-type specific opto- (Knobloch et al., 2012) and pharmacogenetic (Eliava et al., 2016) viral vectors allows us to identify and manipulate OT neurons in freely moving rats. Using these vectors combined with optoelectrode technique (Eliava et al., 2016; Tang et al., 2016 - chapter in Cambridge book) we recorded single OT neuron activity in the paraventricular (PVN) and supraoptic (SON) nuclei in rat hypothalamus during social interaction with unfamiliar conspecifics. Simultaneously we monitored animal behavior by an automated video tracking system (Noldus EthoVision® XT) coupled to recording of ultrasound vocalizations. Our preliminary results show that social interactions induce “miniburst” firing of individual OT neurons (20Hz, 0.5-2sec), which correlates with the distance between interacting rats and their location in the arena, but not correlates with ultrasound vocalizations. In conclusion, the evaluation of intrinsic properties of OT neurons during social interaction might help to dissect sensory pathways controlling OT neuron activity and opens perspectives for translational studies of human psychosocial diseases.

Prosencephalic areas associated to the tonic immobility in pigeons (*Columba livia*): a c-Fos study.

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Tonic immobility (TI) is an innate defensive response to a predator attack. In birds, TI is characterized by immobility of the body and loss of righting reflexes, eventually interrupted by sudden return to standing posture when the danger is perceived as being over. In chickens, the surgical removal of the telencephalon or lesions in the arcopallium (partially comparable to mammalian amygdala) lengthened TI suggesting that prosencephalic mechanisms are relevant to control its duration. TI in pigeons is similar to other birds but the central circuitry involved in its control is poorly known. Here, changes in c-Fos expression were used to examine the prosencephalic structures potentially involved in the control of TI in pigeons. Adult pigeons (8 males and 4 females, 420-500g bw) were assigned to three different conditions (n=4 each): 1- TI; 2- handled control (H); 3- non-TI, non-handled control (C). For TI or H, pigeons were transported from their home cage to a separate room where the TI was induced (TI) or handling was performed for 5 minutes (H). Basal c-Fos activation was examined in pigeons of C group. Ninety minutes after the end of TI or H, pigeons were transcordially perfused under anesthesia (ketamine+xylazine) to brain dissection. One brain section (50 μ m), taken in every 250 μ m, was immunostained to detect c-Fos. Medial hypothalamic areas were double-labeled for c-Fos and corticotrophin releasing hormone (CRH). TI caused significant and selective increase in c-Fos labeling in the intermediate arcopallium (IA), ventral dorsolateral region of the hippocampus (HP-DLv), lateral septal area (SL) and in the medial bed nucleus of the stria terminalis (BSTn-m). Additionally, TI increased the number of double-labeled CRH-c-Fos neurons in the ventral part of hypothalamic paraventricular nucleus similarly in H and TI. Compared to C, higher number of c-Fos cells was also seen in the nucleus Taeniae and dorsal arcopallium in H and TI. Compared to TI and C, the number of c-Fos cells in the posterior nucleus of the arcopallium was higher in H group. These data suggest that TI length, as well other (unknown) attributes of this response in pigeons, may be controlled by a number of prosencephalic areas that are partially comparable to the brain circuits participating in the expression of defensive behaviors in mammalian species. Furthermore, the IA, the HP-DLv, the SL and the BSTn-m areas may contribute to distinctive aspects of the TI in pigeons, while participating (together with hypothalamic CRH cells and other parts of amygdaloid complex) in a more general brain circuit controlling different defensive responses to inescapable stress in this specie. Supported by Capes and CNPq.

PVN Neurons in Mice: Identification, Characterisation, Localisation

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The Paraventricular Nucleus of the Hypothalamus (PVN) is an important autonomic control center in the brain. It contains a heterogeneous population of neuron types that play an important role in regulating autonomic renal and cardiovascular functions, stress responses, and is also crucial for controlling the energy balance. For instance, lesioning the PVN causes hyperphagic obesity in rats (Physiol & Behav 1981; 27(6): 1031-1040) and injection of the melanocortin-4 receptor agonist melanotan-2 into the PVN reduces food intake, while the antagonist agouti-related-protein (and neuropeptide Y) increases food intake (Neuron 1999; 24: 155-163). These results demonstrate, that the PVN is an important integration sites in the hypothalamus for both neuroendocrine and autonomous pathways.

Within the PVN three different types of neurons have previously been identified in rats: Magnocellular neuroendocrine neurons (MC), Parvocellular neurosecretory (NS) and preautonomic (PA) neurons (J Physiol 1991; 434, 271–93). 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 part of the PVN, cell bodies of parvocellular NS and PA neurons are smaller and predominantly located in the anterior and posterior PVN respectively. Furthermore, MC and parvocellular 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. In addition, these neurons differentially express a variety of peptides such as oxytocin, vasopressin, corticotropin-releasing factor, or thyrotropin-releasing hormone (J Comp Neurol 2009; 10(5), 423-41). In rats, PVN neurons can also be identified by their distinct electrophysiological properties (J Physiol 1991; 434, 271–93).

Here we provide a comprehensive electrophysiological characterization of the three PVN neuron types in mice to establish a solid base for future experiments that aim to investigate the modulation of the PVN network under physiological and pathophysiological contexts. By performing perforated patch-clamp recordings in hypothalamic mouse 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 the case of MC neurons, generation of low threshold spikes in parvocellular PA neurons, and typical spike frequency adaptation behavior in parvocellular NS neurons.

Acknowledgments:

We thank H. Wratil for outstanding technical assistance. This work was supported by the Max-Dellbrück-Award and grants from the Deutsche Forschungsgemeinschaft to PK.

Regulation of hypothalamic neuronal function by glucosylceramide synthase (GCS)-derived gangliosides

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Aims:

Neurons of the hypothalamic arcuate nucleus (Arc) are main regulators of energy homeostasis. Neuronal function depends on plasma membrane-located gangliosides. Our studies highlight novel mechanistic concepts for central nervous system regulation of body weight. We have recently demonstrated that leptin and insulin signaling and body weight control depend on glucosylceramide synthase (GCS) in hypothalamic neurons (Nordström et al., 2013, PLoS Biol; Herzer et al., 2015, Diabetes). Further studies shall clarify GCS regulation of hypothalamic NPY and POMC neuronal subpopulations.

Methods:

Body composition, feeding behavior, and metabolism were investigated in mice with hypothalamic GCS deletion. Additionally, receptor signaling in conjunction with gangliosides was analyzed by immune fluorescence, proximity ligation assays and standard biochemical methods in hypothalamic neuronal cell cultures.

Results:

Mice with forebrain-specific GCS deletion display increased body weight and fat accumulation. Fasting mice defend body weight more efficiently. Hypothalamic insulin sensitivity is increased, while leptin sensitivity is impaired. Sympathetic nervous system (SNS) activity and lipolysis are decreased. Increased insulin sensitivity upon pharmacological GCS inhibition has additionally been observed in parallel in vitro experiments in a hypothalamic cell line.

Conclusions:

In a broader context, our results suggest that ganglioside regulation of hypothalamic receptors occurs in a distinct and non-redundant fashion, with individual receptors either being silenced or stimulated. Our data let us surmise that GCS-derived gangliosides are important components for hypothalamic regulation of insulin signaling and subsequent adipose tissue homeostasis. Impaired ganglioside expression in neurons therefore provides a potentially new mechanistic concept for the development of obesity in humans.

SIFamide orchestrates orexigenic and anorexigenic peptidergic signals to promote appetitive and feeding behavior in *Drosophila*

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Animals have evolved interlaced neuronal and humoral systems to control feeding behavior by integrating internal information about metabolic needs with external stimuli signaling the availability and quality of nutrition. Animal behavior is thus not only controlled by neuronal circuits which integrate sensory stimuli and induce appropriate motor responses, but also by physiological parameters whose homeostatic imbalances are signaled to the brain. Here we report that four neurons in the *Drosophila* brain that release the neuropeptide SIFamide integrate orexigenic signals. Activation of SIFamide-releasing cells causes sensitization of olfactory circuits, enhances appetitive behavior and increases food uptake. An anatomical and optophysiological analysis of input- and output of SIFamide-producing cells in the brain of adult *Drosophila* will be presented. This work contributes to the cellular dissection of evolutionarily conserved components that convert peripheral hunger signals into feeding-related behavior.

The role of the endothelial cells in leptin transport into the brain

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Leptin is a 16-kDa peptide produced by the white adipose tissue. After being released in the bloodstream it acts on the brain and controls food intake and energy expenditure. In obese subjects, leptin is no longer able to regulate food seeking behavior although plasma levels are elevated. This phenomenon is called "leptin resistance". A reduced leptin transport through the endothelial cells of the blood-brain barrier (BBB) has been suggested as a cause of leptin resistance. In addition, the choroid plexus and tanycytes are thought to play a role in leptin transport, but until now the mechanisms are still under discussion and the identity of leptin transporters is unclear. Leptin receptor-positive neurons are expressed in several regions of the brain, including hypothalamus and tegmental ventral area (VTA): the first one is the main regulatory center for the so called homeostatic food intake; the second one is involved in the hedonic and motivational value of food intake. In this study, using a specific mouse line with brain endothelial knockout of the leptin receptor ($LepR^{beKO}$), we dissected the two aspects of food intake and the role of this receptor in leptin transport. In particular we found no difference in homeostatic food intake (body weight, food intake, energy expenditure) between $LepR^{beKO}$ and control animals when supplying the mice with high-fat or control diet. Interestingly, the $LepR^{beKO}$ mice showed higher response in a motivational food seeking paradigm. These results suggested that the transport of leptin differs between brain regions. More specifically, tanycytes and choroid plexus may be the key structures regulating the homeostatic value of food intake. According to this concept, brain endothelial cells do not have a role in controlling the peptides transport in areas close to the ventricular system, but they are crucial in leptin transport in midbrain areas like VTA reducing the motivation for food.

The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia

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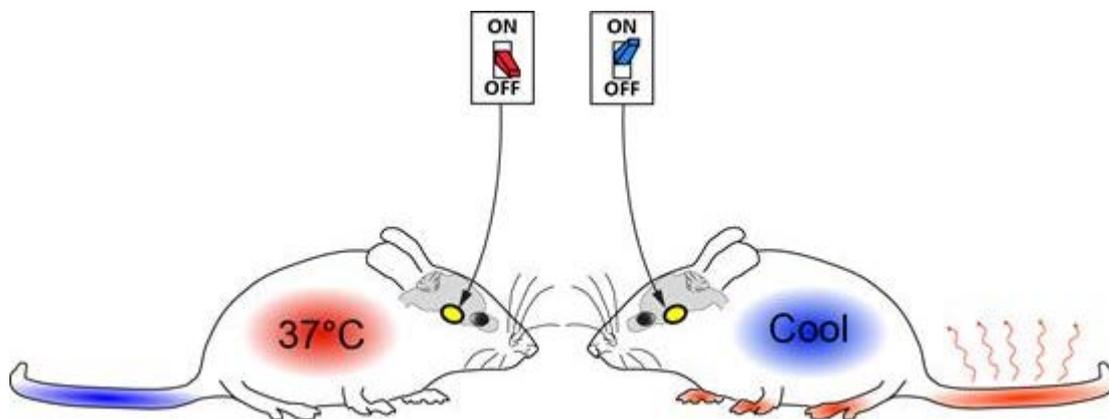
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Body temperature homeostasis is critical for survival and requires precise regulation by the nervous system. The hypothalamus serves as principal thermostat that detects and regulates internal temperature.

We demonstrate that the ion channel TRPM2 is a temperature sensor in a subpopulation of hypothalamic neurons. TRPM2 limits the fever response, and may detect increased temperatures to prevent overheating.

Furthermore, chemogenetic activation or inhibition of hypothalamic TRPM2-expressing neurons in vivo decreased and increased body temperature, respectively. Such manipulation may allow analysis of the beneficial effects of altered body temperature on diverse disease states.

Identification of a functional role for TRP channels in monitoring internal body temperature should promote further analysis of molecular mechanisms governing thermoregulation and foster the genetic dissection of hypothalamic circuits concerned with temperature homeostasis.



Poster Topic

T23: Neural Networks and Rhythm Generators

- [T23-1A](#) Activation and Termination of Rhythmic Activity in a Locomotor Network
Felix Clotten, Carmen R. Smarandache-Wellmann
- [T23-2A](#) Altered Properties of Sharp-Wave-Ripples in the Subiculum of Mice that Underwent Kainate-induced Status Epilepticus
Kristina Lippmann, Anna Maslarova, Zin-Juan Klaf, Seda Salar, Jan-Oliver Hollnagel, Anton Rösler, Uwe Heinemann
- [T23-3A](#) Bistability and Complexity in the Cortical Network *in vivo*
Julia Franziska Weinert, Mattia D'Andola, Lorena Perez-Mendez, Adenauer Casali, Maria V. Sanchez-Vives
- [T23-4A](#) Central and peripheral clocks are coupled by a neuropeptide pathway in *Drosophila*.
Mareike Selcho, Carola Millán, Angelina Palacios-Muñoz, Franziska Ruf, Lilian Ubillo, Jiangtian Chen, Gregor Bergmann, Chihiro Ito, Valeria Silva, John Ewer, Christian Wegener
- [T23-5A](#) Characterization of parvalbumin (PV+) fast-spiking basket cells along the dorso-ventral axis of the medial entorhinal cortex
Sabine Grosser, Federico J Barreda, Sam Booker, Prateep Beed, Dietmar Schmitz, Imre Vida
- [T23-6A](#) Characterization of the daily behavior in single hippocampal neurons
Sinem Meleknur Sertel, Silvio O. Rizzoli
- [T23-7A](#) Circadian pacemaker neurons in the Madeira cockroach *Rhyparobia maderae in vivo* show a prominent evening peak in their electrical activity that is delayed and enhanced via pigment-dispersing factor
Monika Stengl, Julia Gestrich, HongYing Wei
- [T23-8A](#) Comparative analysis of the circadian clock in selected Diptera species.
Pamela Menegazzi, Enrico Bertolini, Marta Beauchamp, Charlotte Helfrich-Foerster
- [T23-9A](#) Components of the molecular circadian clockwork in the cockroach *Rhyparobia maderae*
Achim Werckenthin, Susanne Koziarek, Markus Brand, Monika Stengl
- [T23-1B](#) Consequences of altered dendritic arborization in hippocampal CA1 pyramidal cells—linking molecular signaling, neuronal morphology and electrical signatures
Jana Maurer, Daniela Mauceri, Antonio Yanez, Andreas Draguhn, Hilmar Bading, Martin Both
- [T23-2B](#) Coordinated activity of mitral cells in the olfactory bulb controls the oscillatory entrainment of lateral entorhinal cortex during early development

- [T23-3B](#) Coordinated gamma oscillations in the lateral septum and the lateral hypothalamus drive food seeking
Marta Carus-Cadavieco, Maria Gorbati, Suzanne van der Veldt, Franziska Bender, Natalia Denisova, Franziska Ramm, Karl Deisseroth, Alexey Ponomarenko, Tatiana Korotkova
- [T23-4B](#) Crosstalk between adipokinetic hormone and octopamine to modulate locomotor activity and sleep in *Drosophila melanogaster*
Dennis Pauls, Johanna Räderscheidt, Mareike Selcho, Christiane Hermann-Luibl, Charlotte Förster, Markus Krischke, Martin J. Müller, Christian Wegener
- [T23-5B](#) Determination of the spike discharge pattern in interneurons and pyramidal cells: a proposal for a standardized experimental protocol.
Bernd Sutor, Therese Riedemann
- [T23-6B](#) Differential tuning of neurons coordinating neural oscillators
Anna C. Schneider, Felix Blumenthal, Carmen R. Smarandache-Wellmann
- [T23-7B](#) Electrophysiological and optogenetic methods to trace connections and inputs/outputs of the *Drosophila* clock
Edgar Buhl
- [T23-8B](#) Expression pattern of the neurotransmitter GABA in the circadian clock of the Madeira cockroach *Rhyparobia maderae* with focus on GABA's role in light entrainment
Azar Massah, Monika Stengl
- [T23-1C](#) Gamma-rhythmic input from medial prefrontal cortex to the lateral septum regulates performance in a food-rewarded learning task
Maria Gorbati, Yubin Hu, Marta Carus-Cadavieco, Franziska Bender, Alexey Ponomarenko, Tatiana Korotkova
- [T23-2C](#) Gene Expression in the Plastic Brain of the Pygmy Shrew (*Sorex minutus*)
Moritz Hertel, Javier Lazaro, Marion Muturi, Bernd Timmermann, Dina Dechmann
- [T23-3C](#) Gradient of Synaptic Strength: A Matter of Synapses?
Felix Blumenthal, Carmen R. Smarandache-Wellmann
- [T23-4C](#) Homeostatic scaling of H-current in CA1 interneurons
Dmitri Yousef Yengej, Arnie Boender, Wytse Wadman
- [T23-5C](#) Identification of several somatostatin-expressing interneuron subtypes in the anterior cingulate cortex of the mouse using quantitative classification.
Therese Riedemann, Bernd Sutor
- [T23-6C](#) I_H and I_L involved in rhythm generation and coordination of neuronal activity
Laura Schläger, Carmen R. Smarandache-Wellmann
- [T23-7C](#) Inferring Neuronal Couplings from Dynamic Single-Trial Spiking Data

- [T23-8C](#) Influence of carbachol on firing of dopaminergic neurons lacking NR1 subunit of NMDA receptor
Magdalena Walczak, Kamila Jastrzebska, Jan Rodriguez Parkitna, Tomasz Blasiak
- [T23-1D](#) Intracellular calcium responses to the neurotransmitter GABA in circadian pacemaker neurons of the Madeira cockroach *Rhyparobia maderae*
Maria Giese, Julia Gestrich, HongYing Wei, Monika Stengl
- [T23-2D](#) Neuronal correlates of social behavior in mushroom body extrinsic neurons
Inga Fuchs, Aron Duer, Isabella Hillmer, Benjamin H Paffhausen, Randolph Menzel
- [T23-3D](#) Optogenetic dissection of cellular interactions underlying prefrontal-hippocampal coupling in neonatal mice
Joachim Ahlbeck, Ileana L. Hanganu-Opatz
- [T23-4D](#) Pigment-dispersing factor-immunoreactive neurons in the Madeira cockroach are differentially modulated via their own peptide
Julia Yvonne Gestrich, Wen Shen, Maria Giese, Monika Stengl, HongYing Wei
- [T23-5D](#) Ripples in hippocampal inhibitory networks *in silico* and *in vitro*: Frequency dynamics and response to GABA modulators
Jose R. Donoso, Nikolaus Maier, Dietmar Schmitz, Richard Kempter
- [T23-6D](#) Slowing of theta band activity in the epileptic hippocampal formation
Antje Kiliyas, Ute Häussler, Katharina Heining, Carola A. Haas, Ulrich Egert
- [T23-7D](#) The circadian clock of *C. floridanus*: PER and PDF expression in the brain
Janina Kay, Pamela Menegazzi, Eva Winnebeck, Charlotte Helfrich-Foerster
- T23-8D Retracted
- [T23-9D](#) Using Metabolic Stress for Characterization of Pyramidal Cell Ensembles during Hippocampal Gamma Oscillations
Shehabeldin Elzoheiry, Jan-Oliver Hollnagel, Andrea Lewen, Oliver Kann

Activation and Termination of Rhythmic Activity in a Locomotor Network

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The swimmeret system of the crayfish consists of four paired limbs located on the animal's abdomen and is an easily accessible model for studying locomotion. The segmental organization of the central nervous system provides the opportunity to elucidate the mechanisms of both, the intersegmental coordination of central pattern generators (CPGs) and the coordination of coupled CPGs within one segment (left-right coordination). The general network properties of the swimmeret system and the coordination of CPGs that underlies this coupled activity were previously investigated in detail. Isolated preparations of the abdominal nerve cord are active spontaneously but it is unknown if this activity is caused by an endogenous, excitatory drive or due to the absence of descending, inhibitory input. Consequently it is of great interest to understand the effect of descending input from the brain on the swimmeret system. Both excitatory and inhibitory fibers that either induce or terminate rhythmic activity in the swimmeret system were described at the level of the abdominal nerve cord but so far little information is available about the exact locations within the connectives, the input these fibers receive, or their neural targets within the swimmeret system.

In this comparative study separated axon bundles in the connectives of the abdominal nerve cord were stimulated electrically. Stimulation induced and terminated rhythmic activity in inactive and active preparations, respectively. Histological identification of the stimulation sides confirmed previously described locations of excitatory and inhibitory fibers. In addition, a previously undisclosed location of an inhibitory fiber could be revealed in this study. In the signal crayfish, *Pacifastacus leniusculus*, electrical stimulations always affected both sides of the nervous system in the same manner. Rhythmic activity was initiated or terminated bilaterally to the same extent. In contrast, asymmetric rhythmic activity (i.e. rhythmic activity solely ipsilateral to the stimulated axon bundles) could also be induced in the galician crayfish, *Astacus leptodactylus*. In intact crustaceans this behavior is known as a righting response of the swimmeret system due to spatial movements of the animal. In these experiments, bath application of carbachol, a nicotinic and muscarinic analog of acetylcholine that increases the excitation of the swimmeret system, led to a bilateral activation of the swimmeret system. This suggests that the ipsilateral or bilateral initiation of swimmeret movements depends on the system's excitation level.

With increasing stimulation frequencies the period of the evoked rhythmic activity decreased and more motor neurons were recruited. On an intracellular level, increasing stimulation frequencies led to an increase in the amplitude of membrane oscillation in both neurons of the coordinating networks and motor neurons. These results, in addition with intracellular recordings of motor neurons during sub-threshold stimulations, give evidence that both the swimmeret motor neurons and presynaptic interneurons of the pattern-generating microcircuits are possible targets of command-like fibers within the swimmeret system.

Supported by DFG SM 206/3-1 and UoC ZUK 81/1

Altered Properties of Sharp-Wave-Ripples in the Subiculum of Mice that Underwent Kainate-induced Status Epilepticus

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Sharp-wave ripples (SWRs) are network oscillations in the hippocampus, which occur during rest/slow-wave sleep and represent the replay of firing sequences of place cell assemblies, initially formed during exploratory behavior. SWRs therefore very likely are associated with memory consolidation in the hippocampus. Interictal epileptic events of similar duration and amplitude have been described in the subiculum of epileptic patients in an in-vitro slice preparation, implying a relation and possible transition between these two types of activities.

Using extracellular field potential recordings and intracellular sharp electrode recordings, we investigated the properties of SWRs in acute hippocampal slices from male C57BL/6 mice that underwent status epilepticus, induced by a single injection of kainic acid into the dorsal CA1 region of the hippocampus. SWRs recorded in-vitro from the subiculum of these animals 8 weeks later maintained their usual incidence, duration and amplitude. Similarly to events from control mice, they were sensitive to pharmacological manipulation by glutamate- and GABA-receptor blockers. However, compared to events from naïve mice, they were characterized by an increase in the number of ripples and units/SWR as well as by an increase in ripple and units charge. This change was accompanied with a rise in the ratio of pyramidal neurons that exhibited depolarizing potentials during SWRs (95% vs 70 % in control mice) or fired action potentials during SWRs (47% vs 17%).

These results imply on the one hand side, that SWRs in epileptic specimens might be mistakenly regarded as interictal spikes. The findings may furthermore provide a partial explanation for the pathophysiology of memory impairment in temporal lobe epilepsy.

Bistability and Complexity in the Cortical Network *in vivo*

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Slow oscillations are a class of cortical activity that arise spontaneously during deep sleep and under anesthesia. Slow oscillations appear when the cerebral cortex enters a state of intrinsic cortical bistability, i.e. cortical activity alternating between periods of active neuronal firing (Up states) and silent periods (Down states). Similar to unconsciousness, the cortex shows reduced responsiveness and sensory integration, which has been attributed to an imbalance between functional integration and differentiation. Interestingly, previous studies in humans revealed that the complexity pattern of an evoked cortical response provided a valid measure of functional integration and differentiation, which correlated with the level of consciousness. Furthermore, we have previously shown that causality and complexity were disrupted by bistability in pharmacologically simulated functional states *in vitro*. However, the extent to which the complexity pattern is shaped by ongoing cortical activity is still unknown. Hence, we studied the evoked spatiotemporal complexity pattern of the cortical response to an electrical stimulus *in vivo* 1) under different levels of anesthesia and 2) after activation of the neuromodulatory systems in rats. Our preliminary results show a strong correlation between the complexity of the response and ongoing brain activity. This has implications for the understanding of slow oscillations and their relationship to different states of consciousness, which may provide insights into the mechanisms underlying disorders of consciousness.

Central and peripheral clocks are coupled by a neuropeptide pathway in *Drosophila*.

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Circadian clocks impose daily periodicities to many behaviors and physiological processes. In animals many different tissues are known to contain circadian pacemakers, which have to be synchronized to produce a unified time. The mechanism of clock coupling is poorly understood even though it is highly important for health and performance. In particular, loss of coordination between central and peripheral clocks leads to impaired sleep, jet-lag, depression and is also associated with a number of other diseases.

In *Drosophila* it was shown that the circadian eclosion rhythm depends on a central brain clock and a peripheral clock in the prothoracic gland (PG), which produces the steroid molting hormone, ecdysone. These two clocks restrict the time of adult emergence to a species-specific window within the day. Here we now show by anatomical, behavioral and imaging data that the coupling between the central clock neurons and the PG is mediated by the neuropeptide, sNPF, which transmits time information from the central “master” clock to the prothoracicotrophic hormone (PTTH)-positive non-clock neurons. These peptidergic neurons transmit the information to the “slave” PG clock via the neuropeptide PTTH. This use of two autonomous coupled clocks to control a steroid-dependent process is highly reminiscent of the clock-controlled release of glucocorticoids from the mammalian adrenal gland. Our results indicate that *Drosophila* could serve as a genetically tractable model to dissect the general cellular and neuronal basics of daily steroid rhythms in animals.

This project is funded by the DFG, SFB 1047 “Insect timing”, project B2 (to CW).

Characterization of parvalbumin (PV+) fast-spiking basket cells along the dorso-ventral axis of the medial entorhinal cortex

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The medial entorhinal cortex (MEC), which features spatially modulated grid cells, was found to exhibit a strong inhibitory network in layer II. Although the formation of grid fields is still poorly understood, synaptic inhibition is one of the key factors suggested to coordinate this processes. In fact, in a previous study we found correlation between inhibition and grid field spacing along the dorsoventral axis of the MEC, however, the cellular and synaptic basis of the inhibitory gradient remained unclear. In this study we focused on parvalbumin positive (PV+) fast-spiking basket cells (BC) which represent the majority of interneurons in layer II of the MEC. Using a combined anatomical and in vitro electrophysiological approach, we have characterized their physiological and morphological properties, as well as their synaptic connectivity and output. To facilitate identification of PV+ interneurons for recordings we used transgenic Wistar-VGAT-Venus rats. Our results revealed no differences in the intrinsic properties or morphological feature, including the axonal and dendritic distributions of PV+ BCs between dorsal and ventral levels of the MEC. In contrast, the connectivity between PV+ BCs and closely spaced principal cells was substantially higher in paired patch-clamp recordings in the dorsal vs. the ventral MEC (76.3% vs. 44.2, respectively). Our results thus suggest that a differential convergence of PV+ BC synapses onto principal cells underlie the inhibitory gradient and may explain the scaling of grid fields along the dorso-ventral axis of the MEC.

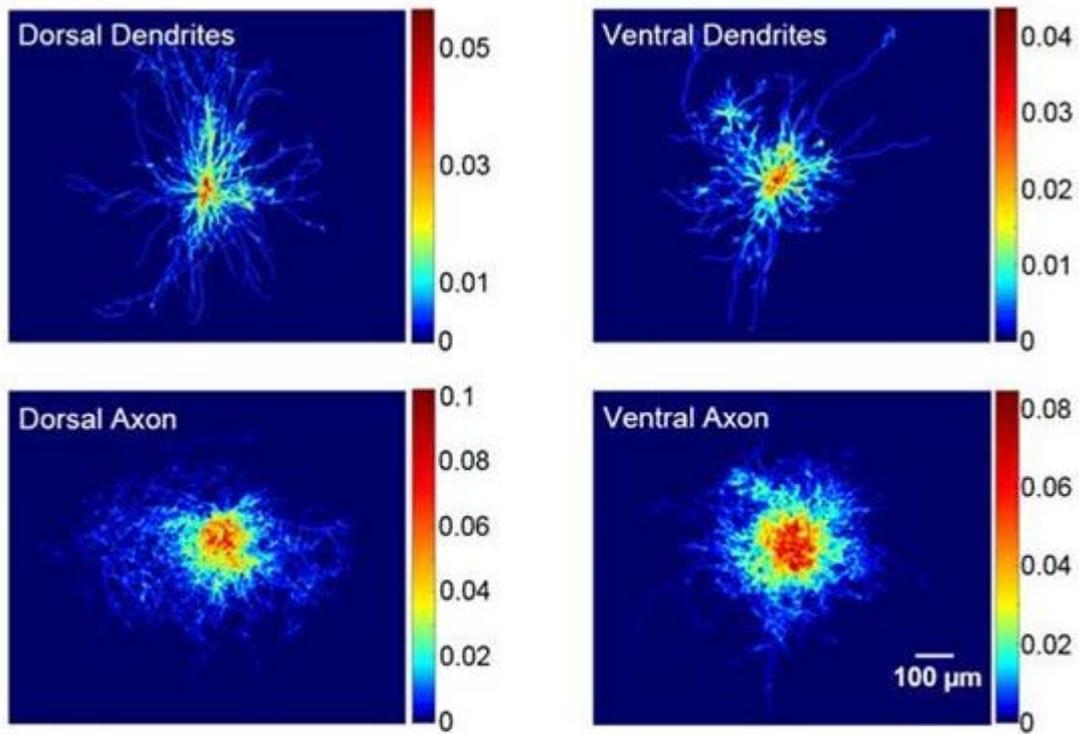


Figure 1: Dendritic and axonal heatmaps of dorsal and ventral PV⁺ fast-spiking BC populations representing the likelihood for each type of neuronal process to be found in a specific location.

Characterization of the daily behavior in single hippocampal neurons

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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 molecular clock rhythm, which is adapted to the cell's function. Molecular as well as cognitive studies have shown that the circadian clock is involved in numerous behavioral and physiological processes. The molecular clock has been shown to have an influence on synaptic vesicle proteins to alter the quantal size of released neurotransmitters, and to induce on ionic channels to generate oscillations in the resting membrane potential. These effects can impact the neuronal depolarization, which is in some cases strong enough to result in long-term changes, including Long-Term Potentiation (LTP). Based on available evidence, it is possible to claim that the molecular clock may interact with memory function by changing the neural activity, especially in the hippocampus, which is the memory consolidation center in the brain, and which has been reported to have oscillations in the expression of the core clock genes. To test whether and how the molecular clock is linked to the activity of single neurons, we monitored the spontaneous neural activity of individual hippocampal neurons in primary cultures, using calcium imaging. The results suggest that neurons show two different states in terms of neural activity: an active or a silent state, which are maintained for a few hours at a time. More importantly, several long-term recordings indicate that the active and inactive states alternate throughout the days. These alternations could be governed by the internal clock, and may be related to the hippocampus function, which will need to be clarified by future efforts.

Circadian pacemaker neurons in the Madeira cockroach *Rhyparobia maderae in vivo* show a prominent evening peak in their electrical activity that is delayed and enhanced via pigment-dispersing factor

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The accessory medulla (AME) with pigment-dispersing factor-immunoreactive (PDF-ir) neurons is the circadian pacemaker center in the Madeira cockroach that controls locomotor activity rhythms. In Noldus-tracking assays the night-active cockroaches exhibit high locomotor activity at night with a peak at dusk and dawn. Furthermore, a short- and a long- period locomotor activity rhythm appeared after lesions of the optic stalk, apparently controlled via two independent neuronal circuits. These findings were reminiscent of the morning- and evening-oscillators that were suggested previously to control bimodal activity patterns in vertebrates and insects alike.

In this study, we searched for the neuronal correlates of morning- and evening-oscillator circuits *in vivo* with extracellular long-term field potential or action potential recordings of the AME of adult male Madeira cockroaches. Since we hypothesize that these two oscillator circuits are recruited via light-dependent PDF-release at dusk and dawn, we analyzed whether application of PDF and/or light affect both morning- and evening-oscillator circuits in phase or amplitude. Furthermore, to examine whether PDF could gate light inputs to the circadian clock we tested whether PDF affects light-responsiveness of AME neurons.

In extracellular long - term recordings from the AME of intact male Madeira cockroaches *in vivo* we found a prominent evening - peak and a small morning - peak in electrical field potentials that were indicative of synchronized synaptic activity at dusk and dawn. Application of PDF increased the amplitude of the evening peak and delayed its occurrence. In addition, during the day PDF application caused AME neurons to switch to bursting activity patterns. Also, light-sensitive neurons in the AME were either activated or inhibited by PDF application and could change their light-responsiveness PDF-dependently. Thus, our findings are consistent with our hypothesis that PDF plays an important role for light-dependent ensemble formation at dusk as well as for gating of light inputs to the clock. [Supported by DFG grants STE 531/18,1-3 and STE 531/25-1 to MS]

Comparative analysis of the circadian clock in selected Diptera species.

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The current state of knowledge of the circadian clock in insects is mostly based on studies of *D. melanogaster* behaviour and genetics. *D. melanogaster* exhibits a strong and reliable daily rhythm in locomotor activity under artificial 12-hours-light : 12-hours-dark regime (LD12:12). This locomotor activity rhythm persists when flies are released into constant darkness (DD), while it is impaired if released into constant light (LL). Specific clusters of neurons (the so-called clock neurons) in the brain of the fly are responsible for driving locomotor activity rhythms. Within these neurons, diverse environmental parameters are translated into rhythmic expression of clock genes and proteins which constitute the molecular machinery underlying the endogenous rhythms.

In diptera species other than *D. melanogaster* the function and structure of the circadian clock have not been so extensively studied. Therefore, we aimed to identify and characterize the specific clock features of selected species of Drosophilidae and Tephritidae flies, in comparison to the well known clock of *D. melanogaster*, both at the neuroanatomical and behavioural levels.

We found that, despite their relatively close phylogenetic distance, insect species within the same order (Diptera) exhibit quite dramatic differences in their circadian clock both at the behavioral and anatomical level. More importantly, it seems that the circadian clock neuroarchitecture is more similar between species that share the same environment rather than between species that are phylogenetically closer to each other.

Components of the molecular circadian clockwork in the cockroach *Rhyparobia maderae*

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Molecular feedback loops are the basis for circadian oscillations that generate rhythms of approximately 24 hours. The molecular circadian machinery of the fruitfly *Drosophila melanogaster* is very well studied, but other insects lack a detailed investigation. The constituents of the molecular circadian clock are mostly conserved in insects, in detail, however, there are major differences. During evolution, some insects have lost or duplicated single genes. For example, the transcriptional repressor *cryptochrome2* (*cry2*) is present in most insects, but absent in *D. melanogaster*, while *timeless* (*tim1*) is absent from the honeybee genome. The function of circadian clock genes was examined in a variety of insects, comprehensive functional molecular studies of basic insects like cockroaches are still sparse though. We used the Madeira cockroach *Rhyparobia maderae*, a well-known circadian model organism, to investigate the function of circadian genes in the molecular feedback-loop system. Transcriptomics, RNA interference (RNAi) and quantitative PCR (qPCR) are the main techniques used in these studies.

Deep-sequencing of brain, antennal and malpighian tubule tissues showed, that all major circadian genes known from *D. melanogaster* and other insects are present in the cockroach *R. maderae*. The core-feedback loop genes *period* (*per*), *tim1*, *clock* (*clk*) and *cycle* (*cyc*), other components of the *clk/cyc* feedback-loop, *vri* (*vri*) and *PAR domain protein 1-ε* (*pdp1ε*) as well as *cryptochrome1* (*cry1*) and *cry2*, *clockwork orange* (*cwo*) and the protein kinases *doubletime* (*dbt*) and *shaggy* (*shg*) are expressed in *R. maderae*. Previously, we could show that *per*, *tim1* and *cry2* oscillate in phase in a circadian manner in the brain of *R. maderae*, and other circadian genes are currently tested for daily oscillations. *Cry1* is only weakly expressed in the brain, but stronger in the antennae and ocelli of the Madeira cockroach. Since cutting the optic nerves renders the animals unable to entrain to circadian rhythms, *cry1* may only be important for the entrainment of peripheral oscillators or be part of the feedback-loop system in those as shown in *D. melanogaster*.

We used RNAi by dsRNA injection to knock-down single or two circadian genes in parallel and investigated the behavior in running-wheels. After the behavioral experiments were completed, the animals were sacrificed and the strength of the knock-down was verified using qPCR. In addition, the effect of the knock-down on other circadian genes was investigated. RNAi works remarkably well in *R. maderae*. Reduction of up to 90% of the transcript was observed one month after injection, and the effect persisted as long as the animals were monitored, up to six months. Neither knockdown of *cry2* nor *tim1* rendered the animals arrhythmic, however, it significantly shortened the period length. *Per* dsRNA injection had various effects, ranging from splitting to arrhythmicity. Even double knock-down of *per/tim1* and *cry2/tim1* did not result in complete arrhythmicity, although both transcripts were strongly reduced. Other circadian genes are currently undergoing investigation. In summary, there appears to be a remarkable plasticity in the Madeira cockroaches' molecular circadian system, and rhythms can still be generated with major components of the circadian clock deactivated. [Supported in part by DFG STE 531/18-1,2 to MS]

Consequences of altered dendritic arborization in hippocampal CA1 pyramidal cells—linking molecular signaling, neuronal morphology and electrical signatures

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Synaptic integration is fundamental for neuronal function and computation. Dendrites are considered to be the input compartment of neurons and they provide powerful options for active modulation and integration of signals arriving at different dendritic segments. The CA1 region of the hippocampus is segmented into several distinct dendritic compartments according to the dendritic morphology and input/output projections. The morphology of dendrites is crucial for integration of signals.

Previous findings showed that the vascular endothelial growth factor D (VEGFD) is involved in the maintenance of dendritic arborization. After down-regulation of VEGFD in hippocampal neurons, a reduction in length and complexity of basal dendrites was observed (Mauceri et al., 2011)¹.

Here, we investigated alterations of information processing due to changes of dendritic arborization in adult mouse hippocampal CA1 pyramidal cells. Following suppression of VEGFD, and thus manipulating dendritic arborization, we performed electrophysiological recordings in vitro in acute hippocampal slices. Additionally, we carried out a detailed anatomical characterization of VEGFD-deficient cells, including 3D reconstructions of apical and basal dendrites and spine density analysis. To link morphological features and electrical signatures, we recorded local field potentials and evoked potentials in vivo throughout all CA1 layers.

Similar to previous findings, basal dendrites became shorter and less complex after suppression of VEGFD. Interestingly, apical dendritic length and complexity was increased. Passive membrane properties and action potential firing properties were unchanged. Further investigations revealed that the elongation of apical dendrites occurs in areas with a high density of synaptic inputs, in particular the stratum radiatum (input from CA3) and stratum lacunosum-moleculare (input from Entorhinal cortex layer III). The spine density in all strata remained unaltered.

In summary, these findings show that the regulation of dendritic geometry by VEGFD follows different mechanisms in apical and basal dendrites. Furthermore, the increase in overall apical spine number indicates stronger synaptic input from Schaffer collaterals and Entorhinal cortex layer III and a decrease in synaptic input to the basal dendrites.

¹ Mauceri D, Freitag HE, Oliveira AM, Bengtson CP, Bading H (2011) Nuclear calcium-VEGFD signaling controls maintenance of dendrite arborization necessary for memory formation. *Neuron*. 71(1):117-130

Coordinated activity of mitral cells in the olfactory bulb controls the oscillatory entrainment of lateral entorhinal cortex during early development

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Coordinated patterns of electrical activity control the maturation of sensory and cognitive abilities. The largely separate investigation of sensory and cognitive ontogeny stems from the fact that most sensory systems are underdeveloped during early life. Thus, their impact on the formation of neuronal networks underlying cognitive processing has been deemed negligible. As a notable exception, the olfactory system reaches full maturity during intrauterine life, controlling mother-offspring interactions and survival. It is, however, still unknown how mitral cells in the olfactory bulb (OB), which directly project to the lateral entorhinal cortex (LEC), shape the functional communication within entorhinal-prefrontal-hippocampal networks during early postnatal development. To fill this knowledge gap, we combined multi-site extracellular recordings from neonatal OB and LEC with patch-clamp recordings from mitral cells *in vivo*. We show that mitral cell firing is timed by discontinuous theta band (4-12 Hz) oscillations and ongoing respiration-related (~ 2.5 Hz) activity in the OB of neonatal mice. These oscillations provide tight coupling by synchrony and directed interactions between OB and LEC. The long-range communication between the two areas is mediated by direct axonal projections from mitral cells to the superficial layers of LEC. These data elucidate the structural and physiological signature of OB-LEC communication during early development. Taking into account the ability of LEC to drive the prefrontal-hippocampal communication at this age, these findings give the first insights into the olfactory control of limbic circuit ontogeny.

Coordinated gamma oscillations in the lateral septum and the lateral hypothalamus drive food seeking

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Lateral hypothalamus (LH) is crucial for the regulation of feeding. Here we studied how LH is regulated by a top-down input from the lateral septum (LS), a key region for governing innate behaviors according to environmental context. To investigate coordination between LS and LH, we combined optogenetics with multisite electrophysiological recordings in behaving mice during spontaneous behavior in the free-access feeding paradigm. We found that LFP in LS and LH displayed prominent gamma oscillations (30-90 Hz) which entrained neuronal activity within and across the two regions. 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, but not the drinking zone. Optogenetic gamma-frequency stimulation of somatostatin-positive (LS-SST) projections efficiently entrained the majority of LH neurons and evoked gamma oscillations in LH. Gamma-frequency stimulation of LS-SST cells or LS-SST-LH projections facilitated food-seeking, i.e. shortened latency to reach the food zone but not the drinking zone or a control zone. It also increased probability of entering the food zone prior to food-free zones, located in other corners of the enclosure. In contrast, food intake was not changed. To explore the necessity of the LS-SST-LH pathway for food-seeking behavior, we optogenetically inhibited the LS-SST-LH pathway during food approach. This behavior-dependent inhibition of LS-SST-LH pathway reduced food seeking. We further identified two function-selective subgroups of LH cells and found that LS-LH gamma-rhythmic input differentially regulate their activity. Our work shows that LS-LH pathway organizes activity of functionally distinct cell groups in hypothalamus and regulates feeding behavior.

We gratefully acknowledge support by the DFG (Exc 257 NeuroCure, TK and AP, SPP1665, AP), The Human Frontier Science Program (HFSP; RGY0076/2012, TK, DB), and GIF (I-1326-421.13/2015, TK).

Crosstalk between adipokinetic hormone and octopamine to modulate locomotor activity and sleep in *Drosophila melanogaster*

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One of the key challenges for every organism is to maintain metabolic homeostasis day after day during the whole life span. Thus, complex circuits exist to integrate neuronal feedback information about energy demands (e.g. based in locomotor activity), metabolic resources and currently available energy based on food intake. Processes dealing with energy storage and remobilization as well as the modulation of behavioral output relies on the action of various neuropeptides and biogenic amines. So far, insect adipokinetic hormone (AKH) is known to function in the regulation of hemolymph lipid and carbohydrate levels and to induce starvation-induced hyperactivity. However, our data suggest that AKH modulates locomotor activity and sleep rather generally than specifically under food deprivation. On top, our work provide functional evidence that the modulation of activity and sleep levels are dependent on the interaction of AKH with neurons expressing octopamine (OA), another important neurotransmitter/neuromodulator in insects, just as well known to modulate both physiological processes and behavioral output. Based on our data we suggest that crosstalk exist between AKH releasing cells and the octopaminergic circuitry, with both additionally signaling onto the fat body, to modulate activity levels and sleep in *Drosophila*.

Determination of the spike discharge pattern in interneurons and pyramidal cells: a proposal for a standardized experimental protocol.

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Within the mammalian central nervous system, there exist more than 20 different subgroups of inhibitory interneurons. These cells have different morphological, biochemical and electrophysiological properties and, hence, in order to classify them, biochemical, morphological and electrophysiological criteria have been established [Ascoli et al., 2008; DeFelipe et al., 2013]. Among the electrophysiological parameters used to classify interneurons, the neuronal discharge patterns obtained in response to suprathreshold current steps are of particular importance. The different types of response patterns are, inter alia, widely used to denominate an interneuron class (e.g. fast-spiking, regular spiking, accommodating, late-spiking, etc.). However, most often, the description of the discharge pattern is qualitative and there are no standardized experimental protocols which can be applied to analyze spike trains. Therefore, the classification schemes display a high degree of variability and it is difficult to compare classifications obtained in different studies. Here, we provide an experimental and analysis protocol, which allows the unique distinction of neurons based on their discharge pattern. Most important, the protocol considers the stochastic nature of neuronal spike train discharges. Specifically, we determined the discharge pattern in an experiment on the basis of the single spike properties, the response behavior following injection of current steps (1 - 2 s) with increasing amplitude ($I = 5 - 10$ pA), the changes of spike parameters during one train, and the stochastic description of the spike train discharge in response to 20 - 30 identical suprathreshold current steps (e.g. interspike intervals [ISI], ISI distribution, relation of mean ISI and its coefficient of variation). In addition, care has been taken to avoid alterations in the discharge pattern by changes in membrane potential, input resistance and/or electrode compensation. The applicability and accuracy of this experimental protocol is demonstrated by means of whole-cell patch-clamp recordings obtained from interneurons and pyramidal cells of the mouse cingulate cortex.

Ascoli GA, et al., Petilla Interneuron Nomenclature Group. Petilla terminology. *Nat. Rev. Neurosci.* 9: 557-568, 2008.

DeFelipe J, et al., New insights into the classification and nomenclature of cortical GABAergic interneurons. *Nat. Rev. Neurosci.* 14:202-216, 2013.

Differential tuning of neurons coordinating neural oscillators

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Neural oscillators need to be coordinated to produce meaningful behavior, e.g. in locomotion. We use the crayfish swimmeret system to study the coordination of distributed neural circuits. The neurons necessary to generate and coordinate locomotion in this system are identified and their connections characterized.

These neurons drive the four pairs of swimmerets in a metachronal wave with approximately 20% phase lag between segments. Each swimmeret is controlled by its own central pattern generator (CPG) located in the corresponding abdominal hemisegment. Ipsilateral CPGs are coordinated by a circuit consisting of three necessary and sufficient neurons per hemisegment: The ascending (ASC_E) and descending (DSC) coordinating neuron, and the non-spiking Commissural Interneuron 1 (ComInt 1). ASC_E and DSC receive the same input from the CPG as the motor neurons. They can thus encode information about their home segment's activity and send it as corollary discharge to the other ganglia. There the information is picked up by ComInt 1. Each action potential of ASC_E and DSC causes a distinct and fast excitatory postsynaptic potential in the target ComInt 1, which integrates the coordinating information in the target CPG.

The intersegmental phase lag is independent of frequency and strength of fictive swimmeret activity. Therefore, we hypothesized that changes in the system's excitation tune the encoding and decoding properties of the coordinating circuit. We used isolated abdominal nerve cords to record intracellularly from ASC_E , DSC or ComInt 1. Excitation was changed by bath application of different carbachol (cholinergic agonist) or edrophonium chloride (acetylcholine esterase inhibitor) concentrations.

We could show that changes in excitation changed ASC_E 's and DSC's tuning curves. To investigate direct and indirect actions of the drugs we measured membrane potential and input resistance (R_{IN}) of these neurons both in the intact network and chemically isolated. R_{IN} of ASC_E decreased, of DSC increased, and of ComInt 1 did not change with increasing system excitation. Only when isolated from the network with TTX in low Ca^{2+} /high Mg^{2+} saline their membrane potential depolarized and drug concentration had the opposite effect on ASC_E 's and DSC's R_{IN} . These opposing effects show that the direct effects of system excitation were usually masked by indirect network effects. ComInt 1 was largely unaffected by changes in excitation. We conclude that the differential direct and indirect effects on the coordinating neurons' tune their encoding properties. As ComInt 1 is not subject to excitation it cannot decode the coordinating information with respect to system excitation but may rather work as hub neuron. Decoding with respect to excitation may then take place in the CPG itself, which is also one source of tuning for the coordinating neurons.

Electrophysiological and optogenetic methods to trace connections and inputs/outputs of the *Drosophila* clock

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To adapt to varying internal and external conditions, all living life forms including insects and humans have evolved circadian clock mechanisms. Circadian clocks regulate changes in behaviour, physiology and metabolism to ensure they occur at certain times during each day allowing adaption to the organism's environment. Disruption of these intrinsic timekeeping processes negatively affects health and well-being and can shorten lifespan. The fly clock consists of 150 neurons grouped into identifiable clusters that sub-serve different circadian functions. Each neuron has a molecular oscillator that switches itself on and then off forming the molecular basis of the circadian clock, which is well conserved from flies to man. In the absence of environmental cycles, the clock free-runs with a period of approximately 24h and is synchronised to the environment mainly by daily changes in light intensity and quality as well as temperature. Individual neurons function as cell autonomous clocks and neurons communicate with each other via electrical and chemical signals and this electrical output signal is important for synchronising these autonomous clocks and for conveying circadian information to the rest of the brain and body. But the precise connections between individual clock neurons, input and output regions are largely unknown. To address this gap and capitalising on fly genetics and pharmacology, I use a combined electrophysiological and optogenetic approach to characterise connections within this highly manipulable and compact clock circuit. I use paired whole-cell recordings in adult fly brains to directly demonstrate connections between the different subsets of clock neurons and characterise the neurotransmitters used by the system. In cases where neurons are not accessible for direct recordings I combine this with optogenetic methods. I can activate a given set of neurons either using light-activated cation channels (ChR2, CsChrimson, ReaChR) or directly by electrophysiological stimulation, while simultaneously either recording from or imaging the membrane potential (ArcLight, Ace2N-2AA-mNeon) of potential postsynaptic neurons.

This novel approach will reveal the connections of the circadian network and lead to a better understanding of the circadian logic, how both sides synchronise, the light and temperature input pathways and finally the output to sleep and locomotor centres.

Expression pattern of the neurotransmitter GABA in the circadian clock of the Madeira cockroach *Rhyparobia maderae* with focus on GABA's role in light entrainment

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The accessory medulla (AME), a small neuropil at the ventromedial edge of the optic lobe's medulla is the circadian pacemaker controlling locomotor activity rhythms in the Madeira cockroach *Rhyparobia maderae*. Neuropeptidergic neurons from 7 soma groups next to the AME innervate the circadian clock. Among them are pigment-dispersing factor (PDF) expressing pacemaker neurons that control circadian locomotor activity rhythms and are also involved in light entrainment in clocks of cockroaches and fruitflies alike. Light entrainment pathways that synchronize the circadian clock with the environment are not well characterized in the Madeira cockroach. So far, no direct connections from histaminergic photoreceptors of the compound eyes to the AME were found. Immunocytochemistry and behavioural assays together with neurotransmitter injections proposed - aminobutyric acid (GABA) as candidate neurotransmitter of photic input pathways. Prominent GABA immunoreactive (-ir) fibers in the distal tract (DT) densely innervate glomeruli of the AME and connect the AME to several layers in the medulla. Therefore, the DT is assumed to relay light information from the medulla to the circadian clock. Consistent with this hypothesis, injections of GABA resulted in a light-like phase response curve. However, it is still unknown where the DT originates from. In addition, many neuropeptides partly colocalized with GABA, i.e. orcokinin, mioinhibitory peptide(MIP), or allatotropin, appear to engage in photic entrainment pathways to the cockroach clock. Here, GABA-ir neurons in the circadian pacemaker network were mapped with focus on the role of GABA in possible light entrainment pathways. Immunocytochemistry revealed that many cells of the six soma groups associated with the AME exhibit GABA immunoreactivity. Five median neurons (MNes), thirteen ventral neurons (VNes), three medial-frontoventral neurons (MFVNes), three distal-frontoventral neuron(DFVNes), three ventromedian (VMNes) and three ventro-posterior neurons(VPNes) exhibit GABA immunostaining. Moreover, double-fluorescence stainings with antisera against GABA and FMRFamide-related peptides and/or the neurotransmitter serotonin revealed that at least one median neuron (MNe) and 2 ventral neurons (VNes) colocalized GABA- and FMRFamide immunoreactivity. In contrast, DT fibers only contained GABA. Furthermore, evaluation of GABA levels in the optic lobe via enzyme-linked immunosorbent assay (ELISA) in the course of a day showed that GABA is increased during the day as compared to the night. Since we hypothesize that light duration regulates neuropeptide/neurotransmitter-synthesis in circadian clock neurons which release these neuropeptides daytime-dependently, we propose that GABA mainly provides light-dependent inhibitions of the AME during the day and at dusk, while it activates/advances the clock at dawn. The DT, in addition, may provide gain control of sensory inputs to the clock. [Supported by DFG grants STE 531/21-1 and 25-1 to MS]

Gamma-rhythmic input from medial prefrontal cortex to the lateral septum regulates performance in a food-rewarded learning task

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Cortical cognitive processing involves gamma oscillations, which support memory, attention, cognitive flexibility and sensory responses. These functions crucially contribute to innate behaviors, including feeding, however, the underlying neural mechanisms are unknown. Combining optogenetics with multisite electrophysiological recordings in behaving mice, we report that gamma oscillations coordinate signaling between medial prefrontal cortex (mPFC) and the lateral septum (LS), a subcortical nucleus, which sends strong projections to hypothalamus. Recordings from mPFC and LS as the animals performed a food-rewarded learning task in the T-maze, revealed that correct choices were associated with an increased count of fast and slow gamma oscillation episodes in mPFC and LS selectively during the choice phase of the task and during the subsequent food approach but not in the start arm of the T-maze. Using excitatory and inhibitory opsins, we further found that mPFC-LS gamma-rhythmic input is causally associated with improved performance in the T-maze: optogenetic stimulation of mPFC-LS projections at gamma frequency led to an increase of the number of correct trials in the T-maze. Further, optostimulation of the mPFC-LS pathway at gamma frequency improved temporal stability of performance, increasing fraction of repeated correct trials compared to repeated incorrect trials. The inhibition of mPFC-LS projections led to an opposite effect, decreasing the performance in the T-maze. This suggests that gamma signaling within the mPFC-LS pathway regulates goal-directed behavior, and contributes to successful performance in a food-rewarded task.

We gratefully acknowledge support by the DFG (Exc 257 NeuroCure, TK and AP, SPP1665, AP), The Human Frontier Science Program (HFSP; RGY0076/2012, TK, DB), and GIF (I-1326-421.13/2015, TK).

Gene Expression in the Plastic Brain of the Pygmy Shrew (*Sorex minutus*)

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Already 1949 August Dehnel published his groundbreaking work on the seasonally shrinking and regrowing skull and brain of some species of the red toothed shrews.

In a population based study he showed that the brain case height is lowered in fall and expanded in early spring again. At the same time the brains of winter animals became lighter and gained weight again in spring. Subsequent studies could show that this changes are not simply due to water content of the tissue but actually reflect substantial differences of some areas in the brain.

We now confirmed that this changes are not only seen on population basis due to a selection process, but are reflecting individual dynamics in skull but also brain morphology.

Here we show that this changes are also reflected in gene expression based on two exemplary brain regions, the hippocampus and the cortex from winter, spring and second year summer animals.

As of abstract deadline samples are sequenced but not analyzed in more detail, for results visit our poster...

Gradient of Synaptic Strength: A Matter of Synapses?

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The coordination of different limb movements to ensure behaviors like walking or swimming is a crucial field of investigation in neuroscience. The swimmeret system of crayfish is an excellent model to understand the neural mechanisms of coordination. During swimming the abdomens four paired swimmerets are coordinated in a metachronal wave propagating from posterior to anterior, whereby each swimmeret is driven by its own module containing a central pattern generator (CPG), four on each side. The coordination of these four ipsilateral, anatomically distributed CPGs is achieved by three neurons located in each hemiganglion forming an intersegmental coordinating circuit. One ascending (ASC_E) and one descending (DSC) coordinating neuron encode the information about the status of their home module and send it to the other abdominal ganglia. The information converges in a nonspiking neuron, Commissural Interneuron 1 (ComInt1), which decodes this information and integrates it into its own CPG. ComInt1 receives these inputs with a gradient of synaptic strength, where the strongest excitatory postsynaptic potential (EPSP) is elicited by the ASC_E from the posterior neighbor. The anterior DSC elicits a weaker EPSP and the most distant coordinating neuron has the weakest input.

One approach to reveal the gradient of synaptic strength onto ComInt1 is to investigate the morphology of synaptic contacts between the three coordinating neurons onto ComInt1. Therefore single coordinating axons were filled with fluorescent dye and the en passant synapses of the coordinating neurons on ComInt1 were marked immunohistochemically with Anti-Synapsin. A triple staining with an intracellular dye filled ComInt1 was so far not successful.

ComInt1 has its soma in one hemisegment, sends its primary neurite dorsally over the midline to the lateral neuropil on the contralateral side where it forms an electrical synapse with one of the CPG neurons. ComInt1 has one ascending and one descending dendritic branch dorsally parallel to the midline. The axons of the coordinating neurons run dorsally, parallel to the midline through each segment, where they have small arborizations at the level of the branches of ComInt1.

At the midline we identified synapses of the coordinating neurons by colocalized presynaptic boutons with the arborizations of the axon of intracellular stained coordinating axons. These colocalizations were dorsally, all along and strictly parallel to the midline of the ganglion. We could calculate areas of colocalizations and count the number of synapses what does not yet explain the three distinct sizes of EPSPs in ComInt1 but it is a first approach to investigate this morphologically.

DFG SM206/3-1

Homeostatic scaling of H-current in CA1 interneurons

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Aims:

Homeostasis and plasticity are of main importance in neuronal networks. Disruptions in either are related to several disorders, including epilepsy. CA1 pyramidal cells can adjust their input resistance and excitability in response to changing inputs by increasing HCN-channels in the membrane (van Welie, van Hooft & Wadman, 2004, Noam, Y. et al., 2010). Here, the input depended H-current scaling and the effect on resonance is examined in interneurons.

Methods:

Whole cell patch-clamp recordings were performed in sagittal slices of 4-6 weeks old male mice brains. H-current was quantified in voltage clamp by hyperpolarizing steps from rest potential to -140mV. Neuronal sub-threshold resonance was measured by injecting a chirp (0.5 – 20Hz) in current clamp. Next, LTX was used to upregulate activity in the slice and thereby upregulate H-current and changes in H-current and resonance were recorded.

Results:

Increased activity upregulates the H-current in CA1 interneurons in a way comparable to the upregulation in pyramidal cells. This furthermore resulted in a change in neuronal resonance in these interneurons. Theta-burst stimulation of the CA1 via the Schaffer-collaterals produced similar results

Conclusions:

In response to increased input HCN-channels are upregulated in interneurons thereby changing the intrinsic excitability and subthreshold resonance. This could contribute to homeostasis and stability of the network

References: Welie, I. van, Hooft, J. A. van, & Wadman, W. J. (2004). Homeostatic scaling of neuronal excitability by synaptic modulation of somatic hyperpolarization-activated Ih channels. *Proceedings of the National Academy of Sciences of the United States of America*, 101(14), 5123-5128.; Noam, Y., Zha, Q., Phan, L., Wu, R. L., Chetkovich, D. M., Wadman, W. J., & Baram, T. Z. (2010). Trafficking and surface expression of hyperpolarization-activated cyclic nucleotide-gated channels in hippocampal neurons. *Journal of Biological Chemistry*, 285(19), 14724-14736.

Identification of several somatostatin-expressing interneuron subtypes in the anterior cingulate cortex of the mouse using quantitative classification.

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A systematic classification of neocortical neurons is indispensable for the understanding of the neuronal circuitry of the neocortex. Modulation of the neuronal circuitry is, for the most part, ensured by inhibitory GABAergic interneurons. Accordingly, malfunction of GABAergic interneurons is associated with many neurological and neuropsychiatric diseases including epilepsy, schizophrenia, major depression and Parkinson's disease. Dysfunction of the GABAergic network in the cingulate cortex in particular has been described in patients suffering from schizophrenia or major depression. In addition, a correlation between the number of somatostatin-expressing (SOM⁺) interneurons in the cingulate cortex and the manifestation of major depression and autism spectrum diseases has been suggested. In contrast to pyramidal output neurons, GABAergic interneurons vary profoundly in terms of their morphological, electrophysiological and molecular properties, adding complexity to the understanding of the neuronal circuitry. Given their enormous heterogeneity, interneuron subtypes are still not well defined. In the study presented here, we focused on the characterization of somatostatin-expressing (SOM⁺) interneurons in the anterior cingulate cortex. Previously, we have shown that SOM⁺ interneurons of the cingulate cortex can be divided into at least seven distinct neurochemical subgroups (Riedemann et al., 2016). The quantitative analysis of SOM⁺ interneurons presented here was performed on 140 biocytin-injected, GFP⁺ interneurons from a SOM⁺ mouse line (FVB-Tg(GadGFP)45704/Swn/J; Oliva et al., 2000). In addition, interneuron properties were compared to 10 biocytin-injected pyramidal neurons. A series of robust physiological, morphological and neurochemical parameters was measured for each biocytin-injected neuron. Next, these data were analyzed to detect possible correlations and SOM⁺ interneuron subgroups. Quantitative classification methods revealed the existence of distinct SOM⁺ interneuron subtypes. Moreover, inclusion of electrophysiological and morphological data led to a regrouping of the previously identified seven SOM⁺ interneuron subgroups. In conclusion, we hypothesize that the properties of a given SOM⁺ interneuron are at least partly shaped by the neuronal circuits that it modulates and we therefore suggest that these interneuron subtypes serve very specific functions within the cortical circuit.

Oliva AA Jr, Jiang M, Lam T, Smith KL, Swann JW. 2006. Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. *J Neurosci.* 20: 3354-3368.

Riedemann T, Schmitz C, Sutor B. 2016. Immunocytochemical heterogeneity of somatostatin-expressing GABAergic interneurons in layers II and III of the mouse cingulate cortex: A combined immunofluorescence/design-based stereologic study. *J Comp Neurol.* 524: 2281-99.

I_H and I_L involved in rhythm generation and coordination of neuronal activity

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The function of nervous systems is based on the interaction of neuronal networks. These networks are built of neurons with individual activity patterns that together regulate and coordinate complex movements and behavior. To better understand the properties of such networks, we investigate the crayfish swimmeret system. Swimmerets are four pairs of abdominal limbs that move in alternating power- and return strokes in a metachronal wave from posterior to anterior. In each hemiganglion a similar subset of neurons can be found that drives this movement. Each microcircuit is composed of five interneurons forming the rhythm generating circuit, three coordinating neurons, and 70 motor neurons (MN). When the system is active, all of these neurons show membrane potential oscillations but with distinct activity patterns. Despite our good understanding of the cellular components and synaptic contacts among them, the intrinsic mechanisms that enable the individual activity pattern still remain unknown. Therefore we are interested in the ionic currents underlying the similar yet different activity patterns of the neurons and how they influence rhythm generation and coordination between segments.

We performed current clamp recordings with sharp electrodes from dendritic arborizations in the isolated abdominal nervous system of the crayfish, *Pacifastacus leniusculus*. To identify and reveal different ionic currents we bath applied selective ion-channel blockers.

After application of channel blockers against the hyperpolarizing activated cation current I_H (ZD7288), the high voltage activated calcium current I_L (Nifedipine) and the transient potassium current I_A (4-AP) we could observe an altered ability of the entire system to produce a steady and coordinated motor rhythm. This led to the conclusion, that the activities of the pattern generating, as well as the coordinating neurons, might be dependent on these currents. To verify this hypothesis cellular properties were started being investigated in a synaptically isolated condition. We detected that some neurons showed the ability to produce a post-inhibitory rebound (PIR). Since PIR has often been shown to be a key mechanism of cells to depolarize upon a phase of inhibition, we wanted to know the ionic basis of the PIR. Despite that none of the investigated neurons showed a sag-potential, likely being induced by I_H , the application of the I_H current blocker ZD7288 reduced the PIR. The additionally application of the I_L current blocker Nifedipine completely abolished the PIR.

These results suggest a special importance of I_H and I_L in generating the well-coordinated rhythmic activity of the system by enabling the neurons to produce a post-inhibitory rebound after receiving inhibitory synaptic input.

Supported by grants from DFG, RTG 1960

Inferring Neuronal Couplings from Dynamic Single-Trial Spiking Data

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Structured spiking activity of neural populations is believed to convey information of sensory signals perceived by an animal. Therefore, to understand the fundamental principles of perception and cognition it is crucial to study the statistical structure of population activity. However, variability and dynamics of recorded activity make this a nontrivial task. Approaches inferring dynamic networks have been proposed that either assume stationarity over repetitions of a behavioral paradigm or reduce the dimensionality of the data. Here, we propose a different approach that does not postulate any stationarity explicitly and still infers a full coupling structure.

Technical description We consider a generative spike train model that generates spike data $\mathbf{S}=\{\mathbf{s}_1,\dots,\mathbf{s}_T\}$ according to a certain state. This state can switch from time to time and hence the statistics of the data change. In detail: We observe a network with N neurons. At each time point t we therefore have a N -dimensional binary vector \mathbf{s}_t . Furthermore, we assume that at each time point the system is in a certain state $z_t=k$. If this is the case the probability of the activity pattern \mathbf{s}_t depends on the coupling matrix \mathbf{J}_k and the previous activity \mathbf{s}_{t-1} . The transition probability is known as the 'kinetic Ising model'. The couplings \mathbf{J} may switch at each time point with probability γ . When the state changes, the next state l is chosen with probability π_l . For a fully Bayesian approach the model assumes priors over all latent parameters $\mathbf{J},\boldsymbol{\pi},\mathbf{Z}$. Thus, the full model describes a Dirichlet process with an additional state switching dynamics.

The exact inference of model parameters is intractable. For approximate inference the state labels \mathbf{Z} are considered to be independent from the rest of the parameters resulting in a variational approach.

We show that inference can be done accurately for artificial data, i.e. couplings \mathbf{J} , state labels \mathbf{Z} and state probabilities $\boldsymbol{\pi}$ are inferred correctly.

Experimental application V4 spiking data recorded while a monkey performed a simple fixation task are analyzed. The model identifies distinct states in ongoing and evoked activity and can differentiate between different stimulus conditions without consideration of trial averages.

The model allows single-trial analysis of neural data while still considering the full connectivity matrix of the network, and thus provides a novel framework to study correlated dynamic spiking data.

Influence of carbachol on firing of dopaminergic neurons lacking NR1 subunit of NMDA receptor

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Dopamine (DA) synthesizing neurons within ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) of the mammalian brain form the core of reward and motivation system. Tonic release of DA into target structures supports animals' basal motivation and motor functions, while phasic increase of DA release signals reward and induces synaptic plasticity. Beside documented engagement of phasic dopamine release in information encoding and memory consolidation, disruption in dopamine release is proposed as one of mechanisms leading to development of depressive-like behaviors. Basal level of DA is mainly maintained by AMPA dependent, tonic or irregular firing of neurons whereas phasic release of DA is induced by NMDA dependent, bursting pattern of firing. The transition between different activity modes is controlled by both excitatory (glutamate) and inhibitory (GABA) inputs, reaching dopaminergic cells. Based on anatomical and electrophysiological results obtained from rodents, also acetylcholine is found to have strong modulatory effect on activity of dopaminergic neurons.

Activation of cholinergic neuronal populations within brain stem nuclei results in phasic, bimodal dopamine release in the striatum and nucleus accumbens. The first, fast onset increase in released dopamine due to activation of nicotinic receptors is followed by sustained increase in amount of dopamine which depends on muscarinic (particularly M5) receptor activation. Thus, both types of cholinergic receptors: nicotinic and muscarinic could be involved in acetylcholine-mediated modulation of dopaminergic neurons activity. It is not known if acetylcholine alone, i.e. without NMDA receptor dependent mechanism, can evoke bursting pattern of firing of DA neurons. Thus, in our study we have extracellularly recorded activity of putative DA neurons in C57BL/6N mice with selective and inducible knock-out of NR1 subunit of NMDA receptor and simultaneously performed iontophoretic application of NMDA and carbachol (CCH). Use of this type of mutation allows to precisely control time and spatial distribution of NR1 subunit deletion, thus giving the possibility to investigate mechanisms of bursting activity independent of NMDA receptors. Both in control and in mutant group similar percentage of recorded neurons displayed robust, prolonged bursting activity during carbachol (non-specific cholinergic receptors agonist) application (7 /42 recorded cells in control group – 17% and 6/54 recorded cells in mutant animals – 11%). However, this type of bursting activity differs to the one observed during NMDA receptor activation.

Our results suggest existence of NMDA-independent mechanism of bursting activity of dopaminergic neurons and bring us closer to understanding the mechanisms responsible for the development of this activity mode.

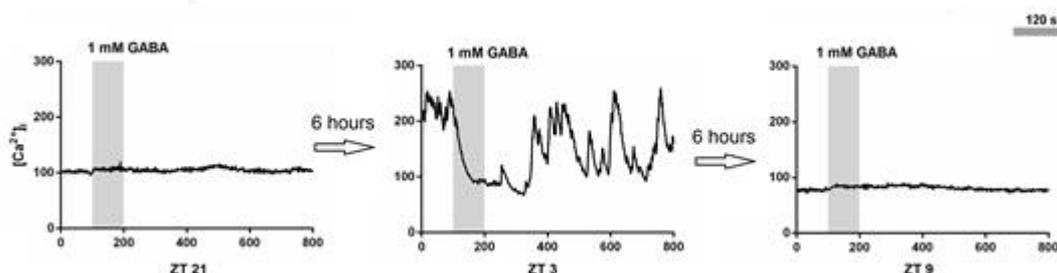
Intracellular calcium responses to the neurotransmitter GABA in circadian pacemaker neurons of the Madeira cockroach *Rhyarobia maderae*

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The accessory medulla (AME) is a self-sustained, synchronized network of neuropeptidergic circadian oscillators that coordinates daily physiological and behavioral rhythms in the Madeira cockroach. Next to an astounding abundance of colocalized neuropeptides, GABA appears to be the main neurotransmitter in the AME. GABA is expressed in inputs, local neurons and outputs of the circadian clock, apparently serving for different functions, such as possibly for light entrainment. At least two GABAergic median neurons that connect the AME with the medulla and the lamina were suggested to be photic entrainment pathways. Also the GABA-ergic distal tract which connects the noduli of the AME with the medulla is a candidate for a light entrainment pathway to the cockroach clock. Consistent with this hypothesis, injection experiments combined with behavioral analysis revealed light-like phase response curves for GABA. Next to photic entrainment, GABA plays also an important role for coupling of circadian pacemaker neurons. Accordingly, extracellular recordings from the AME *in vitro* and *in vivo* revealed that AME neurons are grouped into ensembles of synchronized pacemakers via GABAergic and neuropeptidergic interactions. Here, to analyze possible input signals of GABA into the circadian clock, we employed Fura-2-dependent calcium imaging *in vitro* with primary cell cultures of the AME of adult, male cockroaches. In addition, we also performed extracellular recordings of adult AME to measure GABA-dependent effects *in vivo*. Application of GABA *in vitro* either increased or decreased intracellular calcium levels or spontaneous calcium spikes. As shown *in vivo* GABA could either activate or inactivate spontaneously spiking circadian clock neurons. Interestingly, the same cell could change its response to GABA from inhibition to excitation during a long-term recording. Preliminary pharmacological experiments hint that single circadian pacemaker neurons express chloride transporters allowing for excitatory and inhibitory effects of GABA in the circadian pacemaker center. Indeed, the modulation of intracellular chloride levels mediated via antagonists of GABA-importers and exporters (NKCC1; KCC2) determined the polarity of GABA evoked responses. Future experiments examine whether clock-controlled rhythms in the intracellular chloride concentration is responsible for a daytime-dependent change in the polarity of the GABA response of circadian pacemaker neurons. [Supported by DFG grants STE 531/18-1-3; 25-1]

Figure: Accessory medulla clock neuron in primary cell culture of *Rhyarobia maderae*. Calcium imaging experiments revealed endogenous changes of activity and GABA-sensitivity from ZT 21 to ZT 9.



Neuronal correlates of social behavior in mushroom body extrinsic neurons

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So far no data exist about the neural correlates of social interaction in the honeybee. We record from multiple mushroom body extrinsic neurons during social interaction in a small functioning honeybee colony. The bees cared for the queen, nursed the brood, guarded the exit, cleaned the hive and foraged. The colony used the honeycombs as they would naturally. The recorded bee behaved normal. The weight of the highly flexible twisted triple of wires was counterbalanced by a loose nylon spring. The behavior of both the recorded animal and the hive mates was monitored in infrared by a video camera and tracked. Up to 4 neurons were recorded simultaneously and lasted for up to 47 hours per animal. Spontaneous spike rates were lower than those of similar neurons in harnessed bees. Social interactions, location on the comb and body directions were not encoded by specific neural activities of selected units but rather by the combination of several units.

Neural activity increases frequently during interactions. Furthermore, we find that the variance of spike activity of the units increases suggesting that the neurons sense or control the contacts with other bees. Hints were found that different activity patterns across neurons change with different forms of social interactions. Ongoing analyses including machine learning algorithms are pursued to clarify whether the activity changes are related to, for example, the origin of the approaching bee or the division of labor within the bee colony. The highly variability of neural activity needs further analyses.

Optogenetic dissection of cellular interactions underlying prefrontal-hippocampal coupling in neonatal mice

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Directed oscillatory coupling within prefrontal-hippocampal networks underlies cognitive processing. This communication emerges early in life, long before the maturation of mnemonic abilities, with discontinuous hippocampal theta bursts driving the initial oscillatory entrainment of local prefrontal networks via direct axonal projections. The cellular substrate of this long-range coupling in the developing brain is still poorly understood. To fill this knowledge gap, we optogenetically drove hippocampal CA1 pyramidal neurons while simultaneously recording the local field potential and firing activity within prefrontal-hippocampal networks of neonatal (postnatal day 8-10) mice *in vivo*. Area- (CA1), layer- (stratum pyramidale) and cell-specific (pyramidal neurons) expression of high-efficiency channelrhodopsin mutants was achieved after transfection by *in utero* electroporation. Light stimulation of pyramidal neurons in CA1 area of dorsal hippocampus drives frequency-specific spiking activity and boosts hippocampal oscillatory activity in the theta-beta frequency range. Moreover, selective activation of CA1 pyramidal neurons strengthens oscillatory coupling by synchrony within prefrontal-hippocampal networks. Thus, these data provide the first causal evidence that CA1 pyramidal neurons control the long-range communication between prefrontal cortex and hippocampus in the developing brain.

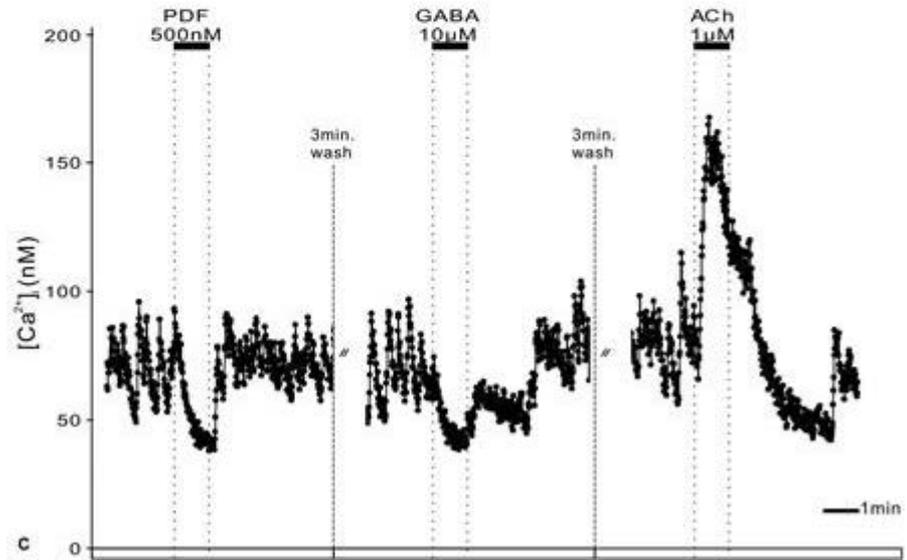
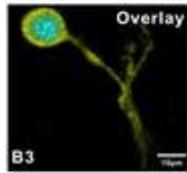
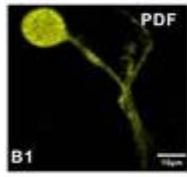
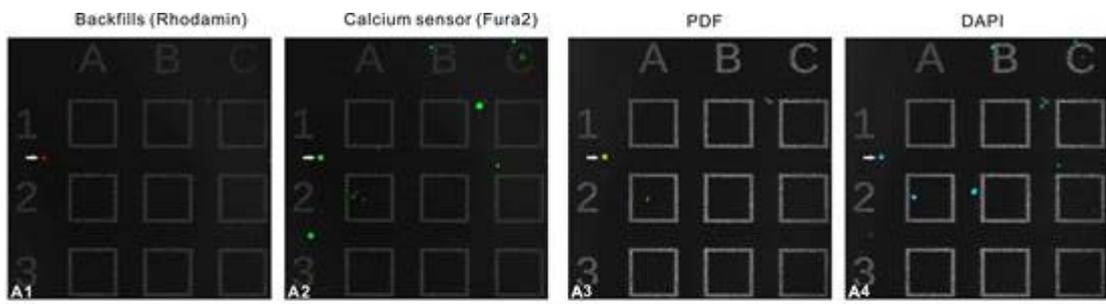
Supported by ERC and DFG.

Pigment-dispersing factor-immunoreactive neurons in the Madeira cockroach are differentially modulated via their own peptide

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Lesion and transplantation studies identified the accessory medulla (AME) with pigment-dispersing factor (PDF) neurons as circadian clock that controls locomotor activity rhythms in the Madeira cockroach *Rhyparobia maderae*. The AME is innervated by about 240 adjacent neurons that are abundant of partly colocalized neuropeptides. Interestingly, often the same neuropeptides such as PDF are present in inputs, outputs, as well as local neurons of the clock. This suggests that PDF-ir neurons connect to each other forming a peptide-dependently "labeled line circuit" via autoreceptor expression. Here, we analyzed the physiological properties and cellular responses of specific PDF-expressing neurons to PDF, GABA, and ACh, focusing on the search for PDF-autoreceptors. Using backfills from the contralateral AME and performing Ca²⁺-imaging and immunocytochemistry on primary cell cultures of the ipsilateral AME we found for the first time PDF-autoreceptors in PDF local and projection neurons of the AME. Only ipsilaterally remaining AME-projection neurons and local PDF neurons of the AME expressed Ca²⁺ level elevations via PDF application. In contrast, all contralaterally projecting PDF-sensitive AME neurons such as the PDF-autoreceptor expressing medium-sized PDF neurons expressed a decrease of spontaneous Ca²⁺ spike-activity after PDF-application. Only the largest PDF neuron did not express PDF autoreceptors. Thus, we found cell-type-specific PDF-signaling. In addition, all PDF-ir neurons receive cholinergic excitatory and GABAergic inhibitory synaptic inputs. Furthermore, using in vivo intracellular recordings we demonstrated that the largest PDF-ir neuron generates bursts of spikes, which could not be regulated by low intensity light during the day. Thus, based on these findings we hypothesize that excitatory PDF-signaling is involved in photic inputs to the ipsilateral circadian pacemaker center, gating ipsilateral clock outputs, while PDF-dependent inhibitions suppress contralateral clock outputs and synchronize both bilaterally symmetric circadian clocks. It remains to be tested, whether the largest PDF neuron regulates rest-activity levels. [Supported by DFG grants STE531/18-1,2,3 and STE 531/25-1 to MS]



Ripples in hippocampal inhibitory networks *in silico* and *in vitro*: Frequency dynamics and response to GABA modulators

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Hippocampal high-frequency ripple oscillations have been implicated in memory consolidation. The network mechanisms underlying the generation of ripples are unclear. Models relying on transiently excited recurrent interneuron networks differ on whether interneurons are predominantly driven directly by Schaffer-collateral input or indirectly via depolarization of local pyramidal cells. Here, we analyzed a physiologically constrained model of the parvalbumin-immunoreactive basket cell network in CA1 under different conditions of excitatory drive to interneurons. Direct drive evoked oscillations constrained to the ripple band (140-220 Hz) that exhibited intra-ripple frequency accommodation (IFA) and frequency resistance to GABA modulators, as *in vitro*. Indirect drive extended the expression of IFA to the fast gamma band (90-140 Hz), as *in vivo*. Our model predicts a maximum oscillation frequency occurring several milliseconds before the peak of excitation in CA1; a trend we confirmed *in vitro*. Taken together, these results unify competing models of rhythm generation by suggesting that ripple and fast gamma episodes are produced by the same interneuron network that is recruited via different excitatory pathways within CA1.

Slowing of theta band activity in the epileptic hippocampal formation

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Mesio-temporal lobe epilepsy (MTLE) is characterized by recurrent spontaneous seizures and histopathological changes in the hippocampal formation. In contrast to the persisting anatomical changes, epileptic activity alternates with periods of putatively normal brain activity including network oscillations such as theta-band activity. In the healthy hippocampus the firing probability of neurons is tightly coupled to that rhythm. We ask how the epilepsy-induced hippocampal pathological reorganization influences properties of network oscillations and the coupling of neuronal firing to these rhythms.

We investigate network oscillations and single cell activity in the intrahippocampal kainate mouse model of MTLE, in which a unilateral injection of kainate into the septal hippocampus induces histopathological changes resembling those in human MTLE. The severity of this restructuring, including mossy fiber sprouting, loss of interneurons and granule cell dispersion in the dentate gyrus (DG), decreases towards the temporal hippocampal pole, but spares the medial entorhinal cortex (MEC). To analyze network activity along this gradient, we implanted electrodes along the axis of the DG and into the MEC to record local field potentials (LFPs) and single cell activity in freely moving epileptic mice.

We show that despite significant changes in connectivity and tissue structure, the theta rhythm and theta-phase modulated firing is present at all septo-temporal positions of the DG in epileptic mice. Theta frequency, however, is systematically decreased throughout DG and MEC, independent of the animal's behavior. Furthermore, theta rhythms in these subregions remain coherent but are shifted in phase. Finally, as in the DG, MEC neurons retain their phase-coupled firing to the theta rhythm.

We conclude that in epileptic mice theta oscillations are prominent LFP patterns that despite pronounced changes in frequency and coupling still entrain neuronal firing.

Support: BMBF (FKZ 01GQ0830) and the Cluster of Excellence BrainLinks-BrainTools (DFG EXC 1086).

The circadian clock of *C. floridanus*: PER and PDF expression in the brain

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As social insects, ants belonging to the species *Camponotus floridanus* are confronted with a number of challenges that need the right timing, like collection of nectar, brood care or mating. To time such behavior, they use an endogenous clock. We investigated the properties of this endogenous clock by recording the locomotor activity of individual *C. floridanus* ants under 12:12h light-dark cycles and constant darkness (DD). We found that most ants were nocturnal and that their activity rhythms free-ran with a period of circa 23 hours under DD. Their period was highly temperature compensated. Immunohistochemical stainings with an antibody against the clock protein PERIOD (PER) revealed PER positive cells in the lateral and dorsal brain. Few PER positive cells in the lateral brain contained the neuropeptide Pigment Dispersing Factor (PDF). The amount of PER cycled significantly in all neurons. PDF-staining furthermore revealed a part of the neuronal network of the endogenous clock in the brain of different morphological castes of *C. floridanus*.

Using Metabolic Stress for Characterization of Pyramidal Cell Ensembles during Hippocampal Gamma Oscillations

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Brain rhythms comprise a broad range of frequencies, which are classified into different frequency bands. The so-called theta, beta and gamma oscillations are examples of brain rhythms, reflecting oscillations in the ranges of 4-7, 14-30 and 30-100 Hz, respectively. Different rhythms are representatives for a wide range of higher cognitive functions such as alertness, exploration etc. We are particularly interested in gamma oscillations, which are known to correlate with sensory perception, motor activity and memory formation and which are associated with high energy expenditure.

In this project, we are using organotypic hippocampal slice cultures to explore the formation and the characteristics of functional ensembles of pyramidal cells during gamma oscillations, including the underlying energy metabolism. To challenge the neuronal ensembles, and particularly fast-spiking GABAergic interneurons, we applied metabolic stress while performing extracellular local field potential recordings. Different oxygen fractions (10%, 5% and 2%) were used in combination with standard (10mM) and low (5mM) glucose concentrations to determine the suitable metabolic stress level for ceasing gamma oscillations, without inducing any pathological activity.

We found that gamma oscillations were persistent with 10% oxygen fraction in standard and low glucose levels, with no significant changes in the power spectrum. A significant reduction in the power of gamma oscillations was observed when 5% oxygen fraction was used. However, gamma oscillations were still persistent, even with low glucose concentration. With 2% oxygen fraction and 5mM glucose, gamma oscillations terminated within 10-15 minutes, without having pathological activity in most of the slice cultures recorded.

We conclude that 2% oxygen fraction in combination with low glucose concentration is a suitable metabolic stress to challenge neuronal ensembles involved in generating gamma oscillations. Currently, this stress level is implemented with tetrode recordings to isolate individual neuronal units.

Poster Topic

T24: Attention, Motivation, Emotion and Cognition

- [T24-1A](#) Ambient noise induces rapid changes in several call parameters in vocalizing marmoset monkeys
Thomas Pomberger, Cordula Gloge, Steffen R. Hage
- [T24-2A](#) Assessing the role of barrel cortex parvalbumin-positive interneurons in whisker detection and discrimination behavior
Nuria Benito, Jens Raymond Vandeveld, Jenq-Wei Yang, Maik C. Stüttgen, Heiko J. Luhmann
- [T24-3A](#) Attention changes firing properties of cells in the Central-complex of freely hunting praying mantises
Anne Wosnitza, Joshua P. Martin, Alan J. Pollack, David J. Bertsch, Roy E. Ritzmann
- [T24-4A](#) Can DC stimulation enhance selective auditory spatial attention in cocktail-party situations? A combined tDCS, ERP and psychophysics study
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Ambient noise induces rapid changes in several call parameters in vocalizing marmoset monkeys

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Any transmission of signals between sender and receiver faces the challenge of being subjected to masking by noise. For acoustic signals, for example, animals have evolved several strategies that aid in increasing the signal-to-noise ratio, thus facilitating signal transmission. One of the most efficient mechanisms is the so-called Lombard effect, i.e., the involuntary rise in call amplitude in response to masking ambient noise. So far, this effect has been observed in several vertebrates, such as birds and mammals. The Lombard effect is often associated with other vocal changes like an increase in call frequency or lengthening of call duration. Recent studies indicate that these changes occur extremely fast and independent of each other suggesting a complex audio-vocal integration system on brainstem level underlying this behavior. In the present study, we further investigated the Lombard effect and its associated vocal changes, fundamental call frequency and call duration in the common marmoset monkey (*Callithrix jacchus*). We investigated how bandpass-filtered noise affected their vocalizations when the band-pass-filtered noise was centered on different frequencies within their hearing range. We found independent changes in call duration, frequency and amplitude related to specific presented ambient noise bands. These different effects that different noise bands had on amplitude, frequency and duration suggest different neural mechanisms underlying these changes.

Assessing the role of barrel cortex parvalbumin-positive interneurons in whisker detection and discrimination behavior

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Primary somatosensory cortex (S1) receives and processes whisker-based touch inputs. In mice, the vibrissae are somatotopically represented by an array of columnar structures referred to as barrel cortex, constituting discrete functional units. The connectivity and functionality of cortical barrel columns have been studied in detail, but the interactions between them, required for intricate sensorimotor behavior, have not been disentangled yet. Recent results from our lab suggest an important role of parvalbumin-expressing (PV) interneurons in lateral inhibition between neighboring barrel columns. PV interneurons constitute about 30% of all GABAergic interneurons in supragranular layers and are known to exhibit *fast-spiking* behavior. Moreover, they electrotonically innervate proximal regions of pyramidal excitatory neurons, where they exert a rapid and powerful inhibitory influence upon action potential initiation (Freund and Katona, *Neuron* 56: 33).

In the present study, we aim to tackle the role of PV inhibitory neurons in lateral inhibition in the context of whisker discrimination behavior. By combining intracortical multichannel electrophysiological recordings with optogenetic modulation of PV interneurons during task performance, we investigate whether this subset of the inhibitory neuron population is involved in tactile discrimination.

Head-fixed PV-Cre mice were trained to emit licks to a water spout following brief whisker deflections (100 ms, 50-Hz pulses), first during a simple detection task (Go/NoGo), and after animals reached success rates >80%, in a discrimination task involving adjacent whiskers. Mice could reliably differentiate the deflection of adjacent whiskers and furthermore were able to reverse the association between the two whiskers and response requirements within only a few hundred trials.

Targeting a specific barrel column *in vivo* by imaging the evoked intrinsic optical signal during vibrissa deflection, we injected pAAV-FLEX-AchT-GFP virus to enable the inactivation of PV cells by shedding light (552 nm wavelength, 159 mW/mm² light power density) onto the specific barrel. After three weeks, the virus expression was checked by epifluorescence microscopy *in vivo*, and silicon probes with 64 recording channels were inserted in S1 covering all cortical layers of two adjacent cortical barrels. Local field potentials and multiunit activity were obtained, and the units sorted in order to identify the active cells and correlate their firing to task performance.

Preliminary results indicate that PV inactivation leads to a slight overall increase of response rate (from 59% to 70%) in the whisker-detection task. Experiments assessing the impact of optogenetic inhibition on whisker discrimination are in progress, as well as recordings of barrel neurons during task performance; together, these experiments will elucidate the mechanism of PV-interneuron mediated intercolumnar lateral inhibition.

Attention changes firing properties of cells in the Central-complex of freely hunting praying mantises

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Complex tasks like hunting a moving prey through an unpredictable environment require high levels of motor sensory integration and decision making. It is crucial to determine when or where to move, to increase chances of finding food while at the same time avoid being detected by a potential predator. A successful hunt also requires the predator to focus its attention on only one of many suitable prey objects, and hence avoid distractions by other movement in its surrounding. In the insect brain, the central complex (CX) is one target area where these complex integrations are likely to take place. Here we performed multi-unit recording in sub-areas of the CX of praying mantises (*Tenodera sinensis*) as they freely hunted several cockroach nymphs. As reported previously for the cockroach CX, we identified populations of cells that showed activity that predicted the animals own movements (Martin et al., 2015, *Curr Biol.* 25:2795-803). However, the praying mantis recordings also tracked the movement of prey. Importantly, our data also revealed activity that monitored movements of one prey while ignoring others. To quantify this effect, simulated prey were oscillated at various angles and distances on an LCD screen positioned below the arena's clear floor. We established altered activity patterns to specific stimuli depending on whether the praying mantis was either focusing its attention on a specific target or was inattentive. These results suggest an important role of the central complex for prey-capture and hence further emphasizes its role in behaviorally- and ecologically-relevant contexts.

Can DC stimulation enhance selective auditory spatial attention in cocktail-party situations? A combined tDCS, ERP and psychophysics study

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So-called *cocktail-party* situations [1], in which a sound source of interest has to be extracted out of multiple distracter sounds, pose a remarkable challenge to the human auditory system. Recently, it has been shown that sound-localization performance under these acoustically complex conditions can be modulated by transcranial direct current stimulation (tDCS) [2]. The aim of the present study was to investigate neurophysiological correlates of the effect of tDCS on selective auditory spatial attention. Human subjects were tested in a free-field multi speaker localization task simulating a cocktail-party situation. In three experimental sessions, anodal, cathodal, and sham tDCS was applied to the right auditory cortex region. Event-related potentials (ERPs) were recorded while subjects performed a task, in which the position of a one-syllable target word, presented simultaneously with three distracter speech stimuli, had to be identified. Data were collected prior to tDCS, immediately after tDCS, and 1 h after tDCS. With reference to sham tDCS, anodal, but not cathodal, tDCS was found to improve the percentage of correct judgments on target location. This effect was restricted to targets in right hemispace. For this condition of auditory stimulation, ERP difference waveforms (anodal minus sham tDCS), obtained in trials with correct responses, showed a broad fronto-central negativity in the time range of the P2-N2 complex. This complex - in particular the N2 component - has been related to the orientation of auditory spatial attention toward a speaker of interest [3,4]. It thus seems possible that tDCS may have modulated processes of selective attention associated with target localization.

This study was supported by the German Federal Ministry of Education and Research (BMBF grant 01GQ1424E) in the framework of the TRAIN-STIM project.

[1] Cherry EC (1953) Some experiments on the recognition of speech, with one and with two ears. *J Acoust Soc Am* 25: 975–979.

[2] Lewald J (2016) Modulation of human auditory spatial scene analysis by transcranial direct current stimulation. *Neuropsychologia* 84: 282–293.

[3] Lewald J, Getzmann S (2015) Electrophysiological correlates of cocktail-party listening. *Behav Brain Res* 292: 157–166.

[4] Lewald J, Hanenberg C, Getzmann S (2016) Brain correlates of the orientation of auditory spatial attention onto speaker location in a "cocktail-party" situation. *Psychophysiology* 53: 1484–1495.

Central amygdala circuit mediates observational transfer of fear

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Emotional contagion, i.e., sharing emotional states between individuals, is considered to be the simplest form of empathy. Even though this phenomenon was reported in several species in behavioral experiments, the neuronal circuits necessary for sharing emotions are poorly understood. Usually similar brain structures that are activated in the subject of emotion ('demonstrator') are also implicated in emotional contagion (in the 'observer') - a phenomenon which is known as activation of 'shared circuits'. However, the functional role of the particular neural circuits involved in emotional contagion is to large extent unknown.

To address this problem we used a rodent model of fear contagion combined with optogenetics and functional anatomical tracing. In our paradigm one of the rats (observer) watched its cage-mate (demonstrator) undergoing contextual fear conditioning. This type of stimulation elicited robust freezing in both rats as well as increased number of 22-kHz (aversive) vocalizations. Moreover, the freezing time in demonstrator predicted the freezing time in observer across individual pairs.

Since the central nucleus of the amygdala (CeA) is critical in fear learning and controls defensive responses, we hypothesized that it also contains the neural circuits controlling observational fear. To test this hypothesis we used optogenetic stimulation of CeA neural circuits activated by observational transfer of fear. A viral vector carrying channelrhodopsin 2 (ChR2) was injected into the CeA of the observer rats. The ChR2 was placed under c-fos promoter to ensure that the protein expression would be limited only to the population of cells activated by the task. Two weeks after the surgery the animals underwent observational transfer of fear; the control group was only exposed to the experimental cage. 24 hours later the rats were placed in a modified version of open field and tested for fear and anxiety. Stimulation of the CeA observational fear circuit resulted in a robust increase of avoidance behavior.

To study the connectivity of cells activated by observational fear we used transgenic Venus-PSD95 rats, in which endogenous green fluorescent Venus protein is expressed under control of c-fos promoter. The rats were injected with anterograde PHA-L tracer into the CeA and subjected to observational transfer of fear. We observed activation of neurons in several brain structures related to fear and anxiety. A large proportion of these cells were innervated by the CeA. The results show that the CeA neural circuits activated by observational fear are involved in control of defensive behaviors.

Cognition, but not personality, is related to faecal stress hormone metabolites in the smallest non-human primate aging model (*Microcebus murinus*)

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It is known from mammals, that individual stress hormone concentrations can be linked to interindividual differences in personality and cognition. The goal of the present study was to examine for the first time the relationship between cognitive abilities, personality traits, and individual mean faecal glucocorticoid-metabolite (FGCM) levels - as a measure for the subject's stress level - in the smallest primate aging model, the grey mouse lemur (*M. murinus*).

In total, a group of 42 individuals (22 females, 20 males; 32 young adults 4 years, 10 aged adults 7 years) was used. Individual personality scores for "anxiety" and "exploration" were determined using a behavioural test battery (i.e. emergence-, open-field-, and novel-object-test) and a subsequent principle component analysis. Learning performance and cognitive flexibility were assessed using a touchscreen-based visual pairwise discrimination/reversal (CANTAB PD/PDR; N=16) paradigm. Faeces were collected every 7 days (3 samples/animal) and analysed using an enzyme immunoassay.

Findings did not reveal sex- or age-differences in mean FGCM concentrations. Neither anxiety nor exploration correlated significantly with FGCM, whereas cognitive flexibility was significantly linked to FGCM: individuals with a low FGCM level made less perseverance errors during reversal learning and were faster in reaching the re-acquisition criterion in the PDR task. Thus, FGCM level predicts cognitive flexibility in a standardized task.

Cognitive deficits in Alzheimer's (AD) patients are also discussed to be linked to stress and personality changes. Since mouse lemurs are used in AD research, FGCM measurements may represent a predictive tool for AD-like neuropathologies in this primate brain aging model.

Project funding was provided by the European Community (FP7/2007-2013; #278486-DEVELAGE)

Comparative characteristics of auditory and visual emotion perception in the primary school age children and their impact on scholastic performance.

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Investigation of the emotion perception specifics is essential for understanding the development of human perceptual-cognitive mechanisms. Studies on the psychophysiological features of recognition of the emotional signals (expressed by tone of voice or by facial expression) suggest the importance of the process for social communication, adjustment to stressful situations, training and academic progress, etc. It has been shown that there are specific features of the relationship between psychophysiological parameters of perception of these two stimuli modalities in adults' population. However, whether and how such features are changing in age course is still not fully clear. Our study considers the comparison of behavioral characteristics of emotions' identification by the primary schoolchildren depending on the type of emotion and the sensory modality used for stimulus presentation and their relationship with scholastic performance.

Materials and methods: Sample consisted of 32 pupils of the second grade of St. Petersburg state school (18 boys and 14 girls, mean age 8.74 ± 0.05). Russian was a mother tongue for all the participants. We have developed the test that includes visual and auditory non-verbal stimuli of four basic emotions (happiness, anger, sadness, fear). In this test, we have used the JACFEE procedure based on emotion recognition of facial expression from photographs and our previous procedure based on recognition of emotional intonation of utterances from the created earlier corpus of speech signals. In procedure we have used the standard method of forced choice: children had to choose one from four answer choices (from four basic emotions). The accuracy of recognition (AR) of nonverbal emotional information for each of the presented emotions in each modality has been calculated. End-of-year grades in language ("reading" and "writing") and math were obtained for each pupil from school offices and ranged for both subjects from 3 to 5. We have used the ANOVA, Mann-Whitney test, correlation and regression analyses to examine the data.

Results. We have found that in visual perception, the identification of emotion is more accurate ($p < 0.05$) and has lower a posteriori probability errors. But though the factor "presentation modality" has a significant impact on the emotion recognition ($p < 0.0005$), the "type of emotion" is the first most important factor ($F = 62.417$, $p < 0.0005$). The interaction of them is highly significant ($p < 0.0005$) as well. The higher accuracy of recognition in the visual modality is achieved through emotions of "joy" (100%) and "anger" as compared to auditory one; the perception accuracy of emotions "sadness" and "fear" is almost equal for both modalities. The obtained variance in recognition accuracy of different emotions confirms the unevenness of perception mechanisms development in primary school children. The lack of correlation between the results of emotions recognition in visual and auditory modalities indicates the independence of processes of emotion recognition in them. We have not found the unequivocal evidence of the impact of the emotion recognition ability on scholastic performance in this age group, though the highly significant relationship between school achievements in "reading" and AR of sad and happy intonations was revealed. It is important to consider this problem further involving the greater samples and school children of different ages.

Changes of the c-fos and p-CREB/CREB ratio in the nucleus accumbens, hippocampus and prefrontal cortex during extinction and reinstatement of morphine-induced conditioned place preference: The role of NMDA receptor

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Glutamate mesocorticolimbic pathway and dopamine (DA) system have a closely relationship in the reward phenomena, enjoyment, arousal and memory. This neurocircuitry comprised from ventral tegmental area (VTA), nucleus accumbens, hippocampus (HIP), amigdala and prefrontal cortex (PFC) that are neuronal regions underlying drug-induced reward and can change the firing frequency of dopaminergic neurons in the reward system. The most studies indicated that alterations in phosphorylated cAMP response element binding protein (p-CREB) and c-fos in the regions associated with reward phenomena is related to drugs exposure. So, we design this study for elucidate the changes in p-CREB and c-fos in NAc, HIP and PFC after intracerebroventricular (ICV) administration of different doses of AP5 or vehicle during extinction period or reinstatement of morphine-induced CPP in rats.

Materials and methods: Forty eight adult male albino Wistar rats weighing 240-290g have done the CPP procedure; after extinction period or reinstatement, we dissected out the NAc, HIP, and PFC regions and estimated the p-CREB/CREB ratio and c-fos level by Western blot analysis.

Results: The results indicated that above factors were dose-dependently decreased in comparison with vehicle group (saline) after ICV administration of different doses of AP5 (except for p-CREB/CREB ratio in pre-reinstatement group in the NAc and c-fos level in pre-extinction-reinstatement groups in HIP). Our findings revealed that antagonism of NMDA receptor decreased p-CREB/CREB ratio and c-fos level in the, NAc, HIP and PFC that are involved reward memory and cell activation, respectively.

Discussion: So, it seems that blockade of the NMDA receptor disrupt connection between drug memory and context may be useful for decrease of extinction period of drug-induced reward and attenuation of cue-induced drug reinstatement.

Comparison of Optogenetic and Electrical Intracranial Self-Stimulation of the VTA in Mice

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Optogenetic methods have revolutionized many fields in neuroscience, due to their cell-type specificity and high temporal fidelity. However, electrical stimulation methods have been used in a large body of existing literature and continue to be important, especially for use in humans. It is therefore necessary to qualitatively and quantitatively compare optogenetic and electrical stimulation. In this study, we imaged the differences between optogenetic and electrical stimulation of the VTA in two different transgenic mouse models (TH::Cre and DAT::Cre mice). We transduced 10 male TH::Cre-positive and 10 male DAT::Cre-positive C57Bl6/J mice with ChR2 and implanted a custom-made optostereotrode into the left VTA. This stereotrode allows for optical and electrical stimulation of the same site in the VTA. In a behavioral task, mice had to lever-press for optogenetic or electrical stimulation using light or current of varying intensity. This procedure allowed us to determine a point, at which electrical and optical stimulation had equal behavioral effects. Using rCBF-sensitive 99mTc-HMPAO-SPECT, we found that the networks active during VTA stimulation differ between the well-established TH::Cre genotype and the DAT::Cre genotype. Applying behaviorally equal stimulus strengths, similar activity patterns in the nucleus accumbens, medial prefrontal cortex, Raphe nuclei and dorsal striatum were observed in result to either optical or electrical stimulation. Our results indicate that at iso-behavioral strengths, optogenetic and electrical stimulation of the VTA give rise to surprisingly similar activity patterns.

Effects of Reward-Associated, Task-Irrelevant Unimodal and Bimodal Distractors on Target-Directed Oculomotor Task

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Our brain is constantly faced with abundant sensory input. In order to efficiently process sensory information, the brain relies on top-down information and allocates more processing resources to the sensory cues that are more important, resulting in stronger sensory representation among the other stimuli. For example, it has been shown that the stimuli associated with high reward value are more salient than those associated with low reward value in an oculomotor task. However, in previous studies, the reward-associated stimuli were presented unimodally (mostly visually), therefore cannot account for the daily scenarios where the sensory information from various modalities are extracted from the same object or event. We consider two alternative hypotheses regarding how reward information could be extracted from such a multimodal object: 1. reward information of various sensory modalities is combined in a purely additive manner, irrespective of the task setting and identity of each sensory modality 2. Integration of reward information occurs in a task and modality-specific manner where the brain prioritizes sensory information detected from a certain modality in order to achieve a relevant, specific goal.

To test these alternatives we designed a behavioral task. Subjects learn reward pairings of two colours and two sounds while performing a simple localization task. In a separate task, they are instructed to make an eye movement towards a target circle, either vertically above or below the fixation point. A unimodal (visual or auditory) or a bimodal (visual and auditory) distractor is presented simultaneously with the target circle. The distractor's colour or sound is previously associated with either high/low reward value. When presented bimodally, the distractor's colour and sound could either be congruent or incongruent in their amount of reward (high/high or low/low versus high/low or low/high, respectively). Our preliminary results reproduce the previous findings showing that unimodal reward information influence oculomotor responses (Hickey and van Zoest, 2012). We hypothesize that the effect of reward is enhanced in bimodal, reward-congruent distractors, thus matching the predictions of an additive integrator. By analyzing the pattern of eye movement deviations in the incongruent condition we can reveal if such a purely additive model can account for bimodal reward information or whether more sophisticated task/identity-specific integration is required.

Electrophysiological signatures of negative and positive polarity processing in German sentence comprehension

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How single words are processed in human language depends on contextual information and accompanying reasoning processes. A prototypical case for how context is used in sentence processing becomes evident in the property of polarity. Negative polarity items (NPIs) such as German *jemals* 'ever' tend only to occur in negative contexts (i), whereas positive polarity items (PPIs) such as German *schon* 'already' tend only to occur in positive contexts (ii).

- i. Kim hat {keinen Kuchen / #den Kuchen} jemals oft gebacken.
- ii. Kim hat {#keinen Kuchen / den Kuchen} schon oft gebacken.

Polarity sensitivity has been a key field of research in generative linguistics, as it is revealing w.r.t. the internal structure of language, i.e. how different aspects of grammar (syntax, semantics) and pragmatics interact with one another (cf. Csipak et al 2013). One of the unresolved questions concerns whether and to what extent NPIs and PPIs are parallel. While there is no consensus on this in theoretical linguistics, previous ERP studies show that the on-line processes of NPIs and PPIs are different. While there is evidence for the occurrence of an N400 component in response to negative polarity violations, the findings for positive polarity violations are inconsistent and include both an N400 and a P600 component, or only a P600 component (Saddy et al 2004 , Yurchenko et al 2013). The present ERP study is aimed at resolving this inconsistency using the German polarity items *jemals* vs. *schon* and an additional control condition that includes the neutral adverb *sehr* 'very'.

The study uses a two factorial design with the factor 'polarity item' (i.e. NPI, PPI, neutral) and 'context' (i.e. negative or affirmative). We are interested in the two critical conditions NPI+affirmative (e.g. #*Kim hat den Kuchen jemals oft gebacken.*) and PPI+negative (e.g. #*Kim hat keinen Kuchen schon oft gebacken.*). Liu (2012) argues that positive polarity violations result from the syntax-semantics interplay. Based on this, we expect that positive polarity violations should elicit both N400 and P600. W.r.t. negative polarity violations, we expect to reduplicate the results of Saddy et al (2004) and Yurchenko et al (2013).

We measured ERPs of 24 adult native speakers of German. The results were inconsistent with both the previous studies and our theory-based hypotheses: Negative polarity violations elicited both N400 and P600, while positive polarity violations elicited only an N400 but no P600 component. The results will be discussed in the light of both linguistic theory and contributing cognitive processes.

Habenula and interpeduncular nucleus differentially modulate odor-induced innate fear behavior: in vivo SPECT-imaging and lesion studies

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Fear is an important evolutionary conserved behavioral system helping humans and animals to survive potentially dangerous situations. Fear-inducing stimuli can be innate and learned. Whereas the neural circuits underlying learned fear are already well investigated, the knowledge about the circuit mediating innate fear is still limited. We here used in vivo single-photon emission computed tomography (SPECT) imaging of cerebral blood flow to determine the spatial patterns of neural activity in unrestrained behaving rats exposed to the predator odor fox urine. Upon odor exposure blood flow increased in a number of brain regions previously associated with innate fear but, unexpectedly, decreased in the interpeduncular nucleus (IPN). Blood flow increased in the habenula (Hb) and correlated with odor effects on defensive strategy. Hb lesions reduced avoidance but increased risk assessment behavior while IPN lesions only reduced avoidance behavior without affecting risk assessment. Our study identifies a new component, the IPN, of the neural circuit mediating odor-induced innate fear behavior in mammals, and suggests that the evolutionarily conserved Hb-IPN system forms an integral part of this circuit. Pathological changes within this system may play also a role in the pathoetiology of psychiatric diseases such as anxiety and mood disorders.

This work was funded by the federal state of Saxony-Anhalt, Project: Center for Behavioral Brain Sciences (CBBS).

Imaging the functional networks activated by optogenetic stimulation of the VTA in rats.

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Dopamine (DA) released from ventral tegmental area (VTA) plays a significant role in addiction, learning and motivation. Dopaminergic projections target a large number of brain structures, such as for example nucleus accumbens, hippocampus, prefrontal cortex or sensory cortex. However, while the anatomical connections are well described, the functional networks recruited during VTA activation are less known. To investigate these networks, we injected a viral vector carrying an opsin into the VTA of wild-type (WT) and TH::Cre rats. In WT rats the opsin was expressed in principal cells, in TH::Cre rats the opsin was expressed only in dopaminergic cells. The animals were trained in an intracranial self-stimulation task (pressing a lever for optogenetic stimulation of the VTA) and no differences in press-rates between WT and TH::Cre rats were observed. We then passively stimulated the animals optogenetically in the fMRI scanner to image the activated networks. In response to stimulation, the BOLD signal increased in the VTA, NAcc, mPFC and tectum in WT rats. This pattern is compatible with the known main projections of the VTA. Surprisingly, in TH::Cre rats, where stimulation was much more dopamine specific, only slight increases of BOLD could be observed. While these results confirm the existence of dopamine-release induced metabolic changes in the brain, the magnitude of the effects is incompatible with the results from human imaging studies. Our findings therefore highlight the importance of exercising care in attributing observed BOLD changes to dopamine release and VTA activity.

In-hive monitoring of social communication by electrostatic fields in common honeybee colonies

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In honeybee colonies communication between foragers and young bees plays a major role for the foraging success of the whole colony. The overall health of the whole colony is reflected in these multiple communication processes. Besides communication via pheromones, movements of the whole body and especially of the wings are used to encode information. Because the body of foraging honeybees charges up electrostatically during flight it is possible to detect any movements of the body by recording the modulated electrostatic fields emanating from the bees and record it via appropriate electric field sensors.

Electrodes aliened on two combs were used to capture the electrostatic signals in different colonies. The main advantage of this technique is that the colony could be kept in natural conditions because no light and space is needed for the recordings. Long term recordings are possible because bee wax doesn't reduce the quality of the signals, a property that offers multiple opportunities and is big advantage over sound recordings.

In this study we have extracted the occurrence of the well-studied waggle dances and stop signals in order to collect information about the activity and health state of the respective colony. Further we quantified the non-communicaton behavior of fanning, which is used to optimize the in-hive climate and to circulate pheromones. The change over time of these signals was correlated to known environmental changes, like temperature and humidity, the time of year and day and special events inside the hive, like parasite treatment, which were documented by collaborating beekeepers.

Interactive effect of menstrual cycle and dopamine baseline levels on Stroop and N-back tasks.

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Sex hormones, particularly estradiol, have extensive effects on the dopaminergic system, affecting the synthesis, release and turnover of dopamine (DA). This neurotransmitter plays an important role for executive control functions in an 'inverted U-shaped' manner with different dopamine optima for different functions. Related to these neuroactive effects, cognitive performance and behaviour change along the menstrual cycle. Consequently, changes in performance are expected to vary according to sex hormones levels and individual differences in DA baseline levels. This has been previously reported for working memory and associated with changes in DLPFC activity (Jacobs and D'Esposito, 2011). Nevertheless, the modulation of sex hormones effects by the DA baseline levels has not been generalized to other tasks so far. DA levels have previously been assessed either peripherally (from blood samples) or genotyping. As a non-invasive alternative, the eye-blink rate (EBR) has been suggested as being indicative of striatal DA levels. Therefore, we aim to replicate and extend the previous findings of DA dependent changes in performance across the menstrual cycle in working memory and inhibitory control functions. Furthermore, we intend to demonstrate the usefulness of the EBR as DA indicator in menstrual cycle research.

In order to study the interactive effect of menstrual cycle phases and DA baseline levels on performance, 36 women with natural menstrual cycle were tested in three different occasions locked to their cycle phases (menses – low progesterone and estradiol; pre-ovulatory – high estradiol; luteal – high progesterone and estradiol) and order counterbalanced. During each session, women performed a verbal N-back task, as measure of working memory, and a Stroop task, as measure of cognitive flexibility. Hormone levels were assessed from saliva samples and spontaneous eye blink rate (EBR) was recorded during menses as an indirect measure of striatal DA levels. Statistical analyses were carried out in R 3.2.2., assessing the possible effect of the factors in each dependant variable through linear mixed models.

Interactive effects between cycle phase and EBR were confirmed. Specifically, in the color condition of the Stroop task, lower accuracy and slower reactions were observed during the luteal phase, specifically in women with higher EBR. This effect was explained by interactive effects of progesterone and EBR on reaction times. In the N-back task, women showed an increased performance in the targets the higher their levels of estradiol, when their EBR during menses was high. On the other hand, for the lure trials, the performance of women with higher EBR during menses was more impaired as the estradiol levels were higher.

This study demonstrates the usefulness of the EBR as dopamine indicator in menstrual cycle research and confirms dopamine dependent changes in executive control functions across the menstrual cycle. Furthermore, different optimal levels of DA for different processes were found, even in the same task, as previously suggested by Cools and D'Esposito (2011). Therefore, we emphasize the importance of taking into account both cycle phase and DA baseline levels when studying these cognitive control functions in women.

Medial orbitofrontal cortex mediates effort-related responding in rats

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Selecting an action from a set of available options based on an evaluation of the costs, such as effort expenditure relative to the benefits of obtaining reward is a fundamental capacity. The nucleus accumbens core (AcbC) and the basolateral amygdala are components of a neural circuit that is critically involved in making decisions on how much effort to invest for rewards. The medial orbitofrontal cortex (mOFC) may be another component, however, its role is still poorly defined.

Here we investigated in rats the effects of mOFC in-/activation on effort-related responding. Furthermore, we analyzed the expression of the immediate early gene c-Fos, a marker for neuronal activity, during effort-related responding. To assess the involvement of the mOFC-AcbC projection in more detail, we used the retrograde tracer fluorogold combined with c-Fos staining.

In Experiment 1, we investigated the effects of excitotoxic mOFC lesions in an effort-based decision making task. In this task, one lever was associated with constant low reward and low effort (1 lever press = 1 pellet) across sessions, the other with constant high reward (4 pellets) but increasing effort across sessions (up to 45 lever presses). Rats with mOFC lesions displayed an increased preference for the high reward-high effort lever across sessions compared with sham controls. Thereafter, we tested rats in a progressive ratio (PR) task. In this task, only one lever was available. The ratio requirements per reward were increased gradually within the test session until an animal ceased lever pressing. The breaking point, i.e. the maximum ratio achieved, was higher in rats with mOFC as in sham controls, i.e. their tendency to work for reward was increased.

In Experiment 2, we investigated the effects of pharmacological mOFC in-/activation on PR responding using microinfusions of muscimol/baclofen or picrotoxin. Results demonstrate that mOFC inhibition increased, while mOFC stimulation decreased breaking points. Relative to appropriate controls, animals that underwent the PR task displayed a larger number of c-Fos positive neurons in the mOFC. There was no selective increase in the activity of mOFC neurons projecting to the AcbC in rats tested for PR responding compared with appropriate controls.

Our results suggest that the mOFC may be part of a neural circuit mediating effort-related responding. The PR task used here demands a comparison between constant reward magnitude and increasing efforts. Obviously, rats with a dysfunctional mOFC miscalculate the appropriate effort relative to the outcome value when responding under a PR schedule. It is well known that the AcbC and the mesoaccumbens dopamine system are crucial in control of effort-related responding. For instance, an intra-accumbens dopamine receptor blockade reduced breaking points in PR responding [1]. Our immunohistochemical data imply that direct mOFC-AcbC projections may play a limited role in mediating PR responding. However, the OFC modulates ventral tegmental area (VTA) firing, e.g. electrical stimulation of the OFC inhibits VTA dopamine neurons [2]. As its output is glutamatergic, OFC stimulation may reduce VTA dopamine neuron activity via inhibitory relays such as the lateral habenula or GABAergic VTA interneurons. Correspondingly, indirect projections from the mOFC to VTA dopamine neurons may be one critical neural substrate that mediates the effects of mOFC manipulations on effort-related responding observed here.

[1] J. E. Aberman, S. J. Ward, and J. D. Salamone, "Effects of Dopamine Antagonists and Accumbens Dopamine Depletions on Time- Constrained Progressive-Ratio Performance," vol. 61, no. 4, pp. 341–348, 1998.

[2] Y. K. Takahashi, M. R. Roesch, R. C. Wilson, K. Toreson, P. O. Donnell, Y. Niv, and G. Schoenbaum, "Expectancy-related changes in firing of dopamine neurons depend on orbitofrontal cortex," *Nat. Neurosci.*, vol. 14, no. 12, pp. 1590–1597, 2011.

Models of the emotional face perception - reproducibility and generalizability

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Introduction

During the last twenty years, neuroimaging studies have identified multiple face- and emotion-sensitive regions in the human brain. Their functional specializations have been intensely reviewed, although many questions are yet to be resolved. In particular, the functional integration of these areas is not understood. By means of network models, many structural and context-dependent interregional couplings between face-selective regions have been proposed. None of them were tested over multiple populations and diverse paradigms yet. However, reproducibility is crucial to assess validity and generalizability of results.

Methods

Four fMRI data sets comprising three different face perception paradigms (Tab. 1) in three populations were used to reproduce two studies (Tab. 1). Time series of face sensitive regions were extracted for each subject. DCMs were created and tested for each data set separately. However, the aim was not a 1:1 reproduction, but to use an - in our opinion - state of the art methodology, as for instance the current DCM version. After model comparison, Bayesian model averaging was conducted over the whole model space to obtain a representative average model for each data set and study.

Results

Model selection in both studies revealed the fullest connected model as the best one amongst the model space. This result was highly consistent over all four investigated data sets. However, results of model selection differed from the results of the original study 1.

Parameter estimates:

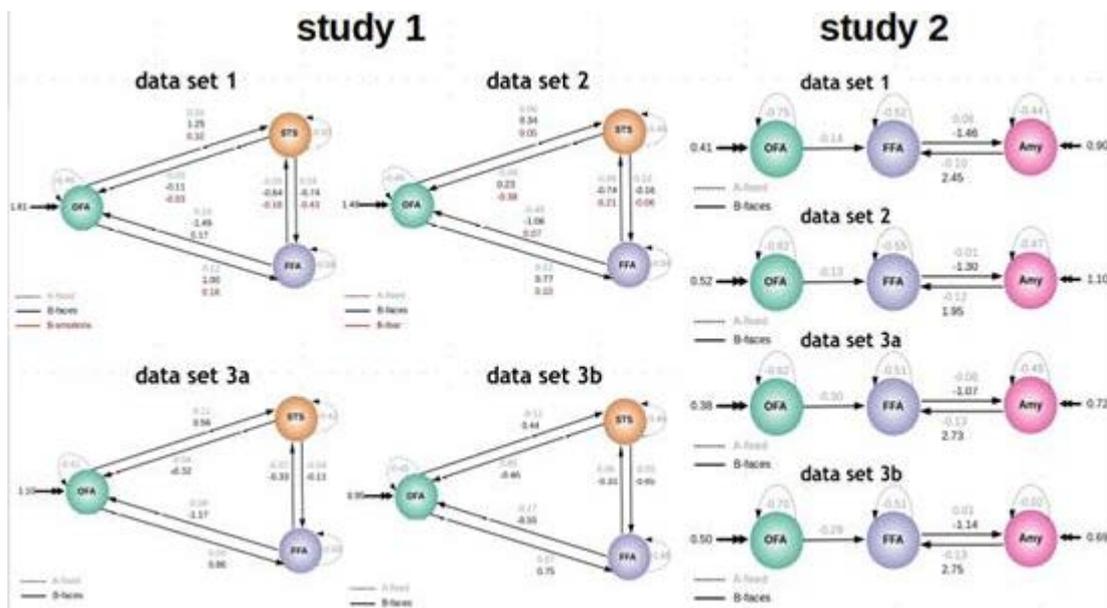
Study 1: Main assumptions about the directionality of regional coupling by an experimental condition (i.e. positive modulation of forward connections from OFA to FFA and OFA to STS by faces and emotions) were present in most data sets (Fig. 1, left). This was in accordance with the original study. Additional connections of each data set's average model, that were not further considered in the original study after a different result of model selection, also revealed high consistency throughout all examined data sets.

Study 2: Faces elicited a positive modulation of the connection from amygdala to FFA. In contrast, they caused a negative modulation of the reverse connection (Fig. 1, right). In the original study, connections in both directions were modulated positively.

Discussion

Diverging results from in model selection (study 1) may be caused by further methodological developments of the past years. However, it underlines the importance for re-evaluation of existing models with the current state of the art. The highly interconnected winning model outperformed all competing models throughout the here examined data sets, and for both studies. Backward connections are likewise important for brain functioning as forward connections, e.g. with regard to predictive coding. Negative backward coupling, as seen on connections between FFA/STS and OFA (study 1, Fig. 1, left) and FFA and amygdala (study 2, Fig. 1, right) play a considerable role in understanding the brain as a highly integrative network.

data set	1	2	3a	3b
participants	25 1:1 sex ratio	35 1:1 sex ratio	18 all female, 2 time points	
paradigm	neutral, happy, angry, and fearful faces vs. houses	neutral and fearful faces vs. houses	neutral faces vs. houses and fourier scrambled faces	
reproduction of face perception DCMs	study 1: Fairhall & Ishai (2007) - Effective connectivity within the distributed cortical network for face perception		study 2: Herrington et al. (2011) - Bidirectional communication between amygdala and fusiform gyrus during facial recognition	
regions	right occipital face area (OFA) right fusiform face area (FFA) right superior temporal sulcus (STS)		right OFA right FFA right amygdala (Amy)	
model space	24 competing models, varying configuration of the comprised regions		6 competing models, varying modulation of the reciprocal connection between FFA and amygdala	



MODIFIED SWIM TEST AS A MODEL OF ENHANCED CONTEXTUAL CONDITIONING DURING DEPRESSION: EXPRESSION OF GSK3 BETA AND EFFECTS OF ANTIDEPRESSANT TREATMENT

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Impaired brain plasticity is a well-established pathophysiologic feature of depression, but little is known about an enhancement of depression-associated cognitive processing. We studied a novel paradigm that potentially models the augmented acquisition of adverse memories during the development of a depressive-like state in mice. We used a modification of the classic two-day protocol of a mouse Porsolt's test with an additional session on Day 5 following the initial swim session. On the last day of testing, floating behavior, a parameter of helplessness, was increased in naïve mice that was accompanied by a reduction in the pGSK3b/GSK3b ratio and increased levels of brain GSK3b mRNA. The increase in GSK3b mRNA in prefrontal cortex during delayed testing session correlated with increases in floating behavior, which is not observed in the classic Porsolt's paradigm. Replacement of the last swim session with exposure to the context of testing resulted in increased GSK3b mRNA level similar to the effect of swimming, while exclusion of the last swim session prevented these changes. The behavioral changes and the alterations in GSK3b gene and protein levels were prevented by 2-week treatment with a low dose of classical antidepressant tricyclic imipramine (7.5 mg/kg/day), or vitamin B1 (thiamine) (200 mg/kg/day), or with the highly bioavailable thiamine precursor benfotiamine (200 mg/kg/day). Our study also demonstrated, for the first time, the antidepressant-like properties of vitamin B1 and its pre-cursor, in a pre-clinical model of depression. Thus, the new forced swim test paradigm models the enhanced contextual conditioning of adverse memories so the individual animals with distinct susceptibility to this syndrome to be differentiated.

Neural activity underlying interval timing in rodent prefrontal cortex

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Neural processing of temporal information is crucial to many cognitive abilities. In mammals, correlates of interval timing have been reported in brain areas such as hippocampus, striatum, prefrontal cortex, and parietal cortex. Single neurons in those areas display various temporal patterns of activity and collectively provide a population signal encoding time. Interval timing is typically investigated with tasks that only test for specific intervals or for discrimination between two groups of intervals. Estimation on a continuum of intervals has rarely been investigated.

We recorded neural activity in the medial prefrontal cortex of Mongolian gerbils (*Meriones unguiculatus*) while the animals measured and reproduced temporal stimuli that were randomly chosen between 3 and 10 seconds. The animals were trained to measure the duration of a visual stimulus, and reproduce it by running for the same duration in a virtual reality.

Single neurons exhibited diverse firing patterns in our timing task. Alike previous studies we found neurons that were transiently active, and neurons that monotonically decreased or increased their firing rate. Responses were adapted to the particular stimulus interval presented in the current trial. Differences among the neurons were also apparent between measurement and reproduction. While some cells were strongly active in only one of the task phases, others responded during both.

Our findings provide the first description of how single neurons in the rodent brain contribute to measuring and reproducing temporal intervals. How these cells act together as a population will be investigated in more detail.

Neural integration of appetitive and aversive outcomes in perceptual decision making in the rat

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A fundamental element of adaptive decision making is the ability to learn from the outcomes of actions, whether those are appetitive (i.e., rewarding) or aversive (i.e., punishing). In uncertain and constantly changing environments, it is necessary for an organism to survive to estimate probabilities of different outcomes and their associated benefits and costs. Even though this is a very basic and crucial mechanism, the computations that the brain is performing to trade off reward and punishment in order to guide behavioral choices toward maximum gain at minimum cost have barely been investigated. To approach this issue, we developed a laboratory model of the uncertainty existing in natural environments, employing varying reward and punishment contingencies. We trained rats to perform a two-alternative auditory discrimination task, in which each trial is initiated by entering the center port of a three-port chamber. This triggers the presentation of one of two sounds (70-ms bursts of band-pass filtered white noise), each indicating the location of the reward on either the left or right side port. Depending on the response of the animal (correct or incorrect), the outcome can be a drop of water (positive reinforcement), a foot shock (positive punishment) or a time out (negative punishment). To get a better idea of how punishment in its different forms impacts adaptive learning, we tested how the rats' choice bias (i.e., the tendency of an animal to prefer one option over the other) changes as a function of punishment intensity (current amplitude for foot shocks, and duration for time-out punishment). Mild foot shock punishment (<0.4 mA) induced a strong decision bias whose magnitude increased monotonically as a function of current amplitude. Time-out punishment also induced a response bias, which however was less pronounced and saturated at longer durations. To investigate the trade-off between reward and punishment, we modified the paradigm such that we manipulated either the probability for reward or the probability for punishment or both in a blockwise manner. This allowed us to track the dynamics of criterion setting as well as the underlying mechanism of reward and punishment processing in isolation as well as their integration. To track the neural computations underlying observed behavior patterns, we recorded single-neuron action potentials in rat medial prefrontal cortex (mPFC) during task performance. Preliminary results show significant neural activity modulation related to block transitions, response direction, and punishment anticipation, with only very few neurons responding to stimulus presentation. Together with experiments employing pharmacological inactivation through intracerebral muscimol infusion, these results implicate mPFC in adaptive choice behavior.

Neural mechanisms of cognitive control: Insights from simultaneous EEG-fMRI recording

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Objective: Long- and short-term goal attainment crucially rely upon behavioural adaptation and corresponding recruitment of cognitive control. Recent studies suggest that active maintenance of contextual information is the core feature of cognitive control, and that it operates in two distinct modes: proactive (i.e. preparatory, context-driven) and reactive control (i.e. corrective, stimulus-driven; Braver, Gray & Burgess, 2007). Past research points to prefrontal and parietal structures, like the dorsolateral prefrontal cortex (DLPFC) or the anterior cingulate cortex (ACC), to be the neural substrates of these mechanisms (Lopez-García et al., 2016; Ardenne et al., 2012). In terms of Event-Related Potentials (ERPs), late fronto-parietal positivity associated with working memory updating and maintenance (i.e. P3a, P3b, Late Positive Potentials), should be observed. **Method:** To highlight the temporal dynamics in the recruitment of these prefrontal-parietal areas, we used simultaneous EEG-fMRI recording with a newly adapted version of the Dot-Pattern-Expectancy-Task (DPX, Henderson et al., 2012). ERPs and continuous fMRI data in a within-subjects design were contrasted for cue-probe-congruency, as well as single cues and probes. **Results:** We found significant activations in the proposed prefrontal-parietal structures, accompanied by late positivity in the EEG. Both activations and ERPs were strongest for incongruent cue-probe combinations and cues with higher predictive power. **Discussion:** Preliminary findings suggest a correspondence between EEG and fMRI results, indicating a goal maintenance network in prefrontal and parietal cortex, as expressed by an evoked positivity peaking at the central parietal scalp.

Neuronal circuits involved in appetitive social interactions

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Both human and animal brains are adjusted to social interactions and constantly shaped by them. The ability to interact with conspecifics is crucial for survival and creates a framework for experiencing and transferring emotional states of different valence. Positive social interactions are essential for proper development, emotional well-being and social learning of animals and are considered to be highly rewarding in most species. Impairments in the ability to attribute positive value to social stimuli characterize several psychiatric disorders, such as autism spectrum disorder, antisocial personality disorder and schizophrenia. Whereas social interactions are relatively well described at the behavioral level, much less is known about neural mechanisms involved in these complex phenomena. To address this question, we decided to set an animal model of elementary appetitive social interaction, in which the interaction was intensified by social deprivation (single housing). Rats which were single-housed, exhibited significantly more social behaviors during interaction compared to pair-housed animals. They also showed more 50-kHz appetitive vocalizations (USVs) than control animals. It has been previously shown that appetitive social interactions evoke activation of several brain structures associated with motivation and reward, including central nucleus of the amygdala (CeA). To investigate the CeA neural circuit activated during positive social interactions, we traced active efferent projections of CeA by injecting transgenic Venus_PSD95 rats with anterograde transport tracer (fluorescently labeled *Phaseolus vulgaris leucoagglutinin*, PHA-L). We identified several brain structures in which neurons receiving inputs from CeA are activated during social appetitive interactions, including ventral tegmental area, substantia nigra, dorsal raphe nucleus and substantia innominata. Moreover, we compared neuronal subpopulations activated by social appetitive interaction and non-social place preference task. We observed that appetitive social and non-social behaviors activated partially distinct populations of cells in CeA. The results suggest that CeA contains neural circuits involved in positive social interaction and that these circuits are, at least partially, distinct from the ones involved in non-social positive emotions.

Philosophical considerations on differences in prey capture behavioural patterns of adult male cuttlefish (*Sepia officinalis*)

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As philosopher Peter Godfrey-Smith claims, Invertebrates such as cephalopods have a special importance in the history of the “mind”, for being an *independent experiment* in the evolution of large and complex nervous systems - in the biological machinery of the mind (Godfrey-Smith, 2013). Eventually, starting from 2014, they are protected as vertebrate species were since 1986. Although the study of animal personality shows consistent between-individual variations in many characteristics, individuality in hunting behaviour has been overlooked, particularly since prey-capture sequences of invertebrates are thought to have a strong automatic “inborn-type” component, making them an invariant. Cuttlefish capture fast moving preys by ejecting their elongated prehensile tentacles. The attack includes three phases: (1) attention score, (2) positionings, and (2) seizures. According to some authors, same as other cephalopods such as Octopuses, cuttlefish have recently demonstrated to display personalities. Some of us analysed in the recent past predation sequences of adult cuttlefish (i) to ascertain within and between individual consistency, and (ii) to test their association with so called ‘personality traits’ under different contexts. Subjects were tested individually for ten days. Predation rate, success rate and hunting latency were significantly correlated with first, second and third Principal Component Analysis factors. Significant correlations among capture patterns and responding nature under the two other contexts were found, highlighting a consistency between personality-measures and prey-capture patterns supposed to harbor little variability. We discuss possible origins and functions of these individual differences, also critically arguing the issue of animal personalities, and its (possible) application on non-vertebrate species.

Probing oxytocin neurons activity in socially interacting rats

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The hypothalamic neuropeptide oxytocin (OT) exerts prominent pro-social effects [3] and hence considered as potential drug for treatment of psychosocial diseases in human patients [5]. Despite numerous publications focused on pro-social effects of OT, it is still unknown how social interaction affects electrical activity of OT neurons.

Recent development of cell-type specific opto- [4] and pharmacogenetic [2] viral vectors allows us to identify and manipulate OT neurons in freely moving rats. Using these vectors combined with optoelectrode technique [1, 2] we recorded single OT neuron activity in the paraventricular (PVN) and supraoptic (SON) nuclei in rat hypothalamus during rest, exploration, and social interaction with unfamiliar conspecifics. Simultaneously we monitored animal behavior by an automated video tracking system (Noldus EthoVision® XT) coupled to recording of ultrasound vocalizations. Our preliminary results show that social interactions induce an increase in firing rate of individual OT neurons which correlates with the distance between interacting rats and their location in the arena.

To analyze how selective responses of OT neurons can emerge from the network dynamics, we built a computational model to draw prediction on how silencing of specific OT modules changes the OT network output, and thus the social behavior, in response to a given input. These predictions are experimentally tested using genetic tagging of OT neurons combined with pharmacogenetic neuronal silencers to inhibit neuronal activity in specific modules of the OT network and to examine the consequent effects in the various social paradigms.

In conclusion, the evaluation of intrinsic properties of OT neurons during social interaction might help to dissect sensory pathways controlling OT neuron activity and opens perspectives for translational studies of human psychosocial diseases.

References:

- [1] Tang Y. et al., *Front.Behavior.Neurosci.*, 2016.
- [2] Eliava M. et al. *Neuron*, 2016.
- [3] Grinevich V. et al., *Biol. Psychiatry*, 2016.
- [4] Knobloch H.S. and Grinevich, V. *Front. Behavior. Neurosci.*, 2014.
- (5) Meyer-Linderberg et al., *Nature Neurosci. Review*, 2011.

Relief learning in rats is mediated by a pmVTA-NAC projection

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Relief learning is the association of a stimulus with the offset of an aversive event. Later, the now conditioned relief stimulus induces appetitive-like behavioral changes, e.g. approach behavior or startle attenuation. We previously demonstrated that NMDA receptors and dopamine receptors within the nucleus accumbens (NAC) are involved in relief learning. Since NAC dopamine is usually released from the dopaminergic projection from the ventral tegmental area (VTA) to the NAC, we hypothesized that the VTA-NAC projection is involved in relief learning. This is supported by literature data showing that some neurons in the posterior medial VTA (pmVTA) are activated by the offset of an aversive event (foot shock).

Here, we present data from three experiments: (A) Injections of the retrograde tracer fluorogold into the NAC were performed. One week later, animals were exposed to foot shocks and then sacrificed. Immunostainings for c-Fos, a marker of neural activity and TH, a marker for dopamine, were performed. Our data show that ca. 12% of the dopaminergic projection neurons from the pmVTA to the NAC are activated by foot shocks. (B) Next, we injected the neurotoxin 6-hydroxydopamine into the pmVTA to induce dopamine-specific lesions of the pmVTA. These lesions blocked relief learning but did not affect safety and fear learning. (C). Last, we injected a DREADD construct into the pmVTA to express an inhibitory designer receptor within the pmVTA projection neurons. CNO which exclusively activate this designer receptor was locally injected into the NAC shell, where the terminals of the pmVTA neurons are located. These CNO injections blocked relief learning.

Taken together, our study indicates that relief learning is mediated by a dopaminergic projection from the pmVTA to the NAC shell.

This study was funded by the Deutsche Forschungsgemeinschaft (SFB779/B13).

Selective attention in tone-in-noise detection in mice

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Spectral processing of the acoustic environment provides important cues for the separation of different sources in complex acoustic scenes. In the mammalian auditory system, sounds of different frequencies are processed in separate filters, each sensitive to a specific frequency range. Adult humans are able to focus on one filter at a time, which helps them to focus on important sounds even in the presence of background noise, a phenomenon typically depicted as 'listening bands'. In consequence, sounds of other frequencies are missed more often. Infants on the other hand have been shown to not focus on any specific filter [1]. Here, we asked whether mice show this basic form of selective attention.

First, the subjects were trained to perform the task to detect a brief tone in noise, where the frequency of the tone was indicated by a sequence of salient priming trials at the beginning of the session. The stimulus was a sound of either 10 kHz or 21 kHz. For initial training, only one frequency was used per session and each frequency was used for the same number of sessions, to ensure that the subjects learned both frequencies, but never experienced a session where both frequencies were mixed. Once the subjects were able to perform the task, we determined the threshold of each subject for each frequency separately.

In the next step, we used the previously determined thresholds for mixed trials containing targets of both frequencies. The subjects were exposed to one frequency, the primer, at a high level at the beginning of the experiment. After 10 trials with the primer frequency, both frequencies were presented to the subject at random. Which frequency was used as the primer was switched in each session. With these results, we aimed to test whether the subjects were better at detecting the primer frequency, which would mean the subjects were focusing at a specific filter.

We found that there were no significant differences between the detection of primer frequency and the detection of the other frequency. This indicates that the subjects in our experimental setting did not focus on any specific filter. In addition, we found that the mice performed better during the mixed-trial experiments compared to the results of the threshold experiments. This and follow-up experiments with a new group of mice showed that perceptual learning was taking place over the course of several weeks. Our results did not yield evidence for selective listening in the time frame of an experiment session after initial priming. However, it is still possible that cues that immediately precede the target lead to focusing on a specific frequency band. We are currently performing a new set of experiments to test this possibility.

[1] J. Y. Bargones and L. A. Werner, "Adults listen selectively; infants do not," *Psychological science*, vol. 5, no. 3, pp. 170–174, Jan. 1994.

Serotonin underlies long-term depression of aggression after chronic social defeat in crickets

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Serotonin is often attributed with a role in suppressing the expression of aggression, but its exact behavioural function and the circumstances when it acts naturally are not known. Our study on male crickets (*Gryllus bimaculatus*) revealed that serotonin plays a key role in establishing a long-term depression of aggression that is first established after chronic social defeat. As in most animals, crickets exhibit a period of suppressed aggressiveness after losing an aggressive contest with another male, which in these insects lasts some 3 h on average (“loser-effect” review: Stevenson and Rillich, *Current Zoology*, 62:265-275, 2016). However, if crickets suffer 6 multiple defeats at 1 hour intervals the losers show a prolonged state of submissiveness that lasts 24-48 h. While treatment with a serotonin synthesis inhibitor (AMTP) or receptor blocker (ketanserin) had no significant effect on initial fighting behaviour, or on the duration of the loser effect after a single defeat, both drugs blocked the development of long-term submissiveness after 6 defeats. The serotonin uptake inhibitor fluoxetine, on the other hand, prolonged the duration of long-term submissiveness after 6 defeats to over 72 hours. Fluoxetine-treated crickets also developed long-term submissiveness after significantly fewer defeats (2 as opposed to at least 4). We have previously shown that the initial decision to retreat and the subsequent loser-effect both result from the action of nitric oxide (Stevenson and Rillich, *Science Advances* 1:e1500060, 2015). Our current findings and ongoing experiments indicate that continued activation of the nitric oxide signalling pathway as a result of repeated subjugation recruits the serotonergic system, resulting in long-term depression of aggressive behaviour. Our work points to a possible mechanism which may also underlying induction of depression after chronic social defeat in mammals.

Supported by the DFG (STE 714/4-1 & RI 2728/2-1)

Sex differences in perspective and strategy during virtual navigation in a new 3D matrix navigation task

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The influence of sex, and in particular a difference in strategy, on spatial navigation was investigated. It has previously been demonstrated that men tend to perform better with instructions from an allocentric perspective (“go North/East/South/West...”) with an Euclidian strategy (“... for 3 Blocks”), while women perform better with instructions from an egocentric perspective (“turn right at...”) with a landmark based strategy (“...at the Bridge”). However, so far perspective and strategy have only been modulated in conjunction and not independently of each other. Furthermore, these instructions were previously applied only to 2D-matrix navigation. However, in computerized versions of the 2D-matrix navigation task the egocentric strategy, which should enhance performance in women, is confounded with mental rotation, for which a male advantage has been demonstrated. In order to dissociate virtual navigation from mental rotation on the one hand and the effects of perspective and strategy on the other hand, a new computer-based 3D navigation task was developed, which allows for testing of navigation abilities under different types of instructions (allocentric Euclidian, allocentric landmark, egocentric Euclidian, egocentric landmark) in a more realistic, yet standardized and controlled environment.

To assess their navigation ability, all 68 participants (43 men, 25 women in their luteal cycle phase) had a first person view of the environment and followed the instructions on the screen, leading them to their destination. To assess their mental rotation ability, participants were asked to identify North after reaching their destination.

It was found that that sex had a significant influence on the perspective effect for reaction times. While reaction times were slower with instructions from an allocentric perspective in all participants, men outperformed women under the allocentric instructions. Furthermore, a significant interaction was found between sex and strategy. Instructions in Euclidian terms were beneficial for men, while instructions in landmark terms were beneficial for women. This was particularly apparent for the more difficult allocentric perspective.

These findings are in line with previous results on sex differences in spatial navigation. Thus, the newly created navigation task shows promising results for the study of different navigation strategies used by men and women.

Sex differences in the Kimchi-Palmer task revisited: A possible role of impulsivity

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Two paradigms have been developed to study global-local processing. The Navon paradigm, and the Kimchi-Palmer task. Both tasks utilize hierarchical stimuli, i.e. large global forms made up of small local forms. In the Navon task, participants have to identify a target at a pre-specified level under time-pressure, while in the Kimchi-Palmer task participants have to judge the subjective similarity between figures that match either at the global or local level. Thus, in the Navon task, the variable investigated is participants' RT to global and local targets, while in the Kimchi Palmer task, studies so far only used participants' choices for global or local matches.

Research, investigating sex differences in global versus local processing is rare, but when administering the Navon task, research indicates a global processing bias for men and a local processing bias for women respectively. Sex differences in the Kimchi-Palmer task can however only be found in children, but not in adults, when considering the number of global or local choices. It has however been demonstrated that decision making in adults is more reflective than in children, such that their choices may not reflect their initial response tendency. Therefore, we set out to investigate, whether sex-differences in global-local processing in the Kimchi-Palmer task may be reflected in choice reaction times rather than in choices in adults. In two studies, we therefore administered a computerized version of the Kimchi Palmer task in adult samples. The first study demonstrates that in a sample where no sex differences can be found in the number of global choices, women showed faster responses to local choices than men, who were faster in making global choices than women. This difference was irrespective of women's menstrual cycle phase. In the second study, these reaction time differences were related participant's impulsivity.

These recent findings may shed new light in sex-dependent research concerning global vs. local processing and according to inconsistencies between children and adults.

The effect of cannabinoid system in the anterior cingulate cortex on effort-based decision making mediates partly via TRPV1 receptors

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Introduction and aim: There is considerable evidence implicating the use of various cannabis derivatives altered cognition and decision making. In addition, it was established that the anterior cingulate cortex (ACC) is one of the important areas involved in effort-based decision making. Therefore, in this study, we examined the involvement of TRPV1 receptors in the ACC on the impairment of effort-based decision making induced by the activation of cannabinoid system.

Materials and methods: We trained different groups of male Wistar rats in an effort-based form of cost-benefit T-maze decision-making task. The animals were bilaterally implanted with two separate cannulae into the ACC. Then, the animals received local injections of vehicle or different doses of ACEA, a cannabinoid type-1 (CB1) receptor agonist, in the ACC bilaterally. Afterward, capsazepine, as a vanilloid receptor antagonist, was injected in the ACC, 5 min before the administration of most effective dose of ACEA. We measured spontaneous locomotor activity following the same treatments. In addition, a separate group received intra-ACC administration of capsazepine to determine if this antagonist alone has any effect on decision-making.

Results: The results showed that the activation of cannabinoid receptors in the ACC impaired effort-based decision making such that rats were less willing to invest physical effort to gain high reward and this effect was dose-dependent. However, intra-ACC administration of capsazepine, 5 min before microinjection of the most effective dose of ACEA resulted in the decrease of ACEA effects. Intra-ACC microinjection of capsazepine alone did not have any effect on effort-based decision making.

Discussion: This finding revealed that the cannabinoid system in the ACC plays a critical role in regulating effort-based decision making and this effect partly mediate via TRPV1 receptors.

The effect of noradrenaline on the interplay between attention and motivation

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The noradrenaline system modulates several cognitive functions - including attention and motivation (Sara, 2009). Yet, to date the role of this neuromodulatory system on the interaction between attention and motivation is poorly understood. The aim of our study was to investigate this issue in monkeys assessing the temporal dynamics of goal-directed behavior while manipulating the level of motivation and enhancing the extracellular levels of noradrenaline (NA) in the brain.

We tested four monkeys in a go/no-go-task. Stimuli were presented sequentially and monkeys had to select one target among distractors. Each session consisted of four runs that lasted 15 min. Within each run, the target was associated with either a high or low level of reward. The level of reward was kept constant across the whole run. In the high reward condition, the monkeys completed more trials, were significantly more accurate and reaction time were less variable compared to the low reward condition. Based on the signal detection theory, we also computed the animals' sensitivity and response bias and investigated their functional relationship using the line of optimal response (LOR; Lynn and Barret, 2014). We found that the high reward condition improved the animals' sensitivity and shifted their response bias bringing their overall performance closer toward the LOR.

We then compared the animals' performance after intramuscular injections of either saline (control) or the NA-reuptake inhibitor atomoxetine (ATX: 0.5 or 1 mg/kg) that enhances the extracellular levels of NA in the brain. Compared to the saline condition, the monkeys completed more trials and were more accurate in the ATX condition. In addition, their reaction time decreased while their movement time increased and both were less variable in the ATX condition compared to the saline condition.

Importantly, enhanced extracellular levels of NA modulated the motivation bias toward the high reward. Under ATX, the animals' performance in low and high reward conditions did not differ significantly, which resulted from improved performance in the low reward condition. However, in the high reward condition, we found that at the highest dose of ATX (1 mg/kg), the monkeys also completed more trials, their sensitivity improved and their performance were closer to the LOR compared to the saline condition. In addition, their reaction time decreased while their movement time increased and were less variable.

To sum up, both high extracellular levels of NA and high level of reward improved performance and reduced response time variability thereby optimizing response strategy. Interestingly, ATX tended to eliminate the motivation bias driven by reward. Yet, a high extracellular level of NA further boosted the improvement of performance driven by the high reward condition on the temporal dynamic of goal-directed behavior. These findings suggest a complex interplay between noradrenaline, attention over time, and motivation, which calls for further investigation.

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- [T25-5B](#) Re-evaluation of learned information in *Drosophila*
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Calcium Imaging of Learning-Induced Plasticity in Single Kenyon Cells in *Drosophila melanogaster*

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Drosophila melanogaster is a key model organism to study neuronal circuits underlying learning and memory formation. Flies are able to associate conditioned stimuli (e.g. odors) and unconditioned stimuli (e.g., electric shocks as punishment or sugar stimuli as reward) during classical conditioning procedures. In a typical differential training paradigm the flies learn to associate one odor (CS+) with a punishment that is presented in temporal coincidence. A second odor (CS-) is presented without punishment. In a subsequent choice situation in which both odors are presented the animals avoid the CS+. The mushroom body of the *Drosophila* central brain has been shown to be the key structure mediating associative odor learning. The intrinsic neurons of the mushroom body (Kenyon cells) are sparsely activated by a given odor stimulus, and dopaminergic neurons projecting to the main output regions of the mushroom bodies, the lobes, mediate punishment. The coincidence of odor-evoked activity of Kenyon cells and dopaminergic input is hypothesized to cause synaptic plasticity of Kenyon cells presynapses, ultimately changing the animals' behavior in response to the learned odor stimulus.

The mushroom body lobes can be subdivided in lobe-specific sub-regions by the innervation of extrinsic neurons (e.g., dopaminergic neurons and postsynaptic "mushroom body output neurons"). These sub-regions were shown to be differentially involved in distinct aspects of learning (e.g., appetitive and aversive olfactory learning). We asked whether neuronal activity within single Kenyon cell axons projecting longitudinally across these sub-regions is modulated in the course of an aversive associative olfactory training procedure, and if yes, whether neuromodulation is restricted to specific sub-regions of the axon. We combine two-photon calcium imaging and a single cell-clone technique (MARCM) to express a DNA-encoded calcium sensor. The animals are trained directly under the microscope and the calcium dynamics within the axons of single Kenyon cells in response to odor stimulations before, during and after training are monitored. First results will be presented.

Desert Ants consider landmark ambiguity

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Information about spatial orientation needs to be evaluated and weighted before a final behavioural decision is taken. To weight this information organisms have to incorporate the reliability and the constant of it. It is shown that the desert ant *Cataglyphis fortis*, a model organism for navigation, includes these features during their long solitary foraging journeys. Returning to their nest the ants use mainly vision-based path integration for long range orientation plus the olfactory and visual surrounding nest cues for rather fine-scale navigation. Former experiments elicit that ants can be trained to olfactory and visual cues to pinpoint their nest entrance which is followed by a stereotype searching behaviour. To test this information weighting process a field experiment in a Tunisian saltpan were performed in which the ants associated an odour blend with their nest entrance in two different training situations: in the first one the odour cues were applied uniquely at the nest entrance whereas in the later one the same odour blends were also applied several times along the foraging path as an omnipresent mark. Do ants consider the ambiguity of the nest defining odour cues in the later situation and would they associate the nest entrance in the single odour situation with higher accuracy? Ants trained with the single nest odour cues exhibited a significantly more focused search around the odour cue than those ants that experienced the nest cues repeatedly along the way. This certainty about their nest entrance position reveals the high rating of reliability in the uniquely applied odour cue situation during the information weighting process.

Discrete gregarising stimuli elicit serotonin release in the metathoracic ganglion of the Desert Locust *Schistocerca gregaria*

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Serotonin (5-hydroxytryptamine, 5-HT) is involved in the rapid (within hours) and reversible transformation of the Desert Locust (*Schistocerca gregaria*) between two distinctly different phenotypes (phases) - a process that is dependent on population density. At low density, locusts develop into their so-called solitary phase, in which they are slower, move mainly at night, and are actively repelled by other locusts. With increased density, however, locusts develop into their gregarious phase in which they become more active and are attracted to other locusts. The concentration of serotonin measured by high performance liquid chromatography (HPLC) in thoracic ganglia is transiently increased during gregarisation, suggesting a role in the transition from solitary to gregarious phase, but not the maintenance of either phase. Furthermore, the somata of specific thoracic neurons show increased serotonin immunofluorescence after locusts are gregarised by repeated mechanosensory stimulation of the hind leg. However, the specific role of serotonin in regulating phase state is yet to be elucidated. We used *in vivo* fast scan cyclic voltammetry (FSCV) to measure, with high temporal resolution, the release of serotonin in the metathoracic ganglia following discrete mechanosensory stimulation. The discrete stimulus consisted of a single paintbrush stroke applied to a stimulation site: hind-, mid- or fore-leg or antenna, to compare responses to gregarising (hind leg) and non-gregarising (other sites) mechanosensory stimuli. The recording electrode was positioned in the medial ventral association centre (mVAC) of the metathoracic ganglia, in which the neurons of interest have projections. A serotonin-specific 'N'-voltage waveform (0 - 1.3 - -0.6 - 0V) was applied to distinguish the serotonin signal from that of other neuromodulators, primarily dopamine. The amplitude of the oxidation peak of the voltammogram and its latency from stimulus onset were used as measures of serotonin release. In both solitary and gregarious animals, hind leg stimulation caused a larger amplitude release of serotonin than did comparable stimulation of other sites. Stimulation of the antenna also elicited release of serotonin in the metathoracic ganglion but the latency of the peak was significantly longer than that for hind leg stimulation. This agrees with the known physiology of the metathoracic mVAC, which receives direct sensory input from the hind leg but has no known direct descending inputs from the antennae. Previous research suggests that thoracic serotonin is involved in the initiation, but not maintenance, of phase change, but our data imply that serotonergic neurons may also remain involved in the transient responses of gregarious locusts to further gregarising stimuli. Our study is the first to show that gregarising stimuli in either phase elicit release (rather than simply change in total amount) of serotonin in the central nervous system.

Familiarity and age interact to affect locomotory hesitation in solitary Desert Locusts (*Schistocerca gregaria*)

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Desert Locusts switch reversibly between two strikingly different phenotypes: a shy and cryptic solitary phase and a more brightly coloured gregarious phase which can form vast swarms. When population density is low, locusts exist as solitary individuals. When food shortage forces them closer together, however, they become gregarised through close contact with conspecifics: they become more active and are attracted to each other. Solitary locusts are less willing to initiate walking, and walk more slowly and intermittently than gregarious locusts.

We asked whether solitary locusts can behave like gregarious locusts without undergoing gregarisation. To what extent does the 'hesitant' behaviour of solitary locusts represent a response to unfamiliar environments that can be overcome by familiarity?

We inserted a single locust into a holding tube, which was placed at one end of a 38cm long wooden beam. At the opposite end was a wheat vial food source behind a screen and a fan served to draw air through the arena towards the locust. The locust was allowed 10 min to cross the beam. Each week locusts were tested 6 times in a row (at 10 min intervals) for a total of 9 weeks. We fitted mixed-effect Cox regression models to analyse the effect of repeated runs, age and phase state on crossing times.

Naïve young solitary locusts (week 1) initially displayed 'hesitant' behaviour, taking much longer to cross the beam than naïve young gregarious locusts. However, their hesitation rapidly decreased over the six runs ($\chi^2 = 11.36$, $df = 3$, $p = 0.01$), which we interpret as a consequence of familiarisation with the arena. Over the following 8 weeks these solitary locusts became progressively less hesitant ($z = 6.66$, $p < 0.001$), eventually matching the shorter crossing times of the naïve gregarious locusts in all but their first run of each week's session. In a separate experiment, naïve age-matched (i.e. 9 week old) solitary locusts had similar crossing times (in their first exposure to the assay at 9 weeks of age) to those of old familiarised solitary locusts, and shorter times than those of gregarious locusts ($z = 2.25$, $p = 0.025$). The naïve old solitary locusts and naïve gregarious locusts were no slower in their first run of the day than in the subsequent runs on the same day. This difference from the behaviour of the familiarised old solitary locusts indicates that age interacted with familiarity to affect the locomotory behaviour of solitary locusts over a range that exceeds phase differences.

The hesitation in the first run of each week's session shown by familiarised old solitary locusts is intriguing. They presumably remember their experience of previous assays, which appears to revert them to their young solitary slow state in their first run. This further exposure to the arena immediately undoes the effect so that they 'remember their age' – they behave like age-matched naïve solitary locusts.

We show that solitary locusts display age-related behavioural plasticity in locomotion which exceeds the phase-related behavioural range. The typical hesitant behaviour of solitary locusts can be overridden by age and experience to result in a locomotory phenotype that is no less hesitant than that found in gregarious locusts.

Genetic Labeling of Memory Engram Cells in Associative Learning

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How does the brain store memories? A learning event is believed to induce biophysical and biochemical alterations in specific populations of neurons, which are often referred to as memory engram cells. Genetic access to label and manipulate the memory engram cells is crucial to dissecting the circuit underlying learning, to enable us to investigate how gene expression changes after learning, and to connect those changes to synaptic plasticity.

The mushroom body is the center of the associative learning in insect brains. In *Drosophila*, the mushroom body receives major sensory input from olfactory projection neurons from the antennal lobes. A given odor activates only ~6% of 2,000 Kenyon cells, major mushroom body intrinsic neurons. The small subsets of Kenyon cells responding to the odor during training have been considered as memory engram cells, because 1) they can encode odor specificity of memory, 2) learning induces synaptic plasticity in their terminals and 3) their output is required for behavioral expression of memory. Punishment and reward activate distinct set of dopaminergic neurons that project to compartmental regions along the parallel axonal fibers of the Kenyon cells. Previous studies have demonstrated that direct activation of a random subset of Kenyon cells can substitute for the odor (Vasmer 2014). Intriguingly, short-term and long-term memories have been mapped on to different set of Kenyon cells, suggesting that existence of different mechanism to induce and maintain synaptic plasticity.

Here we aim to develop a novel technique to create a tool to secure genetic control on a finer subset of memory engram cells (i.e. Kenyon cells) in order to further dissect molecular events induced in different types of Kenyon cells and the underlying circuit after learning. To begin with, we constructed an automated optogenetic olfactory arena, where both activation light and electric shock are delivered to flies for training and memory tests. We used split-GAL4 drivers to express red-shifted channelrhodopsin CsChrimson-mVenus in specific neuron types. Immediately after pairing activation of specific olfactory projection neuron (PN) or olfactory receptor neurons (ORN) with electric shock, flies showed robust avoidance to the activation light. We observed significant long-term aversive memory after repeated training. Flies were also able to form short- and long-term appetitive memory when sugar reward was paired with optogenetic activation of ORN or PNs. After successfully establishing the optogenetic-activation learning paradigm, we are now applying the same method to distinct subsets of Kenyon cells. Using the heat-shock activated Flp-enzyme in order to activate a stochastic subset of Kenyon cells, it was possible to see significant learning by pairing activation of Kenyon cells with an electric shock. Yet, phenotypes were not as robust as in the PN- and OR activation, and we are optimizing the protocol. Preliminary data suggests that the number of activated cells and the activated subtype of Kenyon cells affect the strength of the learning phenotype.

Successful optimization of this method will be an incredibly powerful tool to give us high-resolution insights into the cellular and molecular mechanisms of learning.

Identification and localization of neuropeptides in the brain of *Cataglyphis* desert ants using imaging mass spectrometry

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Desert ants of the genus *Cataglyphis* undergo an age-related polyethism from interior worker to predominantly visually guided foragers. Due to their very distinct behavioral stages and brief transitions, *Cataglyphis* ants provide an excellent model system to investigate the underlying neuronal mechanisms of this remarkable behavioral plasticity. However, the internal regulation of the behavioral transition from interior worker to forager is largely unknown. Recent studies focused on neuropeptides as potential key signals for this behavioral transition in social Hymenoptera, including *Cataglyphis*, and associated changes of neuropeptide levels with the interior-forager transition. In insects, neuropeptides are known to modulate a variety of physiological and behavioral processes including locomotor activity, feeding, and learning and memory. Since some of these processes are likely changing with the interior-forager transition in *Cataglyphis* ants, the neuropeptides associated with these processes might also be involved in the regulation of this transition. Qualitative and quantitative analysis of the distribution of neuropeptides in distinct brain regions might therefore provide deeper insights in the potential role of individual neuropeptides in the behavioral transition. We developed matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) to measure neuroactive peptides in brain cryosections of *C. noda*. MALDI-IMS is a powerful tool to investigate the 2-dimensional distribution of biologically relevant molecules with high resolution but without the need for specific antibodies. We biochemically confirmed 11 neuropeptides in *C. noda* using tandem mass spectrometry. We further compared the distribution pattern for tachykinin (TK) resulting from MALDI-IMS with the distribution pattern revealed using a specific antibody against TK in alternate sections. This approach confirms that MALDI-IMS results represent actual distribution patterns of a specific neuropeptide. Finally, we were able to describe the distribution of all 11 confirmed neuropeptides at a spatial resolution of 30 μm in 14 μm brain cryosections. This includes the three neuropeptide families allatostatin A, short neuropeptide F and TK which were previously studied in *C. fortis*. We conclude that MALDI-IMS represents an appropriate and efficient tool which will allow for a qualitative comparison of individual neuropeptide distributions in the brain of ants of different behavioral stages.

Supported by DFG, SFB 1047 'Insect Timing' (projects B2 and B6) to CW and WR, and DFG PR 766/10-

Induction of associative odor memories by optogenetic activation of Kenyon cells in *Drosophila melanogaster* larvae

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How brains organize behavior based on internal needs on the one hand and changing environmental information on the other is one of the key questions in neuroscience. Learning is defined as a process leading to a lasting alteration in behavior due to experience. Even animals as simple as the *Drosophila* larva are able to form and recall an association of a particular odor with a rewarding stimulus. In the last years it turned out that - similar to the adult fly – the larval mushroom bodies (MB) are required for diverse behavioral functions, including odor learning and memory. The larval MB consists of about 2000 embryonic and larval born Kenyon cells. However, in previous work we could demonstrate that only around 100 embryonic born Kenyon cells are required for associative odor-sugar learning. Furthermore, optogenetic activation of dopaminergic/octopaminergic neurons is sufficient to substitute the unconditional stimulus (US) during conditioning, while optogenetic activation of specific olfactory neurons is sufficient to substitute the conditional stimulus (CS). However, to our knowledge it is still elusive whether the conditional activation of Kenyon cells is sufficient to form memory traces. Thus, we are interested in whether a conditional optogenetic activation of Kenyon cells is, dependent on the set of Kenyon cells included in the Gal4 line, sufficient to induce an appetitive or aversive memory.

Learning and modulation of feeding for amino acids in *Drosophila*

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Organisms have to seek appropriate nutrition and make according decisions to maintain health. Amino acids are important nutrients as they are components of protein, and some of them, essential amino acids, cannot be synthesised; therefore animals need to obtain them from ambient food source. The fruit fly, *Drosophila melanogaster*, have a specific hunger for amino acids, that is, adult flies show increased amino acid feeding behaviour when they are reared on amino acid-free diet (Toshima and Tanimura, 2012). However, the receptors and gustatory neurons sensing amino acids are yet unknown. To identify the gustatory receptor neurons required for amino acid feeding, we performed two-choice preference tests using several mutants and transgenic flies. We found that *poxn* mutant flies, which have no external taste sensory neurons, preferred amino acids over low-concentration sugar, indicating that external gustatory information is not essential for amino acid detection. Additionally, flies in which sugar-sensing neurons were genetically silenced still feed on amino acids, suggesting that sugar-sensing neurons are not necessary for this behaviour.

Given the particular importance of amino acids for growth, we further investigated larval behaviour towards amino acids. For some but not all amino acids, we found that larvae prefer an agar substrate containing amino acids to pure agar without amino acid. In contrast, all amino acids were effective as rewards in an odour-tastant associative learning paradigm (Schleyer et al., 2015). These findings now enable studies to reveal the neuronal circuits for amino acid processing from the sensory periphery towards the brain.

Learning the specific quality of taste reinforcement in larval *Drosophila*

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Reinforcers are the basic motivators of behaviour, and learning to predict them is of essential importance to any animal. But how rich is the content of those learned predictions? We show that larval *Drosophila* compare the memory-predicted reinforcement with the current situation, and translate their memory into behaviour only if doing so promises a gain: either finding the predicted reward, or escaping from a punishment. Importantly, larvae do not only remember the value of reinforcement (How much?), but also its quality (What?), in both the appetitive and the aversive domain [1]. Given that animals as simple as larval *Drosophila*, endowed with only 10,000 neurons, operate with both reinforcement value and quality, we suggest that both are fundamental aspects of mnemonic processing - in any brain.

From the available literature, such nuanced memories for the type of reinforcement are unexpected, and pose a challenge to present models of how insect memory is organized. Four dopaminergic neurons from the PAM-cluster have recently been shown to be crucial to convey an internal reward signal [2]. By optogenetic manipulation of individual neurons from this cluster we ask how the information about reinforcement quality is coded among those dopaminergic neurons. We combine this approach with a high-resolution analysis of the microbehavioural modulations that bring about learned behaviour [3].

References

1. Schleyer et al. 2015. Learning the specific quality of taste reinforcement in larval *Drosophila*. *eLife* 4.
2. Rohwedder et al. 2016. Four individually identified paired dopamine neurons signal reward in larval *Drosophila*. *Curr Biol* 26: 661-669
3. Schleyer et al. 2015. The impact of odor-reward memory on chemotaxis in larval *Drosophila*. *Learn Mem* 22: 267-277.

Locomotor activity and phototaxis are influenced by the neuropeptides allatostatin A and allatotropin and by light exposure in the desert ant *Cataglyphis noda*

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Cataglyphis desert ants show an age-related polyethism that makes them a good model organism for division of labor. Cataglyphis spend the first part of their adult life (interior; ~4 weeks) in complete darkness performing tasks inside the nest. The youngest ants (interior I; first ~14 days) primarily store food for the colony and exhibit a very low level of locomotor activity. With ongoing age ants transition to the interior II stage (~14 days) and perform task like caring for brood and queen, and cleaning and constructing the nest. This later stage exhibits elevated locomotor activity levels compared to interior I ants. Within the following ~2-3 days the ants perform short learning and orientation runs outside of the nest and close to the entrance before they start foraging over distances of up to several hundred meters (foragers; ~6-7 days). The navigation of Cataglyphis foragers is mainly based on visual information in a bright environment. With changing tasks during the interior I-interior II and interior II-forager transition, behavioral aspects like locomotor activity and phototaxis are suggested to alter, too. A novel experimental setup was used to show that the locomotor activity level increases from interior I to interior II to foragers of a laboratory kept *Cataglyphis noda* colony. Furthermore, phototaxis changes from negatively phototactic to more positively phototactic, concomitant with the interior II-forager transition. A previous experiment shows that *C. fortis* callows are more active after light exposure and, in addition, light exposure results in neuronal reorganization in newly emerged ants. Light is therefore considered as an important stimulus, potentially triggering changes associated with behavioral transitions in *Cataglyphis* ants. Therefore, we show in a second approach that interior workers that were preexposed to light show elevated locomotor activity levels and are more positively phototactic. However, their behavior still differs from foragers, suggesting that light exposure itself does not necessarily trigger the interior II-forager transition. Recent studies started to investigate the role of neuropeptides in behavioral transitions of social Hymenoptera. We therefore injected allatostatin A (AstA) and allatotropin (AT) into the hemolymph of the head capsule of *C. noda* workers. In foragers AstA decreases locomotor activity while AT rises it. Furthermore, the injection of AT leads to positive phototaxis in foragers. However, AstA and AT have no influence on the locomotor activity level or phototaxis in interior I ants, suggesting a stage-specific effect. Here we provide first evidence that the neuropeptides AstA and AT affect behavioral traits relevant for behavioral transitions in *C. noda*. We further show that neuropeptides as well as light as an important environmental stimulus have stage-specific effects.

Supported by DFG, SFB 1047 'Insect Timing' (project B6) to WR.

Memory consolidation at the cost of specificity

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Associative memory enables animals to act in anticipation of upcoming significant events. To be useful, associative memory should have an optimal level of specificity. E.g., a too specific cue-punishment memory will be futile, for it will not tolerate the unavoidable sensory noise in nature; whereas an over-generalized cue-punishment memory will create a bewildering state of unfounded fear. Which aspects of a noxious experience influence the specificity of the memory left behind? We systematically addressed this question with fruit fly as a study case. Flies, when trained with an odour that precedes pulses of electric shock, learn to avoid this odour as a predictor for punishment. In such an odour-punishment memory paradigm, we first characterized the dose-effect functions for two different odours and chose odour concentrations that support about equal levels of learning and retrieval. We then used a four group-generalization experiment [1,2]: Two groups of flies were trained with one of the two odours and then tested with that very same odour (i.e. "SAME"). The level of learned avoidance in these groups should reflect the total strength of the memory formed. Two further groups experienced a switch of odours between training and test (i.e. "DIFFERENT"). Any learned avoidance in these groups should reflect a component of the formed memory that is *general* to the respective non-trained odour; whereas the loss of learned avoidance in the DIFFERENT groups as compared to the SAME groups should reflect a memory component that is *specific* to the trained odour. We asked which training and test parameters effect this partitioning of the memory into general and *specific*. Neither repetition of training, nor strength of punishment had an effect. We found however a dramatic effect of the passing of time after training. Namely, once given repetitive training trials with pauses in between, if flies were tested 20 minutes later, the generalization was only partial to the non-trained odour; whereas testing 24 hours later revealed full generalization. This effect was confirmed using another odour-pair. Thus, fly odour-punishment memory seems to consolidate at the cost of specificity. A similar observation had already been made with respect to odour-reward memory [3]. This "cost" of memory consolidation may point to some key properties of the cell-populations harbouring short- versus long-term memory traces as well as the circuit mechanisms of a systems consolidation-like process in the fly. Furthermore, over-generalization of punishment-memory with the passing of time has been observed also in rodents and man and may have implications for post-traumatic psychology [e.g., 4].

1. Guerieri et al., 2005 PLoS Biol 3:e60
2. Niewalda et al., 2011 PLoS ONE 6, e24300
3. Ichinose et al. 2015 Elife 4: e10719
4. Pamplona et al. 2011 J Psychiatr Res 45: 354-360

Neural circuit analyses of relief learning in fruit flies

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A painful event leaves two opponent memories: Cues that precede pain or overlap with it are remembered negatively; while cues that coincide with the relief at pain-offset acquire positive valence. This double-sidedness of memory is strikingly conserved through evolution [Gerber et al. 2014]. Yet, to date no mechanistic account exists in any organism on how these opponent memories are organized with respect to each other. We decided to fill this gap, using as model the fruit fly with a numerically simple, anatomically well-characterized and experimentally accessible brain.

Flies can associate an odour with either the punishing onset or the relieving offset of electric shock, resulting respectively in learned avoidance from or learned approach towards this odour [Tanimoto et al. 2004, Yarali et al. 2008].

Olfactory punishment learning is fairly well studied in the fly. During training, the odour signal and the reinforcement signal, carried by identified dopaminergic neurons, converge on a brain structure called the “mushroom body”. Upon this convergence, mushroom body output synapses towards pre-motor centers are modified to enable conditioned avoidance or approach, respectively.

We found that relief learning, just as punishment learning, relies on mushroom body function, too. Following up on this finding, we now aim at uncovering a minimal mushroom body-centered circuit, within which relief-reinforcement is signaled, relief memories are laid down, stored and retrieved. Previous attempts at identifying a neuronal reinforcement pathway for relief learning had been unsuccessful [Yarali & Gerber 2010], likely due to the inadequacy of the neuronal interference tools used. We now use a set of transgenic tools, which enable us to express either a blocker of action potentials or a light-gated ion channel to optogenetically activate mushroom body-associated neurons, in very small groups at a time, to then screen for effects on relief learning. We present preliminary results with respect to modulatory dopaminergic input neurons and neurons that are efferent to the mushroom bodies, and thus are candidates for “reading out” relief memories.

In the future, detailed fly circuit maps of relief-, punishment- and reward- learning can be integrated into mathematical models, to point to circuit principles that may be evolutionarily conserved, and/ or may be implemented in robotic devices.

1. Gerber et al. 2014. *Learn Mem* 21:232
2. Tanimoto et al. 2004. *Nature* 430:983
3. Yarali et al. 2008. *Anim Behav* 76:1173
4. Yarali & Gerber 2010. *Front Behav Neurosci* 4:189

Operant and classical conditioning of the cockroach *Periplaneta americana* in a forced choice task

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The forced choice paradigm is regularly employed in learning experiments with vertebrates. However, only few successful attempts have been made in insects, most prominently in honeybees. The aim of the present study is to establish a novel forced choice paradigm in *Periplaneta americana* and advertising the cockroach as a model organism for learning and memory. Cockroaches have several advantages and complementary properties in comparison with other well-established insect models for learning and memory. They display a wide behavioral repertoire, show excellent navigation skills, and their robustness and size as well as their relatively large brains support accessibility to neurophysiological *in vivo* investigations.

We developed a novel experimental setup for cockroaches based on a T-maze and established two different forced choice tasks. All experiments were carried out in the dark. The first task uses a paradigm of operant aversive conditioning for spatial learning. Animals had to choose the walking direction (left or right) in the maze and we punished their first choice with an aversive light stimulus. After three learning trials animals were tested in the T-maze after 24h and 48h. The results show a significant learning success and indicate stable individual learning behavior (Pamir et al. 2014).

The second task employs olfactory conditioning. We first tested the initial preference of each animal in the T-maze reproducing that they prefer vanilla over peppermint (Watanabe et al. 2003). We selected those animals with vanilla preference and followed the paradigm of a classical differential conditioning (Watanabe et al. 2003) where vanilla was paired with an aversive stimulus (saline solution) and peppermint was paired with an appetitive stimulus (sucrose solution). Additionally, we used absolute conditioning. For the retention test we transferred the animals to the T-maze one hour after training (de Brito Sanchez et al. 2015). We find that cockroaches accomplished the transfer task with olfactory stimulus.

Our results demonstrate that the cockroach *Periplaneta americana* can be successfully trained in spatial and olfactory forced choice tasks. Future experiments will investigate learning performance with different stimulus modalities and more complex tasks.

References:

de Brito Sanchez, M. G., Serre, M., Avargues-Weber, A., Dyer, A. G. & Giurfa, M. (2015). Learning context modulates aversive taste strength in honey bees. *The Journal of Experimental Biology*, 218: 949-959.

Pamir, E., Szyszka, P., Scheiner, R., & Nawrot, M. P. (2014). Rapid learning dynamics in individual honeybees during classical conditioning. *Frontiers in behavioral neuroscience*, 8.

Watanabe, H., Kobayashi, Y., Sakura, M., Matsumoto, Y. & Mizunami, M. (2003). Classical Olfactory Conditioning in the Cockroach *Periplaneta Americana*. *Zoological Science*, 20(12): 1447-1454.

Principals of olfactory-visual integration to form a common percept

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Many flowers attract their pollinators with diverse color and odor stimuli and finally present nectar and pollen as reward. Thus, the reward associated stimulus for pollinating insects represents a compound of visual and olfactory cues. We will use honeybees to study how the single components (light and odor) might be integrated to form a common percept.

Honeybees can be classically conditioned to a variety of single odor components and monochromatic light stimuli. Their ability to use both modalities to form a common reward association is not tested yet and represents the motivation of our work. To test if the olfactory visual compound is perceived as a unique cue or as the sum of its single components, we apply positive and negative patterning experiments. Furthermore, we aim to test the hypothesis if after associating an olfactory-visual compound with a reward the single component can substitute for the other by initiating the trained response behavior of the compound.

As a first step in investigating these questions, we ensure that the single modality components can be discriminated by the bees. In an initial experiment we have therefore trained honeybees to discriminate different single odor compounds. We tested Geraniol, Farnesol and Citronellol. The bees could differentiate all odor pairs reaching discrimination rates up to 80%. In the next step we establish differential conditioning of monochromatic light stimuli to finally combine both modalities to reach the main goal of the project.

Probing for 'cognitive enhancement' by *Rhodiola rosea*

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Humans traditionally use extracts from *Rhodiola rosea* roots for their 'cognitive-enhancing' remedy. Here, we scrutinize this effect in larval *Drosophila melanogaster*, as well as in adult flies and in the honeybee *Apis mellifera*. We report on experiments using food supplementation with *Rhodiola rosea* and tests for odour-reward learning, as well as tests for task-relevant sensory and motor function. As a next step, candidate bioactive compounds were likewise used and probed for cognitive enhancement effects, in particular in relation to memory acquisition, memory consolidation and age-related memory decline. *Drosophila* as a genetically tractable study case should then allow accelerated analyses of the molecular mechanism(s) that underlie such effects, and targeted follow-up analyses in rodents. To the extent that the molecular determinants of 'cognition' are shared between animals and man, such research may have bearings for humans as well.

Re-evaluation of learned information in *Drosophila*

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Animals constantly reassess the reliability of learned information to optimize their behavior. On retrieval, consolidated long-term memory can be neutralized by extinction if the learned prediction was inaccurate. Alternatively, retrieved memory can be maintained, following a period of lability in which it is reconsolidated. Although extinction and reconsolidation provide opportunities to alleviate problematic human memories, we lack a detailed mechanistic understanding of memory updating processes. Here we identify neural operations underpinning re-evaluation of memory in *Drosophila*. Accuracy of prediction during reactivation of sugar-reinforced olfactory memory can lead to either extinction or reconsolidation. Each process recruits activity in specific parts of the mushroom body output network and different subsets of reinforcing dopaminergic neurons. Memory extinction requires output neurons with dendrites in the α and β lobes of the mushroom body, which drive negatively reinforcing dopaminergic neurons that innervate the same zones. The aversive valence of new extinction memories neutralizes previously learned odor preference. Memory reconsolidation requires the β mushroom body output neurons. This pathway recruits negatively reinforcing dopaminergic neurons innervating the same compartment and re-engages positively reinforcing dopaminergic neurons to reconsolidate the original reward memory. These data establish that recurrent and hierarchical connectivity between mushroom body output neurons and dopaminergic neurons supports memory re-evaluation driven by reward prediction error.

Relief Learning Requires a Coincident Activation of Dopamine D1 and NMDA Receptors within the Nucleus Accumbens.

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Relief learning describes the association of a stimulus with the offset of an aversive event. Later, the now conditioned relief stimulus induces appetitive-like behavioral changes. The nucleus accumbens (NAC) is important for reward learning and it has been shown that this learning is mediated by an interaction of accumbal dopamine and NMDA glutamate receptors. Since conditioned relief has reward-like properties, we hypothesized that acquisition of conditioned relief may also be mediated by a concurrent dopamine D1 and NMDA receptor activation. The present study tested this hypothesis.

Therefore, rats received intra-NAC injections of the dopamine D1 receptor antagonist SCH-23390 and the NMDA antagonist AP-5, either separately or together, at different time points of a relief conditioning procedure. One hundred ninety one male Sprague-Dawley rats, 7 to 9 weeks old at the time of surgery were used. For measurement of acoustic startle reflex, rats were placed in cylinders located inside boxes. The conditional stimulus (CS) was a white light (5 s ca. 1000 lux) presented 2.5 s after an unconditional stimulus (US: 0.5 s; 0.4 mA). Startle stimuli was a 40 ms, 96 dB SPL white noise. For statistical analysis Prism 6.0 were used. The mean startle magnitudes of the startle trials in the absence and in the presence of the CS, as well as their differences were calculated for each animal. The significance level was set at $p < 0.05$ for all statistical tests.

Firstly, we showed that SCH-23390 dose-dependently block both acquisition and expression of conditioned relief. In the second experiment, we injected low doses of SCH-23390 and AP-5 either separately or together into the NAC. Co-injections of SCH-23390 and AP-5 blocked acquisition of relief learning, whereas when injected separately, these low doses had no effects. Notably, the co-injections neither affected consolidation nor expression of conditioned relief. Furthermore, they also did not change the locomotor response to the US suggesting that US perception is not changed.

This data indicates that a co-activation of dopamine D1 and NMDA receptors in the NAC is required for acquisition of relief learning. This D1/NMDA receptor interaction is known to promote synaptic plasticity. Future studies will investigate which intracellular signaling are involve in the synaptic plasticity underlying relief learning.

Role of dorsal hippocampus catecholamine signaling in paired-associates learning and place learning

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Hippocampus-based spatial memory processes include many forms of spatial learning and navigation, i.e. memory of places of events and things in the environment, map-like or paired-associate representations. Emerging findings suggest that hippocampus catecholamine (CA) transmission supports some forms of hippocampus-based spatial learning and memory. However, little is known to date about which forms of hippocampus-mediated spatial learning are modulated by CA signaling in the hippocampus. Therefore, in the current study we examined the effects of 6-hydroxydopamine (6-OHDA)-induced CA depletion in the dorsal hippocampus on two prominent forms of hippocampus-based spatial learning, that is learning of object-location associations (paired-associates learning) as well as learning and choosing actions based on a representation of the context (place learning).

In Experiment 1 we used an automated touch screen paired-associates learning (PAL) task for rodents that demands learning that a particular object, i.e. one out of three symbols, is only correct in a particular location, i.e. one out of three positions on the touchscreen. On a given trial, two symbols are displayed, one in its correct, another one in an incorrect position, and the rat has to respond to the symbol in the correct position. If hippocampal CA signaling supports object-location associations, hippocampus CA depletion should impair task performance. At variance with this hypothesis, results show that rats with CA depletion of the dorsal hippocampus, like sham controls, were able to learn object-location associations in the PAL task. One possibility to explain this negative result is that object-location learning as tested in the touchscreen PAL task seems to require relatively little hippocampal processing.

In Experiment 2, we used a T-maze spatial learning task. This task can be solved either by hippocampus-mediated place learning, i.e. learning and choosing actions based on a representation of the context or allocentric coordinates or by striatum-based response learning that involves body-centered or egocentric coordinates, such as a left-turn response. If hippocampal CA signaling supports learning and choosing actions based on allocentric coordinates, hippocampus CA depletion should induce a preference for a response over a place strategy. Results indicate that, compared to controls, in rats with CA depletion of the dorsal hippocampus the use of a response strategy was facilitated.

Immunohistochemical analysis revealed that control rats displayed sparse tyrosine hydroxylase (TH) positive immunostaining in the dorsal hippocampus. In both experiments, 6-OHDA infusion reduced TH immunoreactivity in the CA1 region. As TH immunoreactivity characterizes both DA and NA fibers, our data indicate that 6-OHDA infusions decreased DA and NA input to the dorsal hippocampus.

Taken together, we suspect that dorsal hippocampus CA depletion could have altered interactions between involved learning systems, i.e. accelerated the shift from the hippocampus-based place learning system to the dorsolateral striatum-dependent response learning system. Thus, our findings provide further support to the notion that hippocampus CA transmission supports at least some forms of hippocampus-based spatial learning and memory.

Role of Nogo-A signaling in regulating spatial learning and memory formation by modulating hippocampal parvalbumin (PV)-interneuron networks

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The neuronal circuitry in the mature brain is characterized by a high degree of stability, thereby providing a correlate for long-term storage of information. On the other hand, learning and memory formation processes involve changes in both the structure and function of synaptic connections. Thus, a set of regulatory molecules are required to tightly control the balance between the plastic changes and the stability of synaptic connections. Nogo-A has been shown to play a crucial role in restricting activity-dependent functional and structural plasticity in the adult CNS. In addition, we could recently show that Nogo-A regulates spatial memory formation and persistence in the adult mouse hippocampus. Learning and memory processes depend on changes in inhibitory network activity. In particular, changes in the activation level of parvalbumin (PV) positive hippocampal interneurons play a crucial role in modulating the distinct phases of hippocampus-dependent learning. During the early phase of spatial learning, the PV expression level of CA3 PV-interneurons decreases resulting in a shift to a low-PV network configuration associated to an enhanced excitation supporting the plastic process of memory formation. On the other hand, during the late phase of spatial learning, the PV expression is increased leading to a shift to a high-PV configuration thereby providing an enhanced inhibition to suppress the activity of pyramidal neurons and to consolidate the formed memory.

Interestingly, we could show that while Nogo-A is expressed at low levels in excitatory hippocampal neurons, it is highly expressed in PV positive interneurons, especially within the CA3 region. We therefore address whether Nogo-A regulates neuronal plasticity in the hippocampus by modulating the activation of hippocampal PV interneurons. Thus, to elucidate a possible correlation between learning-dependent changes in Nogo-A expression and PV network configuration changes in the hippocampus the expression levels of Nogo-A and PV are analyzed during the early learning (day 3) and consolidation (day 10) phases in the Morris water maze training. In addition, we show that Nogo-A negatively regulates excitatory synaptic transmission of CA3 pyramidal neurons on a fast time scale. Upon Nogo-A neutralization using function blocking antibodies we found an increased insertion of AMPA receptors into the membranes of cultured hippocampal neurons along with an increase in the amplitude of miniature excitatory postsynaptic currents (mEPSCs) and of synaptic calcium transients. However, whether this is due to an exclusive effect on excitatory neurons or rather also to an indirect effect via an action on PV interneurons is still unknown. Thus, we are addressing whether Nogo-A might acutely control excitatory synaptic transmission by regulating the activity of hippocampal interneurons. Here, we show that acute neutralization of Nogo-A results in a fast decrease in the amplitude and frequency of miniature inhibitory postsynaptic currents (mIPSCs) in CA3 pyramidal neurons indicating that Nogo-A regulates both excitation and inhibition in the hippocampus.

These results suggest that Nogo-A regulates the activity of inhibitory interneurons. This might in turn control the plasticity levels of the excitatory hippocampal networks thereby modulating hippocampal learning and memory formation. Funded by the DFG (ZA 554/3-1).

Somatostatin-expressing interneurons in the dentate gyrus are required for spatial memory precision.

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The dentate gyrus receives and integrates multiple sensory inputs from the entorhinal cortex to generate spatial representations. At the cellular level this process relies on the activity of granule cells (GC), which in turn is tightly regulated by different types of inhibitory GABAergic interneurons.

Somatostatin (SOM)-expressing interneurons are a distinctive class that synapses onto GC dendrites mainly in the outer molecular layer, regulating entorhinal input through feedback inhibition.

We tested the relevance of SOM-immunopositive interneurons for dentate gyrus-dependent spatial learning by functional inactivation. Our behavioural data suggest that SOM interneuron - mediated inhibition is required for the encoding of precise spatial memories. On the network level, SOM interneurons have been shown to restrict the number of recruited granule cells. We analyze how functional removal of SOM interneurons influences the differential recruitment of GCs during the formation of distinct spatial memories by means of compartmental analysis of IEGs transcription imaged by fluorescence in situ (catFISH).

The Role of full-length Amyloid Precursor Protein-Like (APPL) in *Drosophila* short-term memory formation

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The amyloid precursor protein (APP) has been identified in the search for the causative agent of Alzheimer's disease (AD). Altered proteolytic processing of APP in the brain, leading to increased production of the neurotoxic A β -amyloid peptide and subsequent plaque formation is a hallmark of AD. The APP gene is conserved from nematodes to man but its endogenous function remains elusive by large. The evolutionary conservation of APP genes suggests a beneficial endogenous role for brain integrity and function. The central hypothesis of this work is that the balanced differential processing of the *Drosophila* APP orthologue Amyloid Precursor Protein-Like (APPL) has a crucial role for the function of the central nervous system. Changes in synaptic function and connectivity of neuronal networks are most sensitively monitored by changes in behaviour. Besides APPL, the *Drosophila* genome encodes all types of secretases required for APP/APPL processing. Therefore we tested *Appl* null mutants (*Appl^d*) and heterozygous mutants of all three secretases [*kuzbanian* (*kuz*) = α -secretase, *dBace* = β -secretase and *Presenilin* (*Psn*) = γ -secretase] for a short-term (~4s) memory, the visual orientation memory. The analysis revealed that aged wild-type flies lose the ability to memorize landmarks by the age of four weeks but heterozygous mutant flies for all three secretase surprisingly show an improved memory when compared to age-matched controls (up to six weeks). Notably, *Appl^d* flies have no orientation memory suggesting an important role of APPL and its proteolytic fragments in working memory function. Cell-specific rescue experiments in the R3-ring neuron of the ellipsoid body revealed a requirement of the full-length flAPPL or flAPP for memory formation. However, flAPPL isn't able to rescue, if an ectodomain (E2), which is a well conserved protein binding site, is defect. We also found, that the C-terminal part of the protein is not needed for the visual orientation memory. We therefore suggest, that APPL functions as a ligand by binding via its E2 domain to a yet unknown receptor. To further investigate the APPL fragment distribution in the *Drosophila* central brain we created a fluorescent double-tagged version of APPL and human APP. Expressing dtAPP or dtAPPL specifically in R3-neurons of the ellipsoid body reveals that similar amounts flAPPL/flAPP and secreted sAPPL/sAPP are localized in the axons of the ellipsoid body or the dendritic arborisation in the bulb. Notably, in the cell bodies we find a high variability in the processing and fragment distribution. Some R3 cell bodies are exclusively labelled for the N-terminus or the C-terminus of APPL/APP, respectively, whereas others display considerable amounts of flAPPL/dtAPP together with preprocessed fragment. These results indicate that APPL processing and fragment distribution differs between individual neurons of the same type. Furthermore it could imply that APPL processing differs between neurons of different neurotransmitter signature or mode of activation.

The role of serotonin in behavioural phase transition in the desert locust

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Desert locusts (*Schistocerca gregaria*) transform between two profoundly different phenotypes ('phases') depending on population density. Isolation from conspecifics results in a behaviourally and morphologically cryptic solitary phase, whereas crowding leads to an active and colourful gregarious phase. Behavioural gregarisation can be induced in the laboratory within 2h of crowding, so *S. gregaria* provides a useful model for analysing mechanisms underlying phenotypic plasticity.

Previous studies have reported that the amount of serotonin (5-hydroxytryptamine; 5-HT) in the thoracic ganglia shows a pronounced increase in the first 4 h of gregarization, which correlates with the degree of behavioural gregarization in this time window.

Our attempts to replicate these effects in a different strain of locusts have been unsuccessful. One potential explanation is that strains differ in their propensity to gregarise. To better determine the roles of 5-HT and strain in phase change, we used the same High Performance Liquid Chromatography (HPLC), pharmacological and behavioural techniques in two colonies of locusts: one reared on site for many generations (Leicester strain), and the other a wild-derived colony reared on site for 3-4 generations (Mauritanian strain). Juvenile 5th instar solitary locusts of both strains were either crowded with conspecifics for 4 h to induce gregarisation or left uncrowded (controls). Individual behavioural characteristics were measured in an established arena assay to determine each animal's probability of belonging to the gregarious phase (p.greg). After this, the locusts were immediately frozen and their thoracic ganglia dissected and analysed by HPLC to quantify 5-HT. In a similar experiment, Leicester animals were additionally injected every 2 days over a 5 day period prior to crowding, either with locust saline (controls) or the compound alpha-methyltryptophan (AMTP), previously found to inhibit insect 5-HT synthesis.

Mauritanian animals had significantly higher p.greg values than Leicester animals both before and after crowding ($p=0.029$). As expected, crowding for 4 h significantly increased p.greg in both strains ($p<0.0001$), but there was no interaction between strain and treatment ($p=0.102$). Mauritanian animals had 40% more ganglionic 5-HT than Leicester animals ($p<0.0001$). Surprisingly, 5-HT levels changed by less than 6% after 4 h of crowding in either strain ($p=0.285$). There was no correlation between p.greg and ganglionic 5-HT level in either strain (Mauritanian $r=0.25$, $p=0.12$; Leicester $r=0.06$, $p=0.48$). Furthermore, AMTP only partially reduced gregarisation ($p=0.026$) and depleted 5-HT by just 25% ($p=0.004$).

These results indicate that the Leicester and Mauritanian strains differ subtly in baseline behaviour and 5-HT levels, but have a comparable propensity to gregarise. The absence of strong correlations between 5-HT and behavioural phase state in both strains differs from previous work, as does our failure to observe elevated 5-HT levels after 4 h crowding. It is possible that there are strain-dependent differences in the latency of the 5-HT peak, which would require measuring 5-HT at more time points during gregarisation. The low efficacy of AMTP contrasts with previous work reporting strong effects on behavioural phase state and 5-HT levels. Our future work aims to establish more efficacious methods for depleting 5-HT in the central nervous system to clarify the role of 5-HT in gregarisation.

The timing of the interior-exterior transition in *Camponotus rufipes* ant workers and its underlying neuronal correlates

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In the polymorphic ant *Camponotus rufipes*, media sized workers undergo an age-related division of labor: young ants perform nursing tasks in the nest interior first and then switch to multimodal external foraging tasks in the environment. The duration of the interior period of nurses and therefore the timing of the interior-exterior transition is still unknown for this species. We hypothesize that the worker age at the onset of foraging is a flexible adaptation to the actual colony size and its biological needs. In this study, we established subcolonies, each consisting of initially 100 marked workers and 100 larvae, which were reared in a “nest” incubator (DD 24h, 25°C, 55%rH) with an open access connection to a foraging arena located in a second incubator (LD 12:12h, 25°C, 55%rH, food *ad libitum*). Newly emerged ants were marked individually every day. We continuously videotaped all subcolonies to easily monitor the single workers task affiliation (nursing or foraging) and evaluated the time point of the interior-exterior transition. With 11.500 hours of video recordings, we show that single workers start nursing tasks 1-2 days after eclosion until they leave the nest for foraging with an age up to ~14 days, whereas some ants never switch to foraging tasks within an observation period of 3 month. This suggests that the interior-exterior transition is highly flexible and matches the actual colony needs.

Additionally, we examined whether *C. rufipes* workers express task-related neuronal plasticity within synaptic complexes in mushroom body (MB) input regions at both, the pre- and postsynaptic ultrastructural level. Previous studies in the honeybee, using sectional EM 3D-reconstructions revealed that the interior-exterior transition is correlated with structural changes at the pre- and postsynaptic side of PN boutons in the MB input region (Groh et al, 2012). Implementation of Electron Tomography would be beneficial to increase synaptic resolution for a better comprehension of architectural changes in neuronal plasticity.

Using double tilt series (-70° to 70°), we improved the resolution of single vesicles and the architecture of active zones within 250 nm thin sections. An adequate high resolution is an important requirement for a better understanding of architectural changes within PN boutons in the MB calyx associated with the interior-exterior transition of single *C. rufipes* workers. Supported by DFG SFB 1047 “Insect timing” (B5, B6, C1).

Time-dependent reinforcement effect of dopaminergic neurons

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Animals learn to associate external cues with significant events which subsequently act as a predictor of the event. Associative learning, both regarding reward and punishment, is evolutionarily conserved across phyla including humans, rodents, and also the fruit fly *Drosophila melanogaster* [1]. For an organism to form an associative memory, the timing at which the conditioned stimulus (CS) is presented with respect to a punishing/rewarding event is crucial for the meaning of the stimulus accordingly. In the case of olfactory associative learning in the fruit fly, when an odour is trained such that it precedes a punishment stimulus (i.e., electric shock), then the odour is perceived as a predictor of punishment and later avoided by the fly [2]. Interestingly, when the same odour is presented at the offset of electric shock, it is perceived as a predictor of the end of punishment (relief from punishment) and later approached by the fly. This phenomenon is called relief learning [3] and is also observed in rodents and humans. Very recently, dopaminergic neurons (DANs) were found to timing-dependently mediate both punishment and relief learning in adult *Drosophila* [4]. Also, the larva of the fruit fly is an established model organism for associative learning, offering an even simpler neuronal circuit than the adult stage. Relief learning, however, had not been observed in the larva so far. Importantly, optogenetic activation of DANs is sufficient to establish punishment memory in *Drosophila* larvae [5]. We first confirmed previous studies in finding that a set of dopaminergic neurons projecting from the PPL (protocerebral posterior lateral) cluster to the vertical lobe of the larval association center called mushroom body substitute for punishment in larvae. Then, we investigated the timing-dependent modulatory reinforcement effect of that set of neurons by analyzing the behavioural outcome towards the conditioned odour paired at different interstimulus intervals (ISI) with optogenetic DAN activation. This provides the first systematic analysis of timing-dependent associative learning in larval *Drosophila* and opens the doors for studies of the individual DANs that signal internal reinforcement in a timing-dependent manner.

Reference:

1. Andreatta et al., 2010 Proc Biol Sci 277: 2411–2416
2. Gerber et al., 2014 Learn Mem 21(4): 232–252
3. Yarali et al., 2012 PLoS ONE 7(3): e32885
4. Aso, Y., & Rubin, GM. 2016 ELife 5. pii: e16135.
5. Schroll et al., 2006 Curr Biol 16(17):1741-7

Changes in neuronal plasticity and brain morphology in leptin-deficient (ob/ob) mice

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We were interested in investigating whether obesity has an impact upon neuronal plasticity and/or learning and memory. As an animal model of obesity we used leptin-deficient (ob/ob) mice (4-6 months). We first monitored the increase in body weight over time, as compared to age-matched controls. We could show that the body weight in these mice differed significantly (wt ~24.78g; ob/ob ~57.85g).

Since in humans higher adiposity was consistently found to be associated with cortical atrophy and since in another mouse model of obesity signs of cortical and hippocampal atrophy have been detected, we analyzed the brain morphology by using a micro-CT approach based on inorganic iodine. Scans were analyzed using 3DSlicer-software. In detail, the total volume of the brains and selected brain areas (e.g. hippocampus) and ventricles has been analyzed. For volume measurement of the brains, we also used a method based on a microvolumeter. Our results so far confirmed smaller total brain volumes in ob/ob mice (wt ~0.52ml, ob/ob ~0.48ml).

In order to determine the changes in adult hippocampal neurogenesis in ob/ob mice in detail, we took advantage of stage-specific markers for adult hippocampal neurogenesis. We investigated changes in general cell proliferation using the cell division marker anti-phospho-Histone H3. For the neuronal lineage, including mitotically active neuronal cells and young postmitotic neuronal cells, anti-doublecortin was used. Our results obtained so far indicate an alteration in adult hippocampal neurogenesis in ob/ob mice, leading to fewer newly formed neurons in the dentate gyrus of the hippocampus. Our behavioral analysis concerning the ob/ob mice included open field and Morris water maze testing, which can be used as a readout for altered hippocampus-dependent learning.

Cholinergic regulation of hippocampal network oscillations

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Acetylcholine (ACh) is involved in the transition from hippocampal sharp wave-ripple activity (SPW-Rs) during quiet behaviour to theta and gamma activity characteristic for explorative behaviour.

We use stimulus induced SPW-Rs like activity as a model to investigate the underlying mechanisms involved in switching between SPW-R activity and oscillations in the gamma band.

We observed that application of cholinesterase inhibitor physostigmine (5 μ M) alone or in combination with ACh led to transition from SPW-Rs to gamma oscillations. This switch could be prevented by application of the M1 receptor antagonist pirenzepine.

To further study the differences between these two types of network oscillation we employed multi electrode array recordings. We found that SPW-Rs were associated with preceding multi-unit activity. Large units generated close to the recording electrodes at pyramidal cell layer during epochs of SPW-R activity were rarely observed during gamma oscillations. Cross correlation analysis between different recording sites suggested that the leading sites for SPW-Rs are located in hippocampal area CA3bc, while the leading site for gamma oscillations was likely to be in CA2.

Furthermore, the block of SPW-R activity involves an alteration in probability of transmitter release as indicated by an enhancement of paired pulse index, coefficient of variance analysis of stimulus evoked EPSPs and by a reduction in presynaptic Ca²⁺ entry.

Since ACh levels are elevated during wakefulness it was important to test whether SPW-R activity can be induced in presence of ACh. We therefore tested for facilitated induction of SPW-Rs and found indeed that in presence of physostigmine (2 μ M) alone or in combination with ACh, SPW-Rs could be more readily elicited.

Our data unveil mechanisms that underlie the transition between ripple-dominated vs. gamma-dominated hippocampal network activity.



This work is dedicated to
Uwe Heinemann who left us
last year but inspired many of us

Circuit processing in rodent auditory cortex underlying complex auditory learning

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Auditory cortex plays a fundamental role for auditory learning and the formation of corresponding memory traces. However, few attention has been paid to the distinct cortical circuit mechanisms underlying learned auditory behaviors. Recently, we proposed a recurrent feedback gain circuitry between auditory cortex and thalamus that is under dopaminergic control and promotes the detection of behaviorally important stimuli (Happel et al. 2014). Here, we will present current source density (CSD) data chronically recorded from primary auditory cortex (A1) of Mongolian gerbils (*Meriones unguiculatus*) during different auditory behavioral tasks. We chronically recorded stable CSD data over several weeks to month, while animals successfully performed multiple contingency reversals of a frequency discrimination task. We have used methods for a trial-wise estimation of the choice probability (Deliano et al., 2016) in order to obtain from single-trial CSD data layer-dependent processing modes underlying the behavioral outcome. This allows us to further investigate long-term layer-dependent adaptations of the cortical processing in the context of different forms of learning (detection/discrimination/reversal) and during task-specific changes. Finally, the newly developed approach allows us to discuss causal links between distinct cortical processing modes in sensory cortex, as recurrent feedback gain, and cognitively complex forms of decision making and learning.

Duets in Africa: Wireless microphones on free living white-browed sparrow weavers

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In songbirds, the underlying neuronal substrate for song production is the song system which is present in both the male and female brain. Songbirds learn their stereotyped song pattern early in life and the notion is that in most species the song is produced in males only. A recent study by Odom et al. shows that there are far more species where females sing than supposed (71% of 323 surveyed species).

In the zebra finch, where only the male produces learned song, we found that unlearned calls are used for the communication between male and female of a couple (Termaat et al. 2014), which gives rise to the idea that the song system evolved from a generic communication system that is present in both males and females.

In duetting species both sexes sing and during duetting the songs are performed in concert. The white browed sparrow weaver (*Plocepasser mahali*) is a duetting species. These group-living and cooperatively breeding birds sing alone, in duets as well as in chorus, dependent on their status in the group (Voigt et al. 2007).

Our aim is to study the functioning of the song system of these birds in males and females, and in relation to social context. We have recorded complete groups of white-browed sparrow weavers (2 – 7 group members) in the Kalahari desert, South Africa. Miniature wireless microphone transmitters were used to record each individual of a wild white browed sparrow weaver group at the same time. The temporal relationships between the vocalizations reveal the social relationships in the group and match the behavioral observations of the dominance status.

The data gained in this field study allow us to record song system neurons using implanted electrodes in captive weaver birds that show normal social behavior.

References

Odom et al., Female song is widespread and ancestral in songbirds, *Nature communications*, 3379, 2014

Ter Maat et al., Zebra Finch Mates Use Their Forebrain Song System in Unlearned Call Communication, *Plos One*, 9 (10), 2014

Voigt et al., Socially induced brain differentiation in a cooperatively breeding songbird, *Proceedings of the Royal Society B-Biological Sciences* 274 (1626), 2007

Dynamic computation of hierarchical prediction errors during sequence learning

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Sequence learning is the ability to learn about the temporal patterns of environmental stimuli, and is a crucial ability for much of human cognition and behavior. Many behavioral and neuroscience studies have shown that humans attempt to identify patterns in order to predict future events, even when these patterns do not provide information about future outcomes (1). Neuroimaging research on sequence learning has proceeded largely independently of the research on computational reinforcement learning, because the focus is primarily on learning statistical dependencies of stimulus sequences rather than on optimizing reward-based performance through learning. Here we investigated whether and to what extent mechanisms of reinforcement learning can be also employed in statistical sequence learning.

Standard reinforcement learning model (2) postulates that average rewards are estimated for all available actions and that action selection is biased towards the most rewarding option. It cannot explain, however, how subjects can learn in situations where the average rewards for all the options are equal but the reward received is conditioned on a specific sequence of events. We hypothesized that hierarchical prediction errors are computed to learn the conditional correlations. To test this hypothesis, we developed a Markov decision task where we precisely control the temporal dependencies by first and second-order transition probabilities. 25 participants performed the task while being scanned with fMRI. We analyzed the fMRI data with higher-order reinforcement learning models. We found lower-order prediction errors in the ventral striatum and higher-order prediction errors in the prefrontal and parietal cortices. Our results suggest that the neurobiological mechanism of reinforcement learning is dynamically involved in the processing of temporal dependencies and that the human brain implements reinforcement-learning mechanism to estimate higher-order probabilities in the same manner as estimating the zero-order average rewards.

1. S. A. Huettel, P. B. Mack, G. McCarthy, *Nat. Neurosci.* 5, 485–490 (2002).
2. R. S. Sutton, A. G. Barto, *Reinforcement Learning* (MIT Press, Cambridge, MA, 1998).

Effects of Anodal tDCS on Auditory Learning

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Transcranial direct-current stimulation (tDCS) is a low cost, non-invasive method that has the potential to actively shape neural circuits by sending constant, subthreshold electric direct current through the skull via electrodes placed in a region of interest. This current modulates the resting membrane potential of neurons in cortical layers of the brain. Positive polarity (anodal) at the scalp in general induces excitation of the underlying cortex.

Our research investigates mechanisms underlying the effects of tDCS on cortically-dependent learning and memory. We use rodent GO/NO-GO auditory discrimination with frequency modulated tones, which has been demonstrated to be an auditory cortex dependent task. We tested two experimental groups, an anodal tDCS-stimulated group and a control group that was sham-stimulated. Each group underwent 10 minutes of anodal or sham stimulation directly before each training session. Each animal performed in 60 trial learning sessions during 10 consecutive days, in which they were expected to reach our learning criteria. While analyzing the behavioral data, we realized that animals which learned, whether control or stimulated, could be classified into two distinct strategy groups: the detection first strategy and the discrimination strategy. Our analyses show that there is improved learning in the anodal tDCS group, but this difference is only observed in the animals that follow the discrimination strategy.

Effects of *c-fos* manipulation in the central amygdala on appetitive learning

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In addition to being widely investigated as a marker of neuronal activity, expression of c-Fos has also been shown to be closely linked with synaptic plasticity, learning and memory. Studying c-Fos expression pattern in the brain enables to learn neuronal networks spanning different brain areas that are essential for different behaviors, e.g. appetitive and aversive learning. One of the few brain regions that process those two behaviors is the central nucleus of amygdala (CeA), which is well known for its involvement in aversively motivated learning, e.g. in fear conditioning. However, neurons in the CeA are also essential for reward learning, e.g. place preference tasks. Before, we reported that matrix metalloproteinase-9 (MMP-9, extracellularly operating enzyme) release and activity in the CeA is crucial for appetitive, but not for aversive, learning. Since in activated neurons MMP-9 expression is regulated at the transcriptional level by c-Fos/AP-1, we hypothesized that appetitively motivated learning depends also on learning-driven c-Fos expression in the CeA. To test this hypothesis we injected CeA with a lentiviral vector expressing a short-hairpin (sh) RNA. Blocking *c-fos* expression resulted in impairment of appetitively but not aversively motivated discrimination learning and caused a significant reduction in motivation to seek for a reward. To further characterize c-Fos dependent motivational changes in the CeA we decided to manipulate optogenetically neurons involved in reward learning. Animals were injected with genetic construct, in which channelrhodopsin is placed under control of *c-fos* promoter and trained in an operant conditioning chamber to associate auditory stimulus with contiguous food reinforcement. Learning was defined as an ability to modify the bar-pressing responses during exposure to the signaling stimulus. Subsequent activation of the CeA neurons during the signaling stimulus presentation amplified incentive motivation for food reward reflected by an increase in the number of bar-pressing responses. Such stimulation did not change behavioral responses in the absence of a signaling stimulus. Taken together, the results reveal that the expression of *c-fos* in CeA is necessary for learning motivated by alimentary rewards and that *c-fos* expressing subpopulation of neurons modulate incentive motivation to pursue an associated reward.

Experience induces rapid nucleus-scale movements of chromatin in mouse auditory cortex neurons.

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Dynamic chromatin modifications play a major role in controlling the expression levels of individual genes. Changes in gene expression patterns in response to behavioral experiences crucially contribute to the remodeling of neuronal networks in order to mediate higher cognitive functions such as memory formation. Furthermore, dynamic chromatin movements at the scale of the nucleus have been observed during interphase and are believed to support alterations in gene expression programs. Despite early evidence for nucleus-scale reorganization of chromatin in neurons (Barr et al., *Nature* 1949) up to date, no chronic imaging data of chromatin dynamics in living animals is available. Applying *in vivo* two-photon imaging in a novel transgenic mouse model conditionally expressing a fusion protein of the core histone H2B and a photoactivatable GFP allowed us to chronically photolabel and image chromatin of neurons of the auditory cortex. We show that chromatin organization can undergo rearrangements within minutes. Auditory cued fear conditioning, an associative learning paradigm prompting changes in gene expression patterns in auditory cortex (Peter et al., *Genes Brain Behav.* 2012), induces chromatin remodeling in auditory cortex neurons. Furthermore, we demonstrate in acute brain slices prepared from the same transgenic mouse model that pharmacological manipulation of neuronal activity recapitulates features of the observations made *in vivo*. In summary, we establish a methodology to follow chromatin dynamics chronically *in vivo* and provide a longitudinal description of neuronal chromatin dynamics during behavior.

Extracellular matrix in auditory cortex: impact on remote memory control and learning flexibility in adult rodents

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Recently we have revealed that the extracellular matrix (ECM) in auditory cortex of Mongolian gerbils is in control of the behavioral flexibility underlying cognitively demanding reversal learning tasks (Happel et al., 2014, PNAS). This first study implicated a novel function of the cortical ECM as a potential regulatory switch to adjust the balance between stability and plasticity in the adult, learning brain. However, current research has only started to examine the impact of the ECM on learning-related plasticity, life-long memory re-formation and higher cognitive functions.

Here, we present recent results of intrinsic ECM protein turnover in auditory cortex of mice correlating with individual auditory learning and remote memory retrieval performance. Inherent proteolytically induced ECM remodeling during learning was investigated using post-training quantitative Western blot analysis. Further, enzymatically induced ECM removal in bilateral auditory cortex of mice did not interfere with initial auditory acquisition learning but improved remote memory retrieval. We will discuss our findings with respect to the relationship between learning and long-term memory retrieval and corresponding endogenous or manipulated ECM protein dynamics with respect to individual behavioral performance.

Functional and spatial organization of hippocampal assemblies in vivo

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Diversity of neuronal cell types classically reflects the available repertoire of functions in different brain regions. Despite several studies about interneurons diversity in the hippocampus, only few studies address the possibility that glutamatergic CA1 pyramidal cells may comprise several subtypes with different functional and morphological features (Thome 2014, Mizuseki 2011).

We investigated the relationship between the developmental temporal origin of glutamatergic neurons and their recruitment into behaviorally-relevant network dynamics (SPW-R), specifically the organization of cell assemblies which are known to represent ongoing and remote experiences. We used calcium imaging (GCaMP6-M) to monitor neuronal activity in head-fixed mouse voluntarily running on a cued or un-cued treadmill using 2-photon imaging in vivo. Additionally, we probed the co-occurrence of network oscillations using a contra-laterally implanted field electrode to relate the calcium activity to the network state. Therefore, we were able to characterize the recruitment, the spatial organization and involvement of neurons from different developmental ages.

Injury of GABAergic interneurons and behavioral deficits in mice after postnatal exposure to high oxygen as a model of neuropsychological symptoms in former preterm infants

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Preterm infants often suffer from neurological deficits caused by impaired postnatal brain development. So far, neuropsychological and educational problems of former preterm infants at school age have mainly been ascribed to perinatal white matter damage as an underlying disease. We aimed to investigate whether numbers and function of cortical GABAergic interneurons is perturbed by exposure to oxygen toxicity during early postnatal development. We used a hyperoxia model exposing newborn mice to 80% O₂ for 48h from P5 to P7. The gene expression of markers of the interneuron subtypes Pvalb, Reln, Sst and Vip was analyzed by qPCR at P7. The number of the PVALB+ dominant subtype of GABAergic interneurons was analyzed by immunohistochemistry at P30. To verify an impact of a reduction of GABAergic interneurons on behavior, we performed switched and novel object recognition test, and sociability test in P60-P70 mice.

As a result, hyperoxia exposed mice showed a significant reduction of Pvalb, Reln and Vip gene expression at P7. After recovery in room air the number of PVALB+ cortical GABAergic interneurons was diminished in young adult mice at P30. Mice exposed to hyperoxia showed a significant deficit in memory tasks done by switched and novel object recognition test. The impaired memory was accompanied by a reduced social interaction in the sociability test.

Due to these data, neuropsychological and educational impairments of former preterm infants might be associated with a maldevelopment of the GABAergic interneuronal system in the cortex caused by oxygen toxicity.

Involvement of the prefrontal-thalamic-hippocampal network in a touch screen based working memory task

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Working memory deficits are found in many psychiatric and neurological disorders. Our goal is to combine a sensitive, automated working memory task with electrophysiological recordings to identify key players in prefrontal-hippocampal communication during working memory functioning.

Using Touch Screen Chambers for mice, we designed a highly translational working memory paradigm for mice. Animals are trained to remember specific locations in this automated task, responding by touching light stimuli in order to receive a reward (strawberry milkshake). Once trained, depth electrodes are placed in the relevant brain areas (prefrontal cortex, hippocampal CA1 and thalamic nuclei) to allow for local field potentials and multi-unit recordings while the animals perform the task.

To assure the involvement of the prefrontal cortex, we designed this task at high level of difficulty and unpredictability. Our data show that mice are capable of learning the challenging task, with all mice reaching threshold performance at a 2 second delay, most at 4 seconds and only some with a 6 second delay. Initial analysis indicates that there are specific changes in theta activity during the performance of the task, dependent on correct or incorrect responding. Further, ongoing analysis will confirm the relation between localized neural activity in the prefrontal hippocampal network and working memory performance.

We show activation within this network of specific frequency bands depending on behavioral accuracy. This confirms that the prefrontal-thalamic-hippocampal network plays a crucial role during working memory performance and activation of this network allows for accurate working memory retrieval.

Learning enables view-invariant prediction errors in monkey face patch ML

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Extracting regularities from the environment allows the brain to make predictions about upcoming events. The brain is especially adept at detecting associations between co-occurring stimuli, as exemplified by statistical learning. However, it is currently unclear how these associations are represented and how they may affect sensory processing. We investigated whether learning to associate faces affects face processing at early, view-specific stage of the face processing hierarchy, in face patch ML. To this end, we exposed macaque monkeys to pairs of faces presented in fixed sequence so that the first image in the sequence would become predictive of the second image in the sequence. By pairing faces with particular views (e.g., left face A – right face B), we asked whether the face patch system learns specific-stimulus association (face pairs and their specific view configurations) or instead generalizes across views to learn abstract stimulus pairing at the level of identity (i.e., face A is followed by face B regardless of view). After several weeks of training, we used fMRI-guided single unit recordings to test whether and how the associations between co-occurring faces affected face processing in ML. We found that neurons responded more strongly to facial identities that had not been associated during training relative to trained face pairs throughout the transient and the sustained phase of the response, reminiscent of a prediction error. This effect was not present for pairs in which identities had been associated during training but that were now tested with different views. Thus, learning enables view-tuned neurons in ML to signal view-invariant prediction errors about identity. This indicates that learned associations between faces are already represented at early, sensory stages of face processing and furthermore that they are stored in a generalized, view-invariant format.

Long-term plasticity and fear learning in adult heterozygous BDNF knockout mice

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The neurotrophin BDNF (brain-derived neurotrophic factor) is an important mediator for synaptic plasticity and crucially involved in learning and memory formation. Recently we could demonstrate that heterozygous BDNF knockout (BDNF^{+/-}) mice exhibit an age-dependent learning deficit, when they are 3 months of age or beyond. Furthermore, we could demonstrate that long-term potentiation (LTP) at thalamic input synapses to the lateral amygdala (LA) is already impaired in 4 weeks old animals, while LTP at cortical input synapses remained intact. Thus, we hypothesized that an age-dependent decline in synaptic plasticity at cortico-LA synapses might account for the observed learning deficit. Therefore, we now analyzed LTP at cortico-LA synapses in 3 months old BDNF^{+/-} mice. However, LTP at these as well as at subsequent glutamatergic intra-amygdala synapses was intact. Interestingly, we observed short-term fear memory in BDNF^{+/-} mice up to 6 h after fear conditioning, but the precision of this early fear memory gradually declined. These results suggest an intact acquisition of fear memories in BDNF^{+/-} mice, which is in line with the intact LTP at cortico-LA synapses in these animals.

In addition, we performed *ex vivo* field potential recordings of fear conditioned mice which revealed fear learning induced long-lasting changes at cortico-LA synapses in wildtype but not in BDNF^{+/-} mice. This lack of long-lasting changes at these synapses mirrors the observed deficit in fear memory consolidation in these mice.

In conclusion, our data demonstrate intact LTP at cortico-LA synapses of adult BDNF^{+/-} mice, which is in line with intact acquisition of fear memories. Furthermore, the lack of long-term changes at cortico-LA synapses parallels the observed deficit in fear memory consolidation in BDNF^{+/-} mice.

Supported by DFG, SFB 779/B6

Spatial long-term memory and modulation of NMDA receptor subunit expression in medial septal cholinergic and noncholinergic neurons lesioned rats

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The present study was designed to investigate the effect of selective immunolesions of cholinergic and GABA-ergic SH projection neurons (using 192 IgG-saporin and GAT-1 saporin, respectively) on spatial memory assessed in water maze and the N-methyl-D-aspartate (NMDA) receptor GluN2B subunit expression in the rat hippocampus. We used water maze training protocol with eight training trials. One day after training, probe test with the platform removed was performed to examine long-term spatial memory retrieval. We found that immunolesion of medial septal cholinergic neurons did not affect spatial learning as exhibited by a decreased latency to find the hidden platform across the eight training trials. In contrast, rats with immunolesions of medial septal GABAergic neurons did not show a decreased latency across training trials in water maze. Trained control rats spent significantly longer than chance (15 s) performances such as swimming time in test sector (where the hidden platform was located). Moreover, they spent significantly longer in test sector than in the opposite sector, confirming the establishment of long-term memory. In contrast, the preference for test sector was abolished in medial septal immunolesioned rats. Because Saporin treated rats learned the location of the hidden platform during training, the results suggest that saporin treated rats could not remember the training a day later. We found that the expression level of NR2B subunit of NMDA receptor in the hippocampus was decreased significantly in the GAT-1 treated group compared with the control and saporin treated groups. In conclusion, our findings suggest that immunolesion of medial septal GABAergic neurons can interrupt hippocampus-dependent spatial learning, possibly through modulation of NMDA receptor subunit expression in the hippocampus. Moreover, our finding that selective lesions of medial septal cholinergic neurons affects probe-test performance but not spatial learning, suggests that septohippocampal cholinergic projections are involved specifically in the consolidation or retrieval, but not in the acquisition of long-term spatial memory

The neural basis of sequential behavior in pigeons

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Behavior requires complex motor sequences, some of which are innate, others are learned. One extensively studied sequential behavior is birdsong - a prominent model for vocal learning. Here we investigate the abilities of a non-songbird, the pigeon, to learn complex motor sequences and reliably switch between them by evaluating error signals. Our neurophysiological data allows to test in how far the song system evolved *de novo* or if it is a specialization of a generalized sequential motor-system. Pigeons were trained to execute two distinctly different motor sequences and were able to flexibly and reliably change back and forth between both, depending on visual error feedback. This indicates, that non-songbirds are capable to learn arbitrary motor sequences and are able to apply them depending on environmental demands. Using electrophysiology we recorded neuronal activity of two distinct brain regions, the equivalent of mammalian prefrontal cortex (NCL), and an output structure of the anterior forebrain pathway in the pigeon (NIML). Both regions have previously been shown to participate in sequence execution. Recorded neurons in both areas show specific modulation depending on the executed sequence and element within the sequence. Their specific participation in sequence learning, execution and the flexible switch between different sequences is further analyzed. We thereby shed light on the origins of sequential motor behavior at the neuronal level.

The precuneus is involved in gradual acquisition of non-semantic spatial schemata

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Schema-congruent information has previously been demonstrated to be rapidly and efficiently encoded into explicit memory, and converging evidence from animal research and human neuroimaging suggests that schema-congruent memory traces become independent of the hippocampus in a rather short time. Previous studies have implicated the medial prefrontal cortex (mPFC) in encoding of schema-congruent episodes and, more recently, also in the acquisition of novel schemas via insightful problem solving. However, one criticism of this line of research concerns the fact that schema-dependent encoding has thus far been poorly differentiated from the integration of information into semantic memory.

Here, we investigated the acquisition of novel non-semantic spatial schemas over five days, using functional magnetic resonance imaging (fMRI) in a cohort of 32 young, healthy human volunteers. Participants studied configurations of five icons on a desktop picture over five days. Each configuration was presented three times on a given day, and 30 configurations were kept constant, while in another 30, the icon positions were shuffled every day. Functional MRI was acquired during the learning phase on days 1, 2 and 5. After each training phase consisting of three runs, a recognition memory task was performed in which participants had to retrieve the position of a given icon. Two days after the last training day, the configurations were presented again with one icon substituted for a new one, and the position of this icon was probed afterwards.

Recognition of the icon positions increased steadily from day 1 to day 5 for constant configurations, with the highest learning occurring between day 1 and day 2. No such learning was observed for shuffled configurations. Moreover, constant configurations were associated with better recognition of the substituted icon's position two days after the last training day. Functional MRI revealed a prominent activation of the bilateral precuneus (right>left) during study of constant compared to shuffled configurations. This activation became apparent on day 2, and more prominent on day 5. On the other hand, no robust activations of the mPFC was observed for the study of constant compared to shuffled configurations.

The results will be discussed with respect to the notion that both precuneus and mPFC are part of the Default Mode Network (DMN), which has previously been involved in memory retrieval. We suggest that there might be an anterior-posterior distinction for semantic versus non-semantic schema representations in the DMN.

Touched by the Milkshake: A Rodent Operant Touchscreen Approach to Positive Valence, and Cannabinoid and Vanilloid Pharmacology

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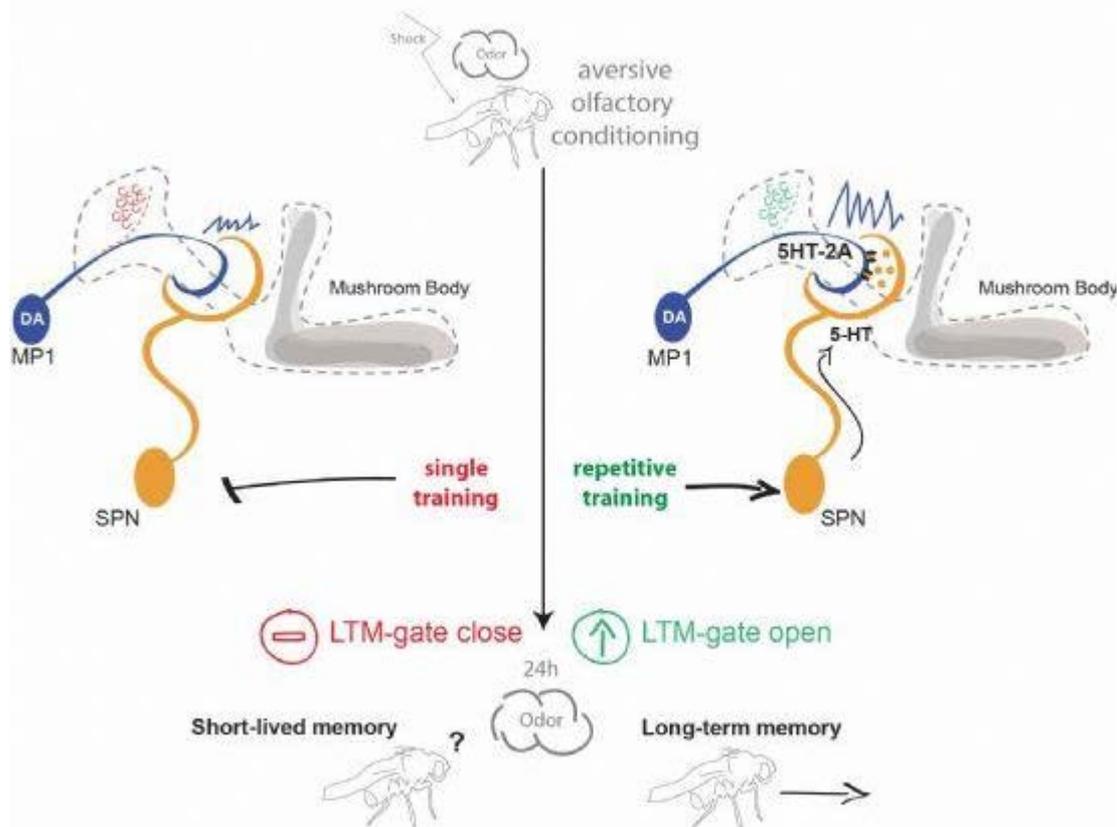
Rodent operant touchscreen testing systems are a modern version of the traditional Skinner box and offer a single test environment for a variety of cognitive assessments. Not until very recently, assessing and pharmacologically manipulating motivational phenotypes in this paradigm has attracted significant interest (Heath et al., 2015, 2016). However, despite its implication in motivational disorders, the endocannabinoid signalling system has rarely been studied in this line of research. Moreover, based on previous research that suggests opposite functions of the TRPV1 and CB1 channel in fear and anxiety (Moreira et al., NS 2012; Casarotto et al., 2012), also the role of the endovanilloid signalling system in regulating appetitive behavior, has yet to be explored. Experiment 1 aimed to evaluate different motivational protocols that either employ food or water deprivation regarding their temporal efficiency, and applied cannabinoid and vanilloid pharmacological treatment to investigate the systems' role in reward learning (treatments: 1) SB366791, 2) SR141617, 3) URB-597, 4) JZL-184, and co-administration of URB-597 with 5) SB366791 and 6) SR141617). Experiment 2 expanded the research to knock-out mouse models and assessed motivational phenotypes in CB1 and TRPV1 wildtype and knock-out mice.

A pair of serotonergic neurons controls long-term memory consolidation in drosophila

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The formation of memory, as an adaptive behavior, is crucial for the fitness and survival of any organism. However, memory and especially long-term memory (LTM), has a cost that can impact the physiology of an animal fundamentally. Therefore, mechanisms that restrict LTM formation are required that integrate the animals' state together with the salience of the received stimulus. We previously showed that in *Drosophila*, such a gate is controlled by a single pair of dopaminergic neuron, MP1, that changes its Ca²⁺ oscillatory activity specifically during LTM consolidation. To date, the identity of regulatory inputs for the oscillatory activity of MP1 is not known. Here we discovered that a bilateral neuron located in the gnathal ganglia (GNG), a brain region associated with gustatory inputs and control of feeding behavior, can modulate MP1 oscillations. Our investigations revealed that the neuron is serotonergic and sends projections toward the mushroom body (MB), the olfactory memory center, where it synapses with MP1 at the level of the MB peduncle. Activation of this serotonergic projection neuron (SPN) induced oscillatory activity in MP1 and facilitated LTM formation. Additionally, conditional knock-down of 5HT in the SPN as well as the 5HT-2A receptor in MP1 interfered with both long-term memory formation and MP1 oscillations. Our study thus identifies a major regulatory input for MP1 that regulates calcium oscillations and LTM formation.



Poster Topic

T26: Computational Neuroscience

- [T26-1A](#) A new automatic multi seed analysis for fMRI resting state data in animal model
Silke Kreitz, Benito de Celis Alonso, Michael Uder, Andreas Hess
- [T26-2A](#) An evaluation of two spike sorting algorithms: Heptode Spike Sorter versus WaveClus
Roman Eppinger, Thomas Schanze
- [T26-3A](#) An open source tool for automatic spatiotemporal assessment of calcium transients and local 'signal-close-to-noise' activity from calcium imaging data
Manju Sasi, Juan Pablo Prada Salcedo
- [T26-4A](#) Analyzing and comparing high-dimensional spatiotemporal cortical activation patterns
Patrick Krauss, Claus Metzner, Achim Schilling, Maximilian Traxdorf, Volker Eulenburg, Holger Schulze
- [T26-5A](#) Approaches to inversely estimate a neuronal source's position with multichannel microelectrodes
Martin Nguyen, Thomas Schanze
- [T26-6A](#) Can the biologically mechanistic model generate pinwheel layouts with common design features ?
Wenqi Wu, Juan Daniel Flórez Weidinger, Fred Wolf
- [T26-7A](#) Coupling of action potentials in primate visual cortex to low frequency local field potentials
Mohammad Zarei, Mohammad Reza Daliri, Mehran Jahed, Stefan Treue, Moein Esghaei
- [T26-1B](#) Combinational Intracortical Decoder of Forelimb Force in Freely Moving Rats
Abed Khorasani Sarcheshmehesmaeilabad, Vahid Shalchyan, Mohammad Reza Daliri
- [T26-2B](#) Detecting Changes in the Intensity and Regularity of Neuronal Spike Trains
Michael Messer, Stefan Albert, Julia Schiemann, Jochen Roeper, Gaby Schneider
- [T26-3B](#) Detection of spike patterns in massively parallel spike trains
Pietro Quaglio, Alper Yegenoglu, Emiliano Torre, Michael Denker, Thomas Brochier, Alexa Riehle, Sonja Grün
- [T26-4B](#) Determinants of spike time precision - differential effects of cell morphology, ion channel voltage dependence and kinetics
Barbara Feulner, Chenfei Zhang, Lenka Vaculciaková, Fred Wolf, Andreas Neef

- [T26-5B](#) Distributions of covariances as a window into the operational regime of neuronal networks
David Dahmen, Markus Diesmann, Moritz Helias
- [T26-6B](#) Elimination of a ligand gating site generates a supersensitive olfactory receptor
Kanika Sharma, Gaurav Ahuja, Ashiq Hussain, Sabine Balfanz, Arnd Baumann, Sigrun I Korsching
- [T26-7B](#) Extending integrate-and-fire model neurons to account for the effects of weak electric fields and input filtering mediated by the dendrite
Florian Aspart, Josef Ladenbauer, Klaus Obermayer
- [T26-8B](#) *In silico* exploration of functional networks underlying behavioral traits
Florian Johann Ganglberger, Joanna Kaczanowska, Josef M. Penninger, Andreas Hess, Katja Bühler, Wulf Haubensak
- [T26-1C](#) Graded persistent activity mediated by ion channel cooperativity
Paul Pfeiffer, Jan-Hendrik Schleimer, Susanne Schreiber
- [T26-2C](#) Implementation of Neural Diversity for Computer Simulations of Neuronal Excitability and Synchronization
Aubin Tchaptchet, Hans Albert Braun
- [T26-3C](#) Joint pausiness in parallel spike trains
Matthias Gärtner, Sevil Duvarci, Jochen Roeper, Gaby Schneider
- [T26-4C](#) Long-term information storage by the collective dynamics of multi-synaptic connections
Christian Tetzlaff, Michael Fauth, Florentin Wörgötter
- [T26-5C](#) Low-dimensional spike rate models derived from networks of adaptive integrate-and-fire neurons: comparison and implementation
Fabian Baumann, Moritz Augustin, Josef Ladenbauer, Klaus Obermayer
- [T26-6C](#) Modeling the Effect of Phase-Triggered Transcranial Magnetic Stimulation on Motor Cortex
Jochen Triesch, Natalie Schaworonkow
- [T26-7C](#) Non-linear computation and establishment of contrast invariance in spatially structured recurrent balanced networks
Laura Bernáez Timón, Sara Konrad, Tatjana Tchumatchenko
- [T26-1D](#) Properties of dendritic trees under different branch ordering schemes
Alexandra Vormberg, Felix Effenberger, Julia Muellerleile, Hermann Cuntz
- [T26-2D](#) Simulating large-scale human brain networks with a mean-field model of EIF neurons: exploring resting state FC and stimulation with electric fields
Caglar Cakan, Josef Ladenbauer, Florian Aspart, Michael Schirner, Simon Rothmeier, Petra Ritter, Klaus Obermayer
- [T26-3D](#) Spike time precision of different neuron classes – influence of morphology and ion channels
Andreas Neef, Carolina León Pinzón, Ricardo Martins Merino, Walter Stühmer, Fred Wolf

[T26-4D](#) Temperature-robust computation with simple network motifs
Pia Rose, Jan-Hendrik Schleimer, Susanne Schreiber

[T26-5D](#) The impact of action potential initiation site separation on fast population encoding
Chenfei Zhang, David Hofmann, Andreas Neef, Fred Wolf

[T26-6D](#) Towards reproducible workflows for electrophysiology data using the *Elephant* analysis framework
Michael Denker, Alper Yegenoglu, Sonja Grün

[T26-7D](#) Transition to chaos in random neural networks in the presence of noise
Sven Goedeke, Jannis Schuecker, Moritz Helias

A new automatic multi seed analysis for fMRI resting state data in animal model

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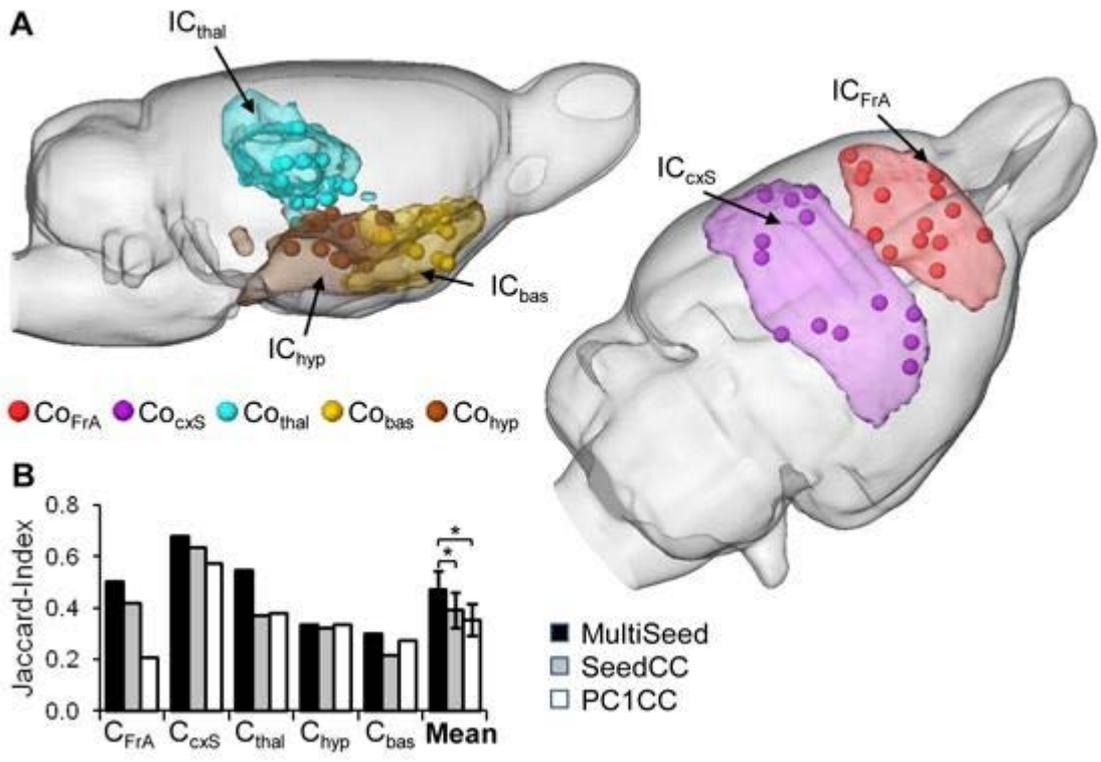
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Resting state connectivity (RS) is increasingly being studied in healthy and diseased brains in humans and animals. There are two major strategies for analysing functional connectivity of the resting brain. The first one is the independent components analysis (ICA), which is data-driven and thus allows data analysis without prior knowledge. However, ICA results are hard to interpret and further quantitative characterization is difficult. The second method is hypothesis driven and includes manually defined regions of interest (ROIs) i.e. seed regions. The seed region approach relies on a priori hypotheses and therefore might lack important information not addressed by the researcher. Additionally, the seed regions are subjected to reproducibility problems due to manual placing. In this study we present a new automatic method based on the seed region approach which overcomes these flaws and provides networks that can be characterized using sophisticated graph theoretical analysis techniques. We validated this multi seed approach in comparison to two other graph theoretical correlation methods and ICA. Finally, the usability of our method was demonstrated by the investigation of short term RS modulation due to whisker stimulation in rats.

Experiments were performed on 25 male rats anesthetized with isoflurane. RS scans were performed on a 4.7 T BioSpec MR (BRUKER, Germany) with a T2*-weighted gradient echo EPI sequence (22 axial slices, 64 x 64 matrix, TR= 2000 ms, TE_{eff}= 24.4 ms, in-plane resolution 391 x 391 μ m, slice thickness 1000 μ m). Functional data were spatially smoothed (FWHM 3px), low-pass filtered at 0.1 Hz and corrected for global signal fluctuations by linear regression of the global mean. A 20 component group ICA was performed using GIFT. For our automatic multi seed correlation method in each rat brain 179 separate brain areas were defined by registering a digital 3D atlas to each dataset. Next, the seed regions were determined automatically in the center of mass of each of these brain areas and the mean time course of each seed region was correlated to all brain voxels. The correlation values per seed region were averaged over each brain area, subsequently resulting in a 179 x 179 asymmetric correlation matrix. The matrices were averaged across animals and used for graph theoretical analysis. Two graph theoretical methods (see Fig. 1) were performed by cross correlating the mean time courses of the seed regions and the complete atlas regions, respectively. For all graph theoretical methods we calculated communities and compared these to ICA components. For the whisker study two RS scans were performed per animal with a 50 min stimulation sequence in between (7 Hz, 10 mm amplitude, 100 stimulations of 8 sec with intermediate rests of 24 sec).

After eliminating noise components 5 ICs representing different cortical and subcortical networks fit well to the network communities (Fig. 1). In comparison to both other graph theoretical methods, the multi seed approach shows the highest similarity to ICA results and the highest reproducibility. Additionally, analyzing the whisker stimulation study using our multi seed approach revealed a large component of modulated connections with statistical significance whereas the other methods failed to detect significantly modulated connectivity components.

Thus, our method is validated and can be used as a new approach to compare and characterize resting state networks from a novel perspective.



An evaluation of two spike sorting algorithms: Heptode Spike Sorter versus WaveClus

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Introduction

Spike sorting is used to sort extracellular neuronal action potentials (EAP), called spikes. The choice of the right spike sorter is not easy, because there are many algorithms that have different properties. An interesting advantage for spike sorting is the use of multichannel electrode recording systems, which often increases spatial resolution and redundancy. To find out the advantage of multichannel electrode recordings for spike sorting we will test two algorithms. The first spike sorting algorithm is the Heptode Spike Sorter (HSS), which is available from Thomas Recording, Giessen, Germany, and which was designed for multichannel electrode recordings, and the second is the WaveClus, a free software package developed by R.Quian (Version 2.0).

Methods

For the evaluation of both spike sorting algorithms, two types of data sets were simulated as extracellular recorded signals: one as tetrode (4 channels) and the other one as a heptode (7 channels) recording. The recordings contain three to five neurons and different signal to noise levels. The neurons were modelled as point sources. EAP amplitudes were generated as a function of distance between neuron and electrode channel, i.e. $V(x) = V(0) \cdot \exp(-x/\lambda)$ with $V(x)$ the potential, x the distance between electrode, $V(0) = 1$ is the amplitude at neuron's position, and $\lambda = 28,42 \mu\text{m}$ is the spatial decay. The spikes waveforms were randomly selected from a portfolio of 37 different multichannel EAP time courses. For comparing the two spike sorting algorithms the detection-threshold was set to four times standard deviation, other WaveClus parameters were set to optimal values. The performance of the algorithms were analysed statistically, i.e. each cluster represents one neuron and the spikes belonging to it were counted as correctly classified spikes, if not they were labeled as incorrectly assigned. To verify the threshold setting, 6 subjects estimated the detection thresholds. These thresholds were compared with the above mentioned four times standard deviation threshold.

Results

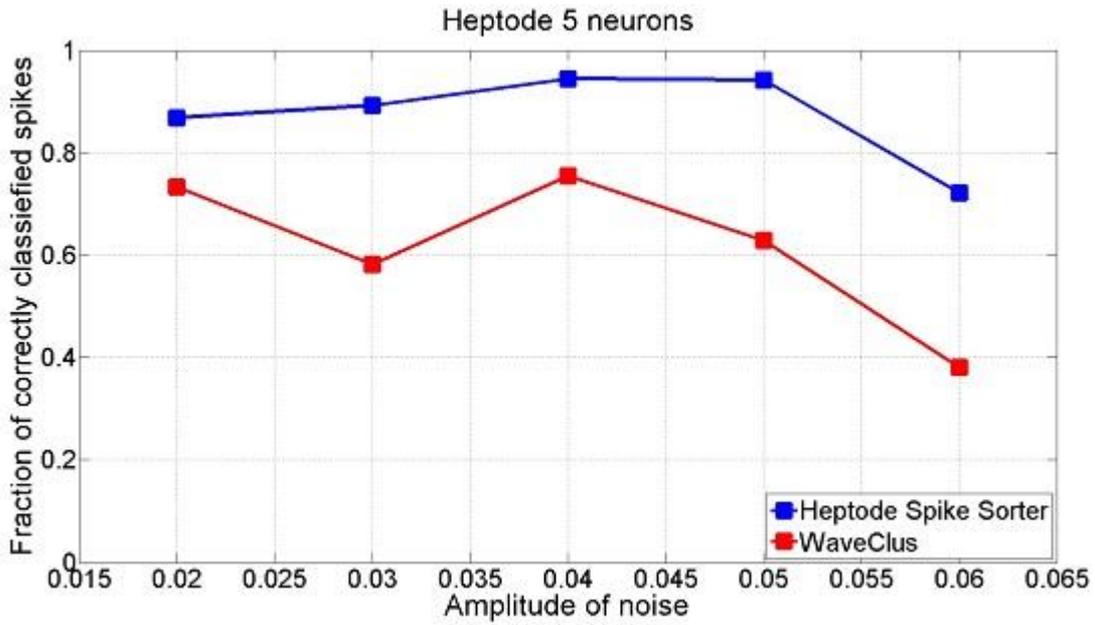
The HSS shows a good performance and achieves better results than WaveClus (Fig. 1). However, both spike sorting algorithms have some trouble with high noise-level data. The performance of both algorithms depend on the number of recording electrode channels used for spike sorting. Here we found simple rule: the more channels, the better the performance. The performances also depend on the number of neurons: the higher the number of neurons, the poorer the performance. However, the four times standard deviation threshold was on average better than manual setting.

Discussion

Our results show that both algorithms are reasonable tools for spike sorting. However, it is not easy to

give a clear recommendation. While the heptode spike-sorting algorithm requires a manual threshold setting the WaveClust algorithm can do this automatically, but it requires a lot of parameters to be adjusted. Thus, for people who are not familiar with spike sorting the HSS could be a good tool. Future work will focus on more detailed statistical evaluations of spike sorting algorithms and on methods to improve them. In addition, we want to develop better signal generating and recording methods and we want to use real data for our spike sorter evaluations.

Figure:
The fraction of correctly classified spikes depending on the amplitude of noise for HSS in blue and WaveClus in red for heptodes.



An open source tool for automatic spatiotemporal assessment of calcium transients and local 'signal-close-to-noise' activity from calcium imaging data

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¹PD Dr. Sibylle Jablonka

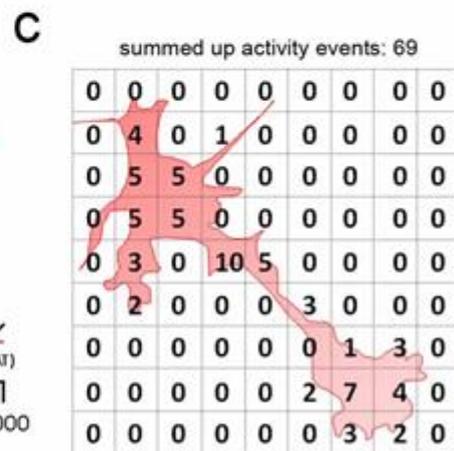
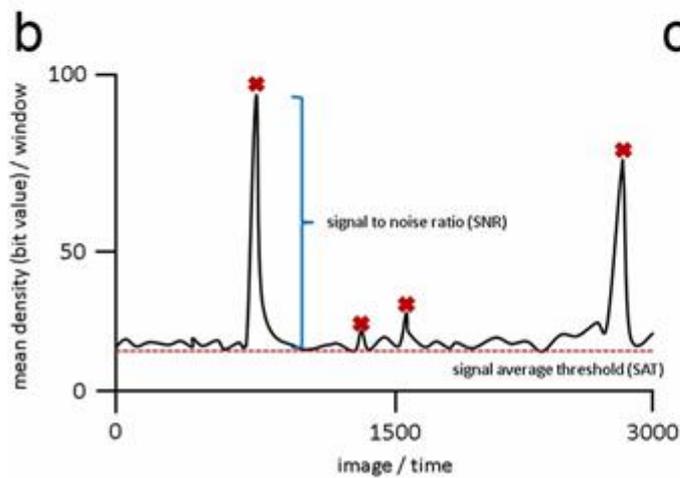
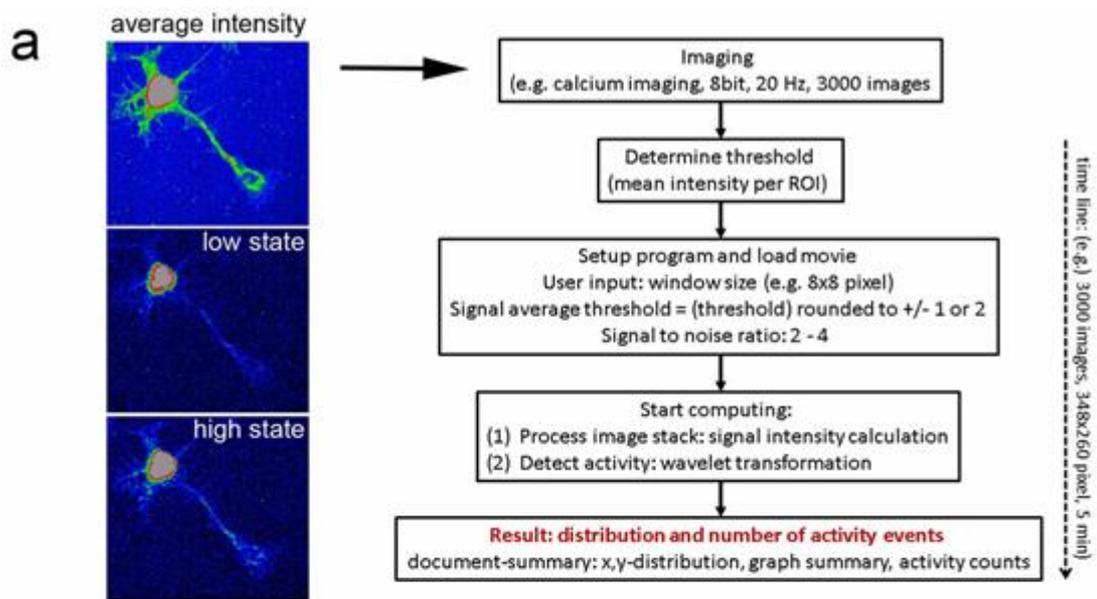
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Fluorescent calcium indicators combined with imaging techniques are used to visualize calcium signals in neurons. Commonly calcium signals are induced, thus making it easy to see the spatiotemporal patterns of calcium signals. Techniques to extract calcium signals from imaging data are commonly region of interest (ROI) analyses. ROIs are typically selected manually or semi-automated. However, spontaneous or cell-autonomous calcium signals may be difficult to assess by a conventional ROI analysis, because they appear in an unpredictable spatiotemporal pattern, and in very small neuronal loci of axons or dendrites. In this study we developed an open source bioinformatical tool on base of 'R' and ImageJ for an unbiased assessment of calcium signals, in x,y-t calcium imaging series. The approach uses a signal average threshold to determine the measuring noise, and the signal to noise ratio to define the stringency of activity detection. Intensity signals are automatically calculated within a x,y grid on the image series. Phases and loci of neuronal activity are calculated by wavelet transformation. Intensity peaks are detected and filtered by their occurrence along the ridges of the transform to discard noise peaks. A dataset is created and shows loci of activity, individual traces of active loci, and calculates the number of activity events. The approach is particularly useful to detect local activity events in neurites which are close to or within the signal noise, however on cost of a little overestimation of activity events. The tool helps to compare activity patterns of neurons, under different physiological or genetic conditions, with minimal user input. We applied the calcium signal detection tool on cultured motoneurons and hippocampal neurons and showed that the tool is powerful to identify local calcium events and 'signal-close-to-noise' activity in distal neurites of hippocampal neurons under spike-block conditions. The tool identifies activity events which are usually filtered out by other tools which include denoising strategies.



Scheme: Assessment of calcium activity events from calcium imaging raw data.

Analyzing and comparing high-dimensional spatiotemporal cortical activation patterns

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We present a new statistical method to analyze multichannel steady-state local field potentials (LFP) recorded within different sensory cortices of different rodent species. Our spatiotemporal cluster analysis (SCA) method enables statistical analyzing and comparing clusters of data points in n-dimensional space. To evaluate the analytical power of our SCA approach, we first tested the method using artificially generated data sets. Subsequently, we demonstrate that using this approach stimulus-specific spatiotemporal activity patterns can be detected and be significantly distinguished from each other during stimulation with long-lasting stimuli. In addition we extend the method to human electroencephalogram (EEG) data and exemplarily show that therewith different REM and non-REM sleep stages may be differentiated, demonstrating the universal applicability of our approach. Our method thereby may be used for the development of new read-out algorithms of brain activity and by that opens new perspectives for the development of brain-computer interfaces (BCI).

Approaches to inversely estimate a neuronal source's position with multichannel microelectrodes

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Introduction

To analyse neuronal activity in small areas of brain tissue, recordings of extracellular action potentials (EAP) with multichannel microelectrodes are often used. This kind of recording offers a high spatial and temporal resolution of neuronal activities in the vicinity of the electrode. Hence, we are developing algorithms to estimate the neuronal source's position and some corresponding electrical parameters with simulated data of EAP-amplitudes. Here, the main focus of our work is to solve ambiguous inverse approximation problems optimally.

Methods

The methodical assumption for a simplification of our simulations is to consider the brain tissue as an infinite, entirely ohmic and ideal homogeneous medium. In addition, the electrode's structure is neglected. Furthermore, the electrode channels and the neuronal sources are represented as position vectors.

To reduce the number of inversely estimated parameters in the algorithm we developed and used several point source models, like monopole, dipole, quadrupole and also complex multipoles from Taylor series expansion.

However, the inverse position estimation is done with the Levenberg-Marquardt algorithm which sometimes leads to ambiguous solutions. Besides to the proper parameters the algorithm computes outliers for poor selected initial values as well. For a statistical processing of solutions the estimation is performed several times with random initial values for one set of EAP-amplitudes, which are assumed to be noise-free.

Here we report on methods to treat the ambiguous problem statistically. Our methods are: 1) to take the mean of the solutions, 2) to sort the solutions and exclude outliers (quantile approach), 3) to eliminate estimates with a large failure by calculating the difference between measured potentials and back-calculated potentials, 4) to take centres from a subtractive clustering procedure, and 5) to omit poor electrode channels in a re-estimation process with subsequent averaging.

Results

The introduced inverse neuronal position estimation algorithm's accuracy is affected from the selection of initial values. Poor selected initial values lead to outliers. To notice is, that closer to the true source parameters selected initial values lead to improved estimation results. In addition, outlier occurrence increases with the source's model complexity.

Anyway, our methods give some effective approaches to estimate the neuronal source's properties more precisely and to discriminate between outliers and true parameters (Fig. 1). Especially the methods 2) to 5) are suitable to use on more complex source models and by larger numbers of recording electrode channels.

Discussion

The developed methods allow a statistical consideration with outlier detection of the position estimation results of different signal source types under ideal recording conditions. Outlier discrimination becomes

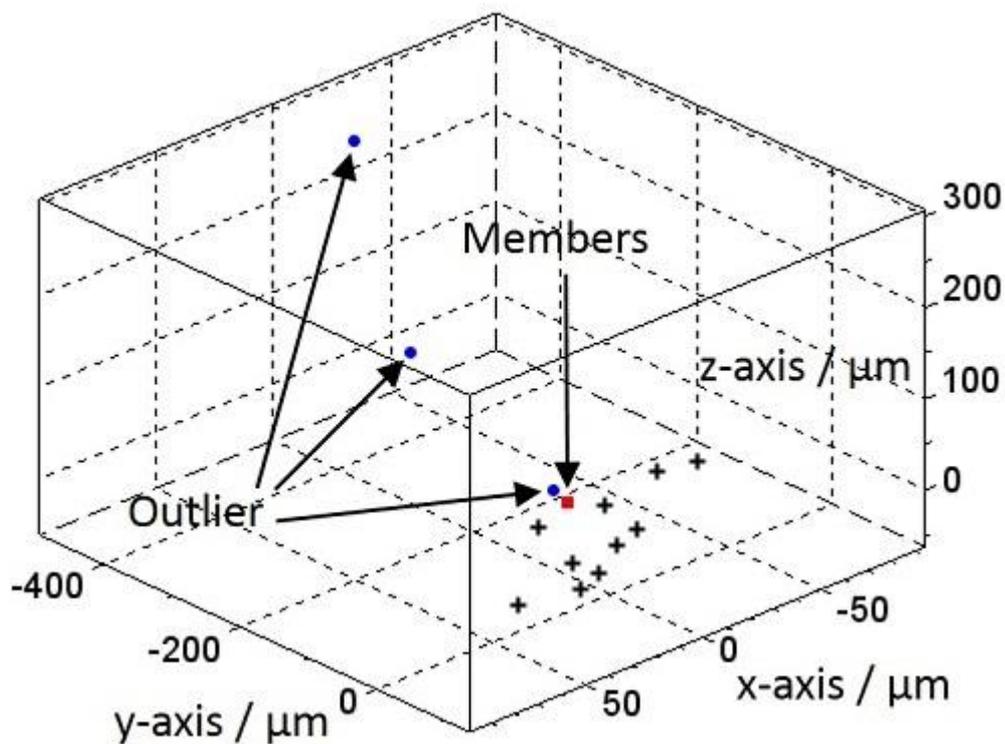
more important at more complex neuronal source model estimations.

However, with our developed methods it is possible to control the ambiguous inverse problem of our recursive neuronal position estimation algorithm. Our methods may also help to improve the assessment of neuronal signal recordings and related applications, e.g. brain-computer-interface.

An important task for future work is to verify the estimation methods and algorithms with multichannel data measured in neuronal tissue samples.

Figure 1: Estimation of a neuronal monopole source's position by using a randomly arranged 10 channel electrode configuration. The black crosses represent the electrode's contacts. The dots indicate the estimated source positions with 1000 different initial values in a hyper-cubic range from -50 to 50. Members (996 properly estimated positions) are plotted with red and outliers with blue dots, while the results are superposed. The expected position of the source, i.e. neuron, was (10, -25, 40) μm .

Note: electrical properties, like the charge (which has a value of 300) of the source, are not shown in the 3D-plot.



Can the biologically mechanistic model generate pinwheel layouts with common design features ?

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The architecture of iso-orientation domains in the primary visual cortex (V1) of primates and carnivores apparently follows species invariant quantitative laws([1],[2]). The emergence of this common design has been explained by an abstract class of mathematical models for neural circuit self-organization([1],[3]). So far no biologically detailed model has been shown to conform with all features of this common design. Recently,[4]made available a biologically mechanistic model which mimics visual pathway and can be trained by natural stimuli. We examine and characterize this biologically mechanistic model in order to understand whether detailed models of Hebbian learning for the formation of nerve cell networks quantitatively match the common design in the visual cortex.

Our results show that, when covering a substantial fraction of the period of juvenile plasticity([5]), the statistics of pinwheel layouts including average pinwheel densities generated by the model are time-dependent and typically drop below experimentally observed values.

We find that, maps in the simulation typically become ordered and crystal-like over time. This process is driven by pinwheel annihilation and creation events. During the time course the ordered and a banded geometry of orientation domain start from the boundary of V1 increasingly dominates the whole simulation area. This indicates that boundary effects strongly influence pinwheel layouts in this model.

We conclude that common design is highly informative and can be used to assess all the validity of biologically detailed model.

Coupling of action potentials in primate visual cortex to low frequency local field potentials

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Synchronous neural activity has been the subject in many recent brain science. One approach to address inter-neuronal synchrony is to investigate the link between (locking) spiking activity to local field potentials (LFPs). In previous studies, an interaction has been observed between the action potentials (spikes) of single neurons and LFPs (which mostly represents synaptic activities) in their low frequency oscillations (<15 Hz). However, the potential role of this locking mechanism in the neural encoding of information is still unknown.

In order to answer this question, two behaving male macaque monkeys were trained to maintain their gaze on a central fixation point on a computer screen while two coherently moving random dot patterns (RDP) were simultaneously presented at eccentric locations, moving linearly in the same direction. One of the two RDPs was presented inside the receptive field of the recorded neuron and moved either in the preferred or anti-preferred direction of the neuron. During the trials LFPs and single unit activity was recorded from visual cortical area MT.

To investigate if any coupling of spikes and LFPs depends on the sensory properties of the visual stimulus inside the receptive field, we measured the interconnection (locking) between spikes and the phase of low frequency oscillations of LFP as a function of the stimulus' motion direction. We found that the locking of spikes to the LFP phase follows a tuning curve based on the direction of the presented stimulus. This tuning curve is inverted compared to the tuning based on the spike rate, i.e., the least spike-LFP coupling occurs for the preferred direction (determined based on the spike rate), while the largest spike-LFP coupling is induced by the anti-preferred direction. This finding suggests that the neural system harnesses spike-LFP coupling in the primate visual cortex to encode visual information. Our results suggest that neural spikes induced by the preferred, rather than the anti-preferred feature of a neuron are distributed more stochastically in time and therefore happen less synchronously with the neighboring neurons. These observations suggest a novel form of information encoding in the neural system.

Combinational Intracortical Decoder of Forelimb Force in Freely Moving Rats

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Brain-machine interface (BMI) systems have been introduced to help paralyzed persons. In the patients with the lost brain-limb connections, this system can be used as an alternative artificial route for transmitting the brain commands to the patient's limbs for movement restoration. The multiunit activity (MUA) recorded with multi-electrode array can provide a high resolution brain signals in both space and time domain. Furthermore, local field potential (LFP) signals recorded from cortex using intracortical microelectrodes can be used as a more robust signal in neural interface devices. Recent studies show that different kinematic parameters such as position and velocity can be inferred from the MUA and LFP as precisely as spiking activities. However, designing a combinational decoders for robust force decoding in freely moving animals has remained an open problem. In this study, we decoded the force amplitude from the combination of MUA and LFP signals and compared the decoding performance of this combinational decoder with MUA-only and LFP- only decoders.

Detecting Changes in the Intensity and Regularity of Neuronal Spike Trains

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Neuronal spike trains often show changes in their firing activity such as changes in the intensity or regularity of spike occurrences. Violating such changes may yield misleading statistical analyses if they require stationarity of the underlying models. Therefore, we are interested in locating 'change points' in spike trains, i.e., points in time where the intensity or regularity of spike occurrences change. With regard to statistical modeling, one can then segment the spike train into regimes that can be assumed approximately stationary.

We propose a multi-filter procedure that aims at the detection of change points that appear on different time scales within the same train. For that, we test the null hypothesis of absence of change points. After rejection of the null, we use a multi-filter algorithm in order to localize change points.

This is a two step procedure where we first analyze changes in the intensity. Second, we investigate changes in the regularity incorporating the information about the estimated intensity.

Our approach is applicable to spike trains modeled by a large class point processes including Poisson- or renewal processes (i.i.d. ISIs), as well as processes with even less restrictive ISIs (renewal processes with varying variance).

This work was supported by the German Federal Ministry of Education and Research (BMBF, Funding number: 01ZX1404B) and by the Priority Program 1665 of the DFG.

Detection of spike patterns in massively parallel spike trains

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Cell assemblies [1] exhibiting neuronal interactions at the millisecond time scale were suggested as building blocks of information processing in the brain [2,3]. Significant patterns of synchronous spikes in electrophysiological recordings are considered as a signature of an active assembly. We recently developed a statistical method - the Spike Pattern Detection and Evaluation (SPADE) analysis [4] - to detect synchronous spike patterns in massively parallel spike data (MPST) on the order of 100 or more neurons. The method deals with a) the combinatorial explosion of the number of patterns to consider by employing a variant of the frequent item set mining technique [5, 6], and with b) the challenge of statistically assessing these patterns due to the multiple testing problem by using Monte-Carlo techniques.

We applied the SPADE analysis method to electrophysiological data recorded from the motor cortex of two monkeys while they executed a delayed reach-to-grasp task. Monkeys were trained to reach and grasp, pull and hold an object by using either a side grip or a precision grip and with high or low force. To allow the monkey to prepare the upcoming movement, the animal is instructed about the grip type at the beginning of a delay period of 1s preceding the GO signal. MPST were recorded by using a 100-electrode Utah array chronically implanted at the MI/PMd border [7]. We hypothesized that different classes of spike patterns occur depending on the behavioral conditions and periods in the trial. To investigate this, we analyzed and compared data from the same set of neurons recorded during the 4 different behavioral conditions (combinations of object load and grip type), and during 6 time epochs of different stages of the task protocol.

We found [8] a variety of significant patterns in specific time epochs and behavioral conditions in both monkeys. We then analyzed the spatial organization of these patterns on the recording array with respect to its cortical location, and found that neurons involved in synchronous patterns were preferentially aligned along the medio-lateral orientation. We also assessed the specificity of the neuronal composition of patterns to different behavioral contexts and found a strong specificity to the grip type and the specific behavioral epoch. These findings provide evidence for the existence of higher-order spike patterns occurring in relation to behavior.

Recently, we have generalized SPADE to detect and evaluate spike patterns of delayed sequence of spikes (spatial-temporal patterns, STP). We dealt with the increased dimensionality of the problem using for prefiltering a measure known from frequent concept analysis ('stability') that allows to efficiently select repeated STPs that are potential significant spike patterns [9]. We validated the performance of the method on a large variety of artificial test data, such that the method can now be applied to experimental data.

Acknowledgements. Supported by Helmholtz Portfolio Theme Supercomputing and Modeling for the Human Brain (SMHB), EU grant 604102 and EU Grant 720270(HBP) (Human Brain Project, HBP), ANR GRASP France.

References

- [1] Hebb, D. O. (1949). *The organization of behavior: A neuropsychological approach*. John Wiley & Sons
- [2] Singer, W., Engel, A. K., Kreiter, A. K., Munk, M. H. J., Neuenschwander, S., Roelfsema, P. R. (1997) Neuronal assemblies: necessity, signature and detectability. *Trends in Cognitive Sciences*. 1, 252-261
- [3] Harris, K. (2005) Neural signatures of cell assembly organization. *Nature Reviews Neuroscience*, 5, 339-407
- [4] Torre E, Picado-Muiño D, Denker M, Borgelt C, Grün S (2013) Statistical evaluation of synchronous spike patterns extracted by frequent item set mining. *Frontiers in Computational Neuroscience* 7:132.
- [5] Picado-Muiño D, Borgelt C, Berger D, Gerstein GL, Grün S (2013) Finding neural assemblies with frequent item set mining. *Frontiers in Neuroinformatics* 7
- [6] Borgelt C, Picado-Muiño D (2014) Simple pattern spectrum estimation for fast pattern ltering with coconad, In Blockeel H, van Leeuwen M, Vinciotti V, editors, *Advances in Intelligent Data Analysis XIII* , Vol. 8819, pp. 3748. Springer International Publishing.
- [7] Riehle A, Wirtssohn S, Grün S, Brochier T (2013) Mapping the spatio-temporal structure of motor cortical lfp and spiking activities during reach-to-grasp movements. *Frontiers in Neural Circuits* 7:48
- [8] Torre, E., Quaglio, P., Denker, M., Brochier, T., Riehle, A., & Grün, S. (2016). Synchronous Spike Patterns in Macaque Motor Cortex during an Instructed-Delay Reach-to-Grasp Task. *The Journal of Neuroscience*, 36(32), 8329-8340.
- [9] Yegenoglu, A., Quaglio, P., Torre, E., Grün, S., & Endres, D. (2016, July). Exploring the Usefulness of Formal Concept Analysis for Robust Detection of Spatio-temporal Spike Patterns in Massively Parallel Spike Trains. In *International Conference on Conceptual Structures* (pp. 3-16). Springer International Publishing.

Determinants of spike time precision - differential effects of cell morphology, ion channel voltage dependence and kinetics

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Cortical neurons represent information as a population in their collective firing rate. The ability of every individual neuron in this population to fire temporally locked to a stimulus can be expressed, in the frequency domain, by a transfer function or dynamic gain (see Tchumatchenko et al 2011). This transfer function captures the population's response properties for the linear case, when small variations in the input current drive small changes in the population firing rate. It represents, in frequency space, the transfer of input current amplitude to firing rate response. We studied the spike time precision, i.e. the neuronal transfer function, in a simplified neuron model that is complex enough to reflect all major influence factors that shape the transfer function in realistic, conductance based neuron models: 1. the passive filtering of input current into membrane voltage (see Eyal et al. 2014), 2. the subthreshold dynamics of ion channels, 3. the voltage dependence and kinetics of the effective depolarizing current at threshold and 4. the electrotonic separation of axonal spike initiation site from the soma 5. The correlation time of the input current.

We found, that each of these parameters can dominate the behavior of the transfer function for the high frequency limit and set the bandwidth of information encoding. Simple dissection of individual contributions is not possible, as the different parameters interact in a complex fashion. For instance, subthreshold potassium currents can lead to a shift in threshold, which entails, a change in the effective voltage dependence of the membrane current at the threshold. Another example is coupling of the correlation time of the input with the dynamics of the subthreshold currents to influence the threshold. Here we broadly define parameter regions in which each of the five influence factors limit spike time precision and discuss relations to physiological conditions.

Distributions of covariances as a window into the operational regime of neuronal networks

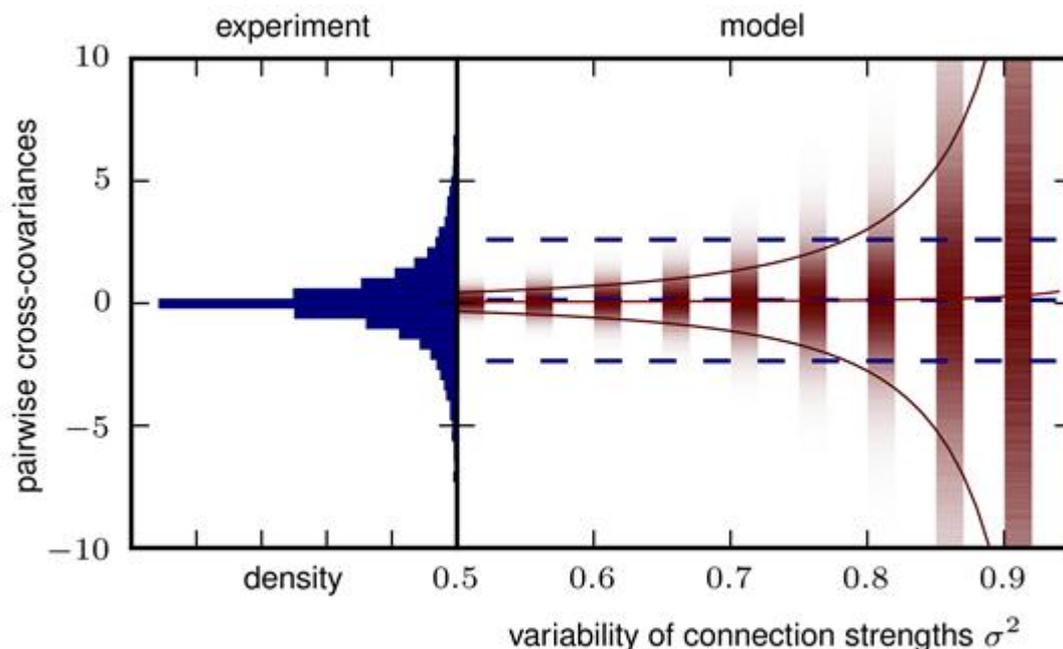
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Massively parallel recordings of spiking activity in cortical networks show that spike count covariances vary widely across pairs of neurons [Ecker et al., *Science* (2010)]. Their low average is well understood [Renart et al., *Science* (2010), Tetzlaff et al., *PLoS CB* (2012)], but an explanation for the wide distribution in relation to the static (quenched) disorder of the connectivity in recurrent random networks was so far elusive. Starting from spin-glass techniques [Sompolinsky and Zippelius, *Phys. Rev. B* (1982)] and a generating function representation for the joint probability distribution of the network activity [Chow and Buice, *J. Math. Neurosci.* (2015)], we derive a finite-size mean-field theory that reduces a disordered to a highly symmetric network with fluctuating auxiliary fields. The exposed analytical relation between the statistics of connections and the statistics of pairwise covariances shows that both, average and dispersion of the latter, diverge at a critical coupling. At this point, a network of nonlinear units transits from regular to chaotic dynamics. Applying these results to recordings from the mammalian brain suggests its operation close to this edge of criticality.



Elimination of a ligand gating site generates a supersensitive olfactory receptor

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The interaction of odors with their cognate receptors constitutes one of the most complex ligand/receptor binding problems in biology due to the sheer quantity of potential odor molecules facing a limited albeit huge number of different olfactory receptors which in some species comprise close to 10% of all proteins. The recognition of the odor molecules by olfactory receptors represents the first stage in odor discrimination. Ligand spectra for olfactory receptors range from extremely broad to monospecific. We have recently deorphanized an olfactory receptor of the trace amine-associated receptor family, TAAR13c, as a specific and sensitive receptor for a bifunctional compound, the death-associated odor cadaverine.

Here we have modeled the cadaverine/TAAR13c interaction. Several predicted binding residues were exchanged by site-directed mutagenesis, and after heterologous expression the functionality of the resulting receptors were compared with wildtype TAAR13c. Two aspartic acid residues, Asp1123.32 and Asp2025.42, in the upper third of transmembrane domains TM3 and TM5, respectively, were found to comprise the bifunctional binding site for cadaverine. Docking predicted an additional binding interaction with Asp2796.58 at the outer surface of TM6, which was lost in docking predictions of D279N, D279A and even D279E.

Unexpectedly, site-directed mutagenesis of D279 to E, N, or A resulted in supersensitive receptors with up to twentyfold increased affinity to cadaverine compared to wildtype TAAR13c. Docking suggests that binding of cadaverine to Asp2796.58 may act as a gating mechanism, limiting ligand access to the internal binding and activation site, thereby causing a downregulation of the receptor's apparent affinity. This would constitute a novel mechanism to fine-tune physiological sensitivity to socially relevant odors. Currently we are further testing the hypothesis preparing double mutants in which external and internal binding sites are mutated concomitantly.

High-affinity olfactory receptor for the death-associated odor cadaverine

Hussain A, Saraiva LR, Ferrero D, Ahuja G, Krishna VS, Liberles SD, Korsching SI (2013) Proc Natl Acad Sci

Elimination of a ligand gating site generates a supersensitive olfactory receptor

Sharma K, Ahuja G, Hussain A, Balfanz S, Baumann A, Korsching SI (2016). Nature Scientific Reports

Extending integrate-and-fire model neurons to account for the effects of weak electric fields and input filtering mediated by the dendrite

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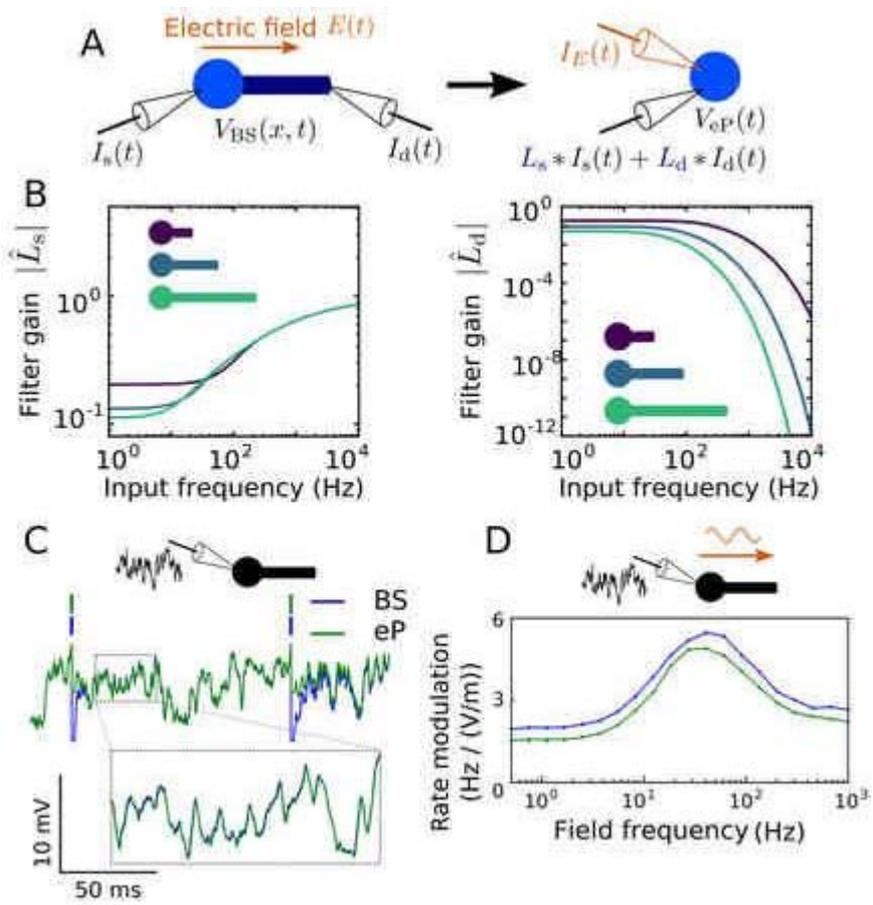
Transcranial brain stimulation and evidence of ephaptic coupling have recently sparked strong interests in understanding the effects of weak electric fields on the dynamics of brain networks and of coupled populations of neurons [1,2]. The collective dynamics of large neuronal populations can be efficiently studied using single-compartment (point) model neurons of the integrate-and-fire (IF) type as their elements. These models, however, lack the dendritic morphology required to biophysically describe the effect of an extracellular electric field on the neuronal membrane voltage. Here, we extend the IF point neuron models to accurately reflect morphology dependent electric field effects extracted from a canonical spatial "ball-and-stick" (BS) neuron model. Even in the absence of an extracellular field, neuronal morphology by itself strongly affects the cellular response properties [3]. We, therefore, derive additional components for leaky and nonlinear IF neuron models to reproduce the subthreshold voltage and spiking dynamics of the BS model exposed to both fluctuating somatic and dendritic inputs and an extracellular electric field. We show that an oscillatory electric field causes spike rate resonance, or equivalently, pronounced spike to field coherence. Its resonance frequency depends on the location of the synaptic background inputs. For somatic inputs the resonance appears in the beta and gamma frequency range, whereas for distal dendritic inputs it is shifted to even higher frequencies. Irrespective of an external electric field, the presence of a dendritic cable attenuates the subthreshold response at the soma to slowly-varying somatic inputs while implementing a low-pass filter for distal dendritic inputs. Our point neuron model extension is straightforward to implement and is computationally much more efficient compared to the original BS model. It is well suited for studying the dynamics of large populations of neurons with heterogeneous dendritic morphology with (and without) the influence of weak external electric fields.

References:

1. Reato et al. (2010), J Neurosci.
2. Anastassiou et al. (2011), Nat Neurosci.
3. Ostojic et al. (2015), J Neurosci.

Figure caption:

A: The extended point (eP) neuron model, derived from a ball-and-stick (BS) model, includes an input current equivalent to the field effect and two synaptic input filters. B: Filters gain. C: Voltage response of the two models to synaptic input. D: Spike rate modulation amplitude due to a field.



***In silico* exploration of functional networks underlying behavioral traits**

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Linking genetic information to brain anatomy allows for the computational exploration of molecular-to-systems level organization of brain function. We have developed a novel algorithm that fuses genetic, transcriptomic and connectomic information from brain and genomic initiatives for the quick functional exploration of the brain *in silico*. We found that, in the brain, functionally related genes are not distributed at random but synergize in specific networks which recapture meaningful functional anatomical annotation of actual functional networks from fMRI. When applied to gene sets known from behavioral genetics (e.g. stress, reward or social behavior related genes), we demonstrate that our workflow can extract and rank order their putative effector networks. The algorithm can be used to extend our knowledge about functional networks associated with conditions such autism or anxiety, and concomitantly reveals entry points for functional circuit dissection with e.g. optogenetic and pharmacogenetic manipulations. Moreover, the voxel-wise gene expression information assembled in the process will provide a basis for further investigating the interactions of functionally related genetic with neuronal networks. Importantly, our strategy can also be applied to other neural systems (e. g. human) for which genetic information, transcriptomes and connectomes are available.

Graded persistent activity mediated by ion channel cooperativity

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Ion channels control the flow of charges across the membrane of a cell and shape electrical signalling properties of neurons like the generation of action potentials. A main assumption for ion channels' functioning as well as modelling of electrical activity has been that channels gate independently. In other words, opening and closing of ion channels is determined by external parameters, such as membrane voltage or calcium concentration, but channels are presumed to be not interacting i.e. not influence each other's state transitions. Experimental observations, however, show that certain channels violate the independency supposition; gating depends on the state of neighbouring channels. For example, CaV1.3 channels in hippocampal neurons have recently been demonstrated to gate cooperatively [1]. Despite multifaceted implications of ion channel cooperativity for the electrical activity of neurons and their computations, so far the functional role of channel cooperativity has received relatively little attention [2,3].

Here, we show that ion channel cooperativity can lead to bistable gating and trigger persistent activity in individual neurons. Specifically, we propose that clusters of cooperative ion channels can generate graded persistent activity, as observed by Egorov and colleagues [4] in the entorhinal cortex. Such activity leads to firing rates persisting even beyond transient input pulses; rates also further increment with each new pulse. Based on numerical simulations of a conductance-based neuron model, we demonstrate that cooperativity of a small fraction of ion channels (that do not directly contribute to action-potential generation) is sufficient to explain the experimental observations in entorhinal cortex [4]. Our model predicts the long-term stability of the persistent firing and allows us to characterise further firing statistics, like spike timing noise from parameters of channel cooperativity. Altogether, we hypothesise that clusters of cooperative ion channels provide a physiological mechanism for cell-intrinsic persistent activity that does not require a network contribution via reverberating connections. Other mechanisms explaining the graded persistent activity in cells of the entorhinal cortex [4] have been proposed [5]. Whether the cooperative mechanism actually underlies persistent activity in these cells remains to be explored in experiment. We conclude that, on a wider scope, cooperative gating of even relatively small groups of ion channels can substantially enlarge the computational repertoire of single neurons, making channel cooperativity a highly interesting biophysical mechanism for many cells in the brain.

Acknowledgements

This work was supported by the German Federal Ministry of Education and Research (01GQ0901, 01GQ1403) and the Volkswagen Foundation (SmartStart).

References

- 1: Moreno, C. M. et al. Ca²⁺ entry into neurons is facilitated by cooperative gating of clustered Cav1.3 channels. *Elife* 5, 1–26 (2016).
- 2: Naundorf, B., Wolf, F. & Volgushev, M. Unique features of action potential initiation in cortical neurons. *Nature* 440, 1060–3 (2006).
- 3: Zarubin, D., Zhuchkova, E. & Schreiber, S. Effects of cooperative ion-channel interactions on the

dynamics of excitable membranes. *Phys. Rev. E - Stat. Nonlinear, Soft Matter Phys.* 85, (2012).

4: Egorov, A. V, Hamam, B. N., Fransén, E., Hasselmo, M. E. & Alonso, A. A. Graded persistent activity in entorhinal cortex neurons. *Nature* 420, 173–8 (2002).

5: Fransén, E., Tahvildari, B., Egorov, A. V., Hasselmo, M. E. & Alonso, A. A. Mechanism of graded persistent cellular activity of entorhinal cortex layer V neurons. *Neuron* 49, 735–746 (2006).

Implementation of Neural Diversity for Computer Simulations of Neuronal Excitability and Synchronization

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Neurons can exhibit an enormous diversity what each experimental neurophysiologist very well knows but often is neglected in computer simulations. A major obstacle towards the implementation of neuronal diversity might be that conventional tools like the widely used Box-Müller transform for Gaussian distribution are not always appropriate for the implementation of physiologically adequate randomness. Here we present a novel strategy for the implementation of randomness in a Hodgkin-Huxley type neuron model which considers the experimentally most relevant parameters – from ion, concentrations to equilibrium potentials, to leak conductances, and voltages to voltage dependent conductances and activation variables, including membrane capacitances according to the neurons' different sizes.

We can demonstrate a manifold of physiologically meaningful distributions of neuronal parameters achieved from uniformly distributed random numbers of the computer by means of physiologically appropriate parameterization of our algorithms. Examples are given in Fig. 1.

Such randomization generates a broad diversity of model neurons of different excitability and sensitivity as can be seen, for example, in the virtual "SimNeuron" laboratories (fully functioning demo versions can be downloaded from www.virtual-physiology.com). Additionally, we will show that neuronal diversity will have significant effects on neuronal synchronizations, e.g. allowing to selectively activate specific parts of a neuronal network (Fig.2).

Fig. 1: Randomized distribution of several neuron parameters.

Fig. 2: Partial synchronization in a network of 100 nearest neighbor gap-junction coupled neurons (coupling strength 0.01 μ S) with randomized parameter settings. Current injection (A) to one of the neurons (no. 66: white triangle) leads to the induction of action potentials (C, recorded from the neurons indicated by white dots). Transiently observable fluctuations in the global field potential (B) during current injection, disappear after current offset leaving traveling waves spreading over part of the networks neurons.

Fig. 1

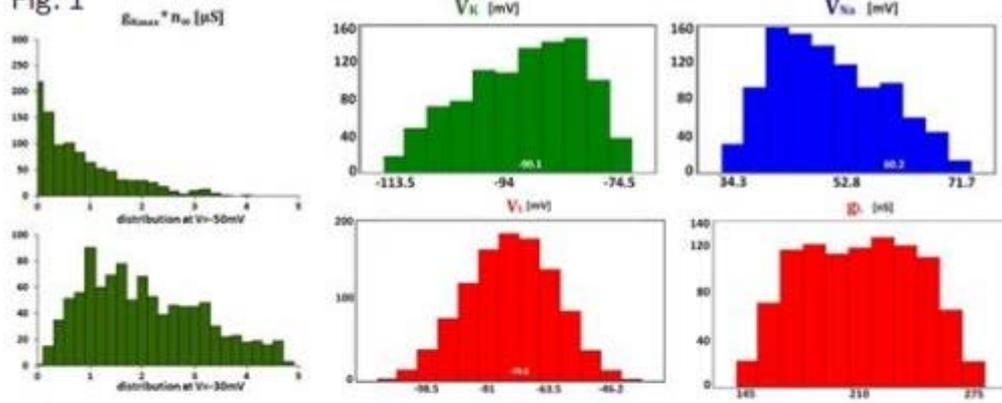
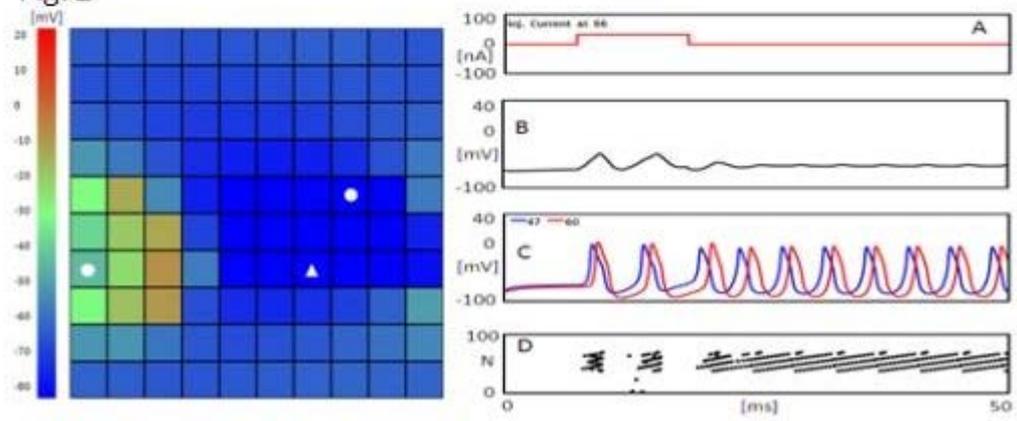


Fig. 2



Joint pausiness in parallel spike trains

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So-called 'pauses', i.e., periods with surprisingly few spikes, have recently gained increasing attention in the analysis of parallel spike trains of dopaminergic (DA) and Purkinje cells, in particular concerning simultaneity of pausing activity. The analysis of simultaneous pauses is usually based on the pauses identified in the separate spike trains. As a consequence, such techniques can suffer from the local definition of a pause within one spike train and can thus fail to identify joint pauses across spike trains that are easily detectable by eye. In addition, they crucially depend on the algorithm used for pause detection.

In order to tackle this problem, we present a new statistical method for the detection of synchronous pauses that focuses on typical characteristics of time periods showing synchronous pauses in parallel spike trains, and introduce a new measure for synchronous pausiness in parallel spike trains. We apply the technique to a data set of parallel DA neurons recorded from the VTA in freely moving mice. Interestingly, pausiness can be significantly increased in parallel spike trains as compared to individual processes or processes shifted by small time lags. This observation is robust and practically independent from the algorithm used for pause detection.

Acknowledgements

This work was supported by the Priority Program 1665 of the DFG (SD, MG, JR, GS) and by the German Federal Ministry of Education and Research (BMBF, Funding number: 01ZX1404B; GS).

Long-term information storage by the collective dynamics of multi-synaptic connections

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The majority of excitatory synapses in cortex typically reside on dendritic spines. Although cortical synapses play an important role in long-term memory, these dendritic spines undergo a continuous turnover [1,2]. This implies the question how information can be stored as long-term memory on a variable substrate as spines or synapses. Here, we propose that the collective dynamics of multiple synapses yields the stable storage and retention of information on such long timescales. The impact of such collective dynamics can already be seen between two neurons being connected by multiple synapses. More precisely, the experimentally measured distribution of the number of synapses between pairs of neurons, which is bimodal with peaks at zero and multiple synapses, can only emerge from the collective dynamics of the involved synapses [3]. Theoretical studies indicate that these collective dynamics emerge from the interaction of synaptic and structural plasticity [4,5] and, furthermore, that these dynamics are sensitive to changes in the external stimulation such that the neurons become either unconnected or connected by multiple synapses [5].

Here, we investigate the information storage and retention of these collective dynamics with a simple stochastic model of structural plasticity; new synapses are formed with a constant probability and removed with a probability depending on the number of existing synapses and the external stimulation. Using information theoretic measures, we show that the collective dynamics, yielding the bimodal distributions of the number of synapses, enables information retention on timescales orders of magnitude longer than the typical lifetime of a synapse. Thus, the conflict of spine turnover and long-term memory can be resolved by storing information in the collective dynamics of multiple synapses instead of single synapses.

Yet, at different external stimulation levels, where the collective dynamics yields distributions with a single peak either at zero or at multiple synapses, information about the initial condition is forgotten fast. This, however, implies that these stimulations can be used to learn new information orders of magnitude faster than it is forgotten. We confirm this by using these stimulations to store an image in a population of multi-synaptic connections. Indeed, this image can be retained orders of magnitude longer than it took to store it. Thus, learning can be faster than forgetting, which is also a necessary prerequisite to solve the plasticity-stability dilemma in learning and memory on the timescale of structural changes.

References:

- 1 - Yang G, Pan F, Gan WB (2009) Stably maintained dendritic spines are associated with lifelong memories. *Nature* 462: 920-924.
- 2 - Xu T, Yu X, Perlik AJ, Tobin WF, Zweig JA, et al. (2009) Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature* 462: 915-919
- 3 - Fares T, Stepanyants A (2009) Cooperative synapse formation in the neocortex. *Proceedings of the National Academy of Sciences*. 106:16463–16468.
- 4 - Deger M, Helias M, Rotter S, Diesmann M. (2012) Spike-timing dependence of structural plasticity explains cooperative synapse formation in the neocortex. *PLoS Comput Biol*. 8:e1002689.
- 5 – Fauth M, Wörgötter F, Tetzlaff C (2015) The formation of multi-synaptic connections by the interaction of synaptic and structural plasticity and their functional consequences, *PLOS Comput Biol*. 11(1):e1004031

Low-dimensional spike rate models derived from networks of adaptive integrate-and-fire neurons: comparison and implementation

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The spiking activity of single neurons can be well described by a nonlinear integrate-and-fire model that includes somatic adaptation [1]. When exposed to fluctuating inputs sparsely coupled populations of these model neurons exhibit stochastic collective dynamics that can be effectively characterized using the Fokker-Planck equation. This approach, however, leads to a model with an infinite-dimensional state space and non-standard boundary conditions [2-5]. Here we derive from that description four simple models for the spike rate dynamics in terms of low-dimensional ordinary differential equations using two different reduction techniques: one uses the spectral decomposition of the Fokker-Planck operator [3], the other is based on a cascade of two linear filters and a nonlinearity, which are determined from the Fokker-Planck equation and semi-analytically approximated [6]. We evaluate the reduced models for a wide range of biologically plausible input statistics and find that both approximation approaches lead to spike rate models that accurately reproduce the spiking behavior of the underlying adaptive integrate-and-fire population. Particularly the cascade-based models are overall most accurate and robust, especially in the sensitive region of rapidly changing input. For the mean-driven regime, when input fluctuations are not too strong and fast, however, the best performing model is based on the spectral decomposition. The low-dimensional models also well reproduce stable oscillatory spike rate dynamics that is generated by recurrent synaptic excitation and neuronal adaptation. The computational demands of the reduced models are very low but the implementation complexity differs between the different model variants. Therefore we have made available implementations that allow to numerically integrate the low-dimensional spike rate models as well as the Fokker-Planck partial differential equation in efficient ways for arbitrary model parametrizations as open source software. The derived spike rate descriptions retain a direct link to the properties of single neurons, allow for convenient mathematical analyses of network states, and are well suited for application in neural mass/mean-field based brain network models.

1. R. Brette et al., J. Neurophysiol. 94 (2005)
2. M. Augustin et al., Front. Comput. Neurosci. 7 (2013)
3. M. Mattia et al., PRE 66 (2002)
4. G. Gigante et al., PRL 98 (2007)
5. N. Brunel et al., J. Comput. Neurosci. 8 (2000)
6. S. Ostojic et al., PLOS Comput. Biol. 7 (2011)

Modeling the Effect of Phase-Triggered Transcranial Magnetic Stimulation on Motor Cortex

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Introduction. Responses to transcranial magnetic stimulation show large variability, between subjects as well as within subjects across trials. At least part of this variability may be explained by prominent oscillatory cortical rhythms, such as the μ -rhythm of motor cortex or the α -rhythm of visual cortex. In line with this hypothesis, recent studies have shown a dependence of TMS responses on the oscillation phase at stimulation onset [1,2].

Objectives. Our previous network model of the effects of TMS on motor cortex [3] displays several characteristics of so-called D&I-waves. Here we extend this model to incorporate oscillatory background activity. We test if triggering the TMS pulse at different phases of the background μ -rhythm results in similar systematic changes in the model's responses as observed experimentally.

Materials & Methods. The model consists of 250 multi-compartmental layer 5 (L5) cells (with identical dendritic tree morphology) each receiving input from 300 excitatory and inhibitory layer 2/3 (L2/3) point neurons. The model output is the pooled activity of the 250 L5 cells. The L2/3 cells receive input from a sine wave modulated Poisson process. TMS is modeled as a current injection into the L2/3 somata and L5 axons. TMS pulse onset was varied with respect to the phase of a 10 Hz oscillatory L2/3 background activity. We use a single-pulse protocol and a paired-pulse protocol, with varying inter-stimulus intervals (ISIs), with a subthreshold conditioning stimulation and a suprathreshold test stimulation.

Results. The model shows a clear modulation of L5 firing rate depending on the phase at which the TMS pulse is applied, as well as a systematic effect on amplitude and number of I-waves. The largest response can be elicited when applying the pulse in a phase when the L5 neurons are in a depolarized state, with the difference between peak and trough response regulated by stimulation intensity. Notably, depending on the pulse strength, the amplitude and latency of I-waves is modulated by the level of background activity at the time of the TMS pulse, due to short-term synaptic depression. For the paired-pulse protocol, averaging over different phases of the background activity, our model reproduces the physiological result that short ISIs between the two pulses lead to a depression and long ISIs lead to a facilitation of I-wave amplitudes. But when considering individual phases, a differential pattern arises, revealing interactions between ongoing activity and synaptic short-term depression.

Conclusion. Our extended network model including oscillatory background activity displays phase-dependency of TMS responses and can parsimoniously explain how cortical rhythms contribute to the variability of TMS effects.

[1] Dugué, L, Marque, P, VanRullen, R (2011). The phase of ongoing oscillations mediates the causal relation between brain excitation and visual perception. *J Neurosci*, 31(33), 11889–93.

[2] Triesch, J, Zrenner, C, Ziemann, U (2015). Modeling TMS-induced I-waves in human motor cortex. *Prog Brain Res* (222), 105–124.

[3] Rusu, CV, Murakami, M, Ziemann, U, Triesch, J (2014). A model of TMS-induced I-waves in motor cortex. *Brain Stimul*, 7 (3), 401–414.

Non-linear computation and establishment of contrast invariance in spatially structured recurrent balanced networks

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Visual objects can be recognized over a wide range of illuminations and contrasts. The mechanism underlying contrast invariant perception may be grounded in the ability of the neurons in the visual cortex to maintain their selectivity for sensory stimulus orientation across wide variations in stimulus contrast. This important property of visual circuits is called contrast invariance, and it is probably one of the most remarkable mechanisms involved in visual perception. A yet open question is how contrast invariant responses arise in the presence of complex feed-forward and recurrent connections and how invariance can be sustained despite non-linear neural computation¹. In the present work, we have considered a balanced recurrent network model of the visual cortex² and included spatially structured and non-structured connectivity as well as activity dependent synaptic plasticity, as consistent with experimental data.

Our results show that plasticity introduces nonlinearities in computation and that recurrent neural circuits can switch between sub- or superlinearity for different stimuli via subtle changes of synaptic strengths. We also found that contrast invariance is present in a recurrent network model which lacks spatial distribution of synaptic connections. However, when we combined spatial structure with synaptic plasticity we found that contrast invariance was weakened. Our results can help explain why some neurons show contrast invariance while others lack it³.

¹ M. Carandini, "Melting the Iceberg: Contrast Invariance in Visual Cortex", *Neuron*, vol. 54, no. 1, pp. 11-13, 2007.

² C. van Vreeswijk and H. Sompolinsky. Chaos in neuronal networks with balanced excitatory and inhibitory activity. *Science*, 274:1724-1726, 1996.

³ Y. Li, W. Ma, L. Li, L. Ibrahim, S. Wang and H. Tao, "Broadening of Inhibitory Tuning Underlies Contrast-Dependent Sharpening of Orientation Selectivity in Mouse Visual Cortex", *Journal of Neuroscience*, vol. 32, no. 46, pp. 16466-16477, 2012.

Properties of dendritic trees under different branch ordering schemes

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Dendrite morphology varies greatly between different cell types and is known to influence neuronal function. We examine general dendritic tree features in reconstructed dendrites and in synthetically generated model dendrites as a function of different branch ordering schemes.

The classical centrifugal ordering (CO) starts at the root of the tree and increases order at every branch point. However, CO performs badly as a hierarchical system to identify primary, secondary or tertiary branches as classified by human experts. Also, local branching statistics do not tend to correlate strongly with CO.

By contrast, the Horton-Strahler order (SO) is a centripetal branch ordering system originally developed to describe river networks [1,2] where order assignments increase from the terminal branches with order 1 towards the root of the tree. When two branches of the same order meet, the order of the parent branch is increased by 1, otherwise the higher order is passed on [2]. We find that even though these measures are currently used in neuroscience, topological branching statistics such as branch and segment numbers decay exponentially with SO in a manner that is universal for all binary trees and therefore have no descriptive power for dendrite morphology. However, other SO-sorted metrics seem to be cell type-specific, rendering them potential candidates for categorising dendritic tree structures. Interestingly, branch diameters correlate faithfully with centripetal but not with centrifugal branch ordering, indicating a functional importance of SO for dendritic morphology and growth.

In this work, we furthermore explore a novel scheme to order branches hierarchically that is inspired by the known developmental growth process of dendritic trees. In this scheme, long primary branches span the extent of the final coverage area of the dendrite, then secondary branches are the longest branches that emanate from the primary ones, etc. Preliminary results show that branches cluster into separate classes of characteristic lengths, indicating possible steps in the dendrite growth process.

Acknowledgements

The work was supported by a BMBF grant (No. 01GQ1406 – Bernstein Award 2013 to H.C.).

References

1. Horton, R. E. (1945). Erosional development of streams and their drainage basins; Hydrophysical approach to quantitative morphology. *Bulletin of the Geological Society of America*, 56(3), 275–370.
2. Strahler, A. N. (1957). Quantitative analysis of watershed geomorphology. *Transactions American Geophysical Union*, 38(6), 913–920.

Simulating large-scale human brain networks with a mean-field model of EIF neurons: exploring resting state FC and stimulation with electric fields

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The use of whole-brain networks for understanding the dynamics of the interaction between brain regions has experienced a rise in popularity in the last few years. Here, we calibrate a whole-brain network model to human resting state data, and use it to explore the effects of weak electric fields on the network dynamics.

The structural connectivity of the brain network is extracted from parcellated brain scans using an atlas with 68 regions (Desikan et al., 2006) and DTI tractography of long-range axons to estimate coupling strengths and delays between regions averaged over 48 individuals (Ritter et al. 2013, Schirner et al. 2015). The mean activity of each brain region is described by a mean-field population model of EIF neurons (Ladenbauer, 2015). After fitting local parameters such as recurrent coupling strengths and delays and the global parameters coupling strength, axonal signal transmission speed and external noise intensity, our model can produce simulated BOLD functional connectivity (FC) with high Pearson correlation (mean .55, max/min .78/.25) to the empirical 20 minute resting state BOLD FC of these individuals. A local model with a limit cycle at gamma frequencies and a bistability with a low and a high-activity fixed point was found to produce good fits.

A range of global parameters can produce good grand average FC fits. However, the FC in the resting state is not stationary. To capture the brain's dynamical properties in the resting state, the FC fit is complemented by a fit of the FCD matrix (Hansen et al, 2015). We show that the simulated FCD matrix is well comparable to empirical data (Kolmogorov distance around 0.1) on several timescales. Clustering of the power spectra of the local nodes shows that nodes of the brain graph can be divided into two sets with dominant alpha and gamma frequencies respectively and that contralateral regions end up in the same cluster.

Lastly, we present results of modeling the effect of external tACS-like brain stimulation on the global network activity. By modifying the dynamics of a subset of nodes, the global dynamics of the brain network can be shaped. We stimulate the bilateral entorhinal cortices, the main interface of the cortex to Hippocampus. We show that on the global network level, transitions from a DOWN state to an UP state tend to lock on the onset of oscillatory stimulation and relate these results to experimental findings in the rat brain conducted in Ref. (Battaglia et al, 2004).

Spike time precision of different neuron classes – influence of morphology and ion channels

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Cortical neurons operate in a fluctuation driven regime, they receive thousands of synaptic inputs every second but fire only few spikes during this time. A population of neurons encodes information in the population firing rate. In such a population, always a small fraction is close to firing threshold. Therefore, the population firing rate can reflect changes in the input with very short delay, on the order of a millisecond. This temporal precision can also be captured, in the frequency domain, using the transfer function or 'dynamic gain' that captures the transfer from input current amplitude to population firing rate. We studied temporal precision of spiking in a number of different neuron classes: in pyramidal cells (PC) of layer 2/3 and layer 5 and in the interneurons spiny stellate cells (SSC) of layer 4, basket cells and chandelier cells of layer 2/3. Excitatory cells, this is PC and SSC, were characterized by their location within the cortex and their electrophysiological properties. Inhibitory interneurons were identified in mice that expressed a fluorophore either in parvalbumin positive neurons or in Nkx2.1Cre mice crossed with Ai9 reporters (both from the Jackson laboratories). We patched the cells in coronal brain slices from mice. In the current clamp mode we injected a fluctuating current, characterized by its mean, standard deviation and correlation time. This fluctuating current leads to a fluctuating somatic membrane potential and irregular spiking and thereby emulates the fluctuation driven regime observed in-vivo. We adjusted mean and standard deviation to achieve a firing rate of around 5 Hz and studied the relation between current input and spike output, constructing the transfer function for each neuron.

For different excitatory neurons of the same class, we found similar transfer functions. In the GABAergic interneurons, transfer functions seemed to be more variable. However, also the electrophysiological properties showed no clear separation of those cell types. We found differences in the shape of the transfer function in both the low frequency region, as well as the high frequency limit that governs the temporal precision of spike timing. The differences in the transfer functions at low frequencies ($f < 30$ Hz) could be accounted for by the action of sub-threshold, voltage dependent ion channels and the passive filter properties due to the different impedances of the dendritic tree. The latter are of particular impact in layer 5 pyramidal cells, where the low frequency suppression of the large dendritic tree leads to an apparent boost of high frequencies in the transfer function.

In excitatory neurons we blocked potassium channels KCNQ7.2/3 and Kv1, predominantly located in the axon initial segment, and found that these potassium channels co-determine the bandwidth of the transfer function at high frequencies. Potassium channel blockade lead to a decrease of the transfer function at frequencies above 50 Hz. The magnitude of this effect depends on neuronal type and the correlation time of the input. Slow input correlations, such as correlation time constants of 10 or 20 ms, result in a larger influence of the potassium channels. This influence is the largest in the small spiny stellate cells but smaller in pyramidal neurons. We present numerical simulations that explain the combined impact of input correlation time, potassium channels and cellular morphology on the shape of the transfer function.

Temperature-robust computation with simple network motifs

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Temperature substantially affects physiological processes including those in the nervous system. As a consequence of changes in temperature, well-coordinated dynamics of neurons in networks as well as cell-intrinsic dynamics may get out of balance and nervous system function could be put at risk.

While temperature variations in the nervous system of (warm blooded) endotherms may seem relatively mild (due to regulation of central body heat), in (cold blooded) ectotherms the whole nervous system is exposed to substantial temperature variations of several 10°C mirroring those of the environment. In evolution, temperature robustness must hence have been a severe constraint and neural design should reflect functional invariance to temperature changes, at least in the physiological range. Mechanism fostering such an invariance could be implemented on the cellular level, see for example [1,2,3], but must also be ensured on the network level, in particular as synaptic transmission depends on temperature.

Here, we use simple mathematical models of neuronal network motifs and explore their temperature robustness. In particular, a parallel pathway of excitation and inhibition, as it is often encountered in vertebrate [4] and invertebrate systems [5], promises benefits for temperature robustness due to a balancing of opposing forces. We explore this hypothesis in small networks of conductance-based neurons including temperature-dependent ion channel dynamics as well as a phenomenological temperature-dependence of synaptic transmission and identify temperature robustness in the context of different neural functions.

This work was supported by the German Federal Ministry of Education and Research (01GQ0901, 01GQ1403).

References:

- [1] F.A. Römschied, et al. "Cell-intrinsic mechanisms of temperature compensation in a grasshopper sensory receptor neuron." *eLife* 3 (2014): e02078.
- [2] F.A. Römschied. "Neural Mechanisms of Temperature Compensation in an Insect Auditory System." Diss. (2016).
- [3] A.Rinberg, et al. "The Effects of Temperature on the Stability of a Neuronal Oscillator." *PLoS Comput Biol* 9.1 (2013): e1002857.
- [4] M. Okun and I. Lampl. "Instantaneous correlation of excitation and inhibition during ongoing and sensory-evoked activities." *Nature neuroscience* 11.5 (2008): 535-537.
- [5] V. Marquart "Local interneurons mediating excitation and inhibition onto ascending neurons in the auditory pathway of grasshoppers." *Naturwissenschaften* 72.1 (1985): 42-44.

The impact of action potential initiation site separation on fast population encoding

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It has been discovered that cortical neurons can realize fast population encoding. The underlying biophysical mechanisms however are not well understood. Although it has been shown that reducing action potential onset rapidness can impair encoding ability, it is not clear whether onset rapidness and encoding ability are tightly connected with each other. Several biophysical hypotheses have been proposed. One hypothesis states that rapid action potential onset is caused by the separation of the action potential initiation site and the soma. And this separation causes the fast population encoding. Here we examine this hypothesis with the linear response function. We found that the initiation site separation hypothesis alone is not sufficient to reproduce the high cutoff frequency observed in cortical neurons. Also, the linear response functions at high frequencies are insensitive to the temporal correlation of the inputs. This disagrees with previous experiments as well. By tuning the slope of the sodium current activation function, we also examine the cooperative gating hypothesis under this multi-compartment framework. The cooperative gating hypothesis proposes that when emitting spikes, sodium channels are more likely to open together. The sodium current activation function is like a step function. When the cooperative gating of the sodium channels is introduced, both discrepancies above are resolved. We further compare the action potential dynamics under the two hypotheses, and propose an explanation for these discrepancies. We examine a simplified model of the multi-compartment model with cooperative gating. With some approximations, we obtain the linear response function for this model analytically. The analytic result indicates that a smaller soma can help to improve the encoding ability. In summary, our study shows that the separation of the action potential initiation site from the soma alone is not sufficient to realize fast population encoding. Adding cooperative gating of the sodium channels to the model can reproduce this property. In the cooperative gating model, the separation of the action potential initiation site from the soma plays a limited role in determining high cutoff frequency.

Towards reproducible workflows for electrophysiology data using the *Elephant* analysis framework

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The degree of complexity when working with data from electrophysiological experiments has reached a level where well-structured and defined workflows for data and metadata acquisition, pre-processing, and subsequent analysis are becoming a necessity. The implementations of such workflows are often heterogeneous across researchers and experiments, dependent on custom-written codes, and far from being automatized. As such, they place a high burden and workload on the individual researchers in charge of defining the workflows and translating them into software. While in the meantime a number of generic software solutions to support some parts of such workflows are under development, software covering other aspects of the workflow are still lacking. This situation has serious consequences regarding the degree of reproducibility of data capture and data analysis in that it leads to ineffective and unsustainable science. Our aim is therefore to create and refine guidelines and tools that facilitate transparent and accessible workflows for managing and analyzing electrophysiological data.

Here we outline how already today existing software tools can be combined to construct partial workflows that are capable of addressing some of the resulting challenges facing researchers, as summarized in [1]. To this end, we introduce a case study that links emerging software tools to form a reproducible analysis workflow based on the Python programming language. At the heart of this workflow we identified and partly developed three open-source software tools that represent the scaffold from which the analysis is built. First, we demonstrate how data of different origins can be represented in a standard form using the *Neo* framework [2]. Second, we demonstrate how the complex metadata accumulating in an electrophysiological experiment [3] can be gathered and stored using the open metadata markup language (*odML*) for metadata management [4]. These metadata are suitable to be combined with the actual data in the *Neo* framework, leading to a common representation of both data and metadata for subsequent use in the analysis workflow. Finally, as the key component of such workflows, we introduce the Electrophysiology Analysis Toolkit (*Elephant*, <http://neuralensemble.org/elephant/>) as a recent community-centered initiative to develop an analysis framework for multi-scale activity data based on this data representation. As such, *Elephant* represents a modular software component that provides generic library functions to perform standard and advanced analysis processes. In an outlook, we outline how this workflow can be extended by additional tools and technologies to handle access to high-performance computing, provenance tracking of the results, and work in a highly collaborative environment (see also [5]). In part, the work presented in this abstract is detailed in [6].

References:

- [1] Badia, R., Davison, A., Denker, M., Giesler, A., Gosh, S., Goble, C., Grewe, J., Grün, S., Hatsopoulos, N., LeFranc, Y., Muller, J., Pröpper, R., Teeters, J., Wachtler, T., Weeks, M. & Zehl, L. (2015) INCF Program on Standards for data sharing: new perspectives on workflows and data management for the analysis of electrophysiological data. Techn. Report, International Neuroinformatics Coordination Facility (INCF). <https://www.incf.org/about-us/history/incf-scientific-workshops>
- [2] Garcia, S., Guarino, D., Jaillet, F., Jennings, T., Pröpper, R., Rautenberg, P.L., Rodgers, C.C.,

- Sobolev, A., Wachtler, T., Yger, P. & Davison, A. (2014) Neo: an object model for handling electrophysiology data in multiple formats. *Frontiers in Neuroinformatics* 8, 10.
- [3] Zehl, L., Jaillet, F., Stoewer, A., Grewe, J., Sobolev, A., Wachtler, T., Brochier, T., Riehle, A., Denker, M. & Grün, S. (2016) Handling Metadata in a Neurophysiology Laboratory. *Frontiers in Neuroinformatics*, 10, 26.
- [4] Grewe, J., Wachtler, T. & Benda, J. (2011) A Bottom-up Approach to Data Annotation in Neurophysiology. *Frontiers in Neuroinformatics* 5, 16.
- [5] Yegenoglu, A., Senk, S., Amblet, O., Brukau, Y., Davison, A., Lester, D., Lührs, A., Quaglio, P., Rostami, V., Rowley, A., Schuller, B., Stokes, A, van Albada, S.J., Zielasko, D., Diesmann, M., Weyers, B., Denker, M. & Grün, S. An Exemplary Collaborative Simulation-Analysis Workflow for Computational Neuroscience using HPC, JARA-HPC-Symposium (2016), Springer Series Lecture Notes in Computer Science (in press).
- [6] Denker, M. & Grün, S. Designing workflows for the reproducible Analysis of Electrophysiological Data. In: *Brain Inspired Computing*, eds: Katrin Amunts, Lucio Grandinetti, Thomas Lippert, Nicolai Petkov. Lecture Notes in Computer Science, Springer (in press).

Acknowledgements: Helmholtz Portfolio Theme Supercomputing and Modeling for the Human Brain (SMHB), EU grant 720270 (Human Brain Project, HBP).

Transition to chaos in random neural networks in the presence of noise

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Networks of randomly coupled rate neurons display a transition to chaos at a critical coupling strength [1]. These networks exhibit optimal information processing capabilities close to the transition – at the edge of chaos – and have been a focus both in neuroscience and machine learning. In the absence of noise or time-dependent inputs the transition is well understood by a dynamical mean-field theory describing the fluctuations of a single unit [1]. In particular, the transition to chaos occurs exactly when the trivial fixed point becomes unstable and hence can be predicted by linear stability analysis. Moreover, chaos is uniquely indicated by a decaying autocorrelation function. However, in nature and technology, these networks operate in the presence of noise or time-dependent inputs, rendering their dynamics stochastic or nonautonomous.

Previously it was found that noise shifts the transition to chaos to larger coupling strengths in simpler discrete-time systems [2], where the critical coupling corresponds to the point at which the system becomes locally unstable. The absence of temporal correlations on the one hand greatly simplifies the analysis, but on the other hand makes it impossible to transfer the results to continuous-time systems considered here. Kadmon et al. [3] studied the effect of small noise on the autocorrelation function and showed that noise smooths the transition. However, the fundamental mechanism by which noise affects the maximum Lyapunov exponent and the location of the transition are not understood.

Here, we investigate the effect of additive white noise on the transition to chaos. We develop the corresponding dynamical mean-field theory yielding the self-consistent autocorrelation function. As expected, the autocorrelation function always decays due to the noise and cannot be used as an indicator for chaotic dynamics. A transition to chaos must therefore be qualitatively different from the noiseless case. To find the transition we determine the maximum Lyapunov exponent, which describes the asymptotic growth of infinitesimal perturbations also for stochastic dynamics. We derive an exact condition for the transition from stable to chaotic dynamics. The transition is shifted to significantly larger coupling strengths than predicted by linear stability analysis of the local Jacobian matrix. This hints toward a dynamic mechanism by which noise suppresses chaos in continuous-time systems [4].

Acknowledgements

Jannis Schuecker and Sven Goedeke contributed equally to this work. Partly supported by Helmholtz association: VH-NG-1028 and SMHB; EU Grant 604102 (HBP).

References

1. Sompolinsky, H., Crisanti, A., & Sommers, H. J. (1988). Chaos in random neural networks. *Physical Review Letters*, 61(3), 259.
2. Molgedey, L., Schuchhardt, J., & Schuster, H. G. (1992). Suppressing chaos in neural networks by

noise. Physical review letters, 69(26), 3717.

3. Kadmon, J., & Sompolinsky, H. (2015). Transition to chaos in random neuronal networks. *Physical Review X*, 5(4), 041030.
4. Goedeke, S., Schuecker, J., & Helias, M. (2016). Noise dynamically suppresses chaos in random neural networks. arXiv preprint arXiv:1603.01880.

Poster Topic

T27: Techniques and Demonstrations

- [T27-1A](#) A genetically encoded system with high spatiotemporal resolution for modification of neuronal network activity patterns *in vivo*
Firat Terzi, Johannes Knabbe, Hongwei Zheng, Niklas Schneider, Sidney Cambridge
- [T27-2A](#) A new approach for ratiometric calcium imaging of intact microglia *in vivo*
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Bart R.H. Geurten, Simon P. Schäfer, Heribert Gras
- [T27-4A](#) Assessing cortical cellular composition and volume changes by longitudinal *in vivo* imaging of cell nuclei
Livia Asan, Fred Hamprecht, Thomas Kuner, Johannes Knabbe
- [T27-5A](#) Coating of fluorescent PLGA-Dil nanoparticles with poloxamer 188 leads to enhanced duration and intensity of the fluorescence signal in rat retinal endothelium
Enqi Zhang, Nadya Osipova, Olga Maksimenko, Bernhard Sabel, Svetlana Gelperina, Petra Henrich-Noack
- [T27-6A](#) Data organization made easy: Safe and efficient data management for neuroscience
Michael Sonntag, Achilleas Koutsou, Christian Garbers, Christian J. Kellner, Adrian Stoewer, Jan Grewe, Thomas Wachtler
- [T27-1B](#) Evaluation of brain pharmacokinetic properties in awake animals: from rodents to non-human primates
Marcel van Gaalen, Gunnar Flik, Joost Folgering, Arash Rassoulpour, Minha Choi, Robert Stratford, Thomas Cremers
- [T27-2B](#) Efficient isolation of viable primary neural cells from adult murine brain tissue based on a novel automated tissue dissociation protocol
Hui Zhang
- [T27-3B](#) Fast Imaging And Probabilistic Reconstruction of Light-Evoked Activity in the Mouse Retina
Luke Edward Rogerson, Katrin Franke, André Maia Chagas, Zhijian Zhao, Philipp Berens, Thomas Euler
- [T27-4B](#) Functional characterization of new, flexible multi-contact silicon probes for chronic intra-cortical recording and stimulation

- [T27-5B](#) Impact of the insertion speed of the recording probe on the quality of neural recordings in acute experiments
Richárd Fiáth, Adrienn Márton, Silke Musa, Alexandru Andrei, Carolina Mora Lopez, István Ulbert
- [T27-6B](#) KCC2 dependent steady state chloride levels in mouse layer 2/3 cortical neurons *in vivo*.
Juan Carlos Boffi, Johannes Knabbe, Michaela Kaiser, Thomas Kuner
- [T27-7B](#) CRISPR-Cas9 Lipid Nanoparticles as an Efficient Delivery Tool in Primary Neural Cultures_Proof of Concept
Nadia Tagnaouti, Anitha Thomas, Rebecca De Souza, Grace Tharmarajah, Oscar Seira, Jie Liu, Wolfram Tetzlaff, Peter Deng, Jan A. Nolte, Kyle D. Fink, David J. Segal, R. James Taylor, Euan Ramsay
- [T27-1C](#) Manipulation of Neurons with Precisely Controlled Illumination in Space and Time Using Two-Photon Lasers and Spatial Light Modulators
Gert Rapp, Susanne Holzmeister, Manuela Fichte, Alexander Heckel, Oliver Wendt, Stephan Junek
- [T27-2C](#) Low cost open source hardware and software in behavioral and electrophysiological experiments: Arduino & Raspberry Pi
Benjamin Hans Paffhausen
- [T27-3C](#) Multi-scale detection of rate changes in spike trains with weak dependencies
Gaby Schneider, Kaue M. Costa, Jochen Roeper, Michael Messer
- [T27-4C](#) odML-tables Providing a graphical interface for odML based metadata management
Julia Sprenger, Lyuba Zehl, Jana Pick, Carlos Canova, Sonja Grün, Michael Denker
- [T27-5C](#) Optical activation of neurons through two-photon excitation of gold nanoparticles
Jan Hirtz, Wieteke de Boer, Mercè Izquierdo-Serra, Shuting Han, Yuri Shymkiv, Christophe Dupre, Rafael Yuste
- [T27-6C](#) Quantitative detection of intracellular sodium using FLIM with CoroNa-Green
Jan Meyer, Verena Untiet, Christoph Fahlke, Thomas Gensch, Christine R. Rose
- [T27-7C](#) Fast and accurate spike sorting in vitro and in vivo for up to thousands of electrodes
Olivier Marre, Pierre Yger, Giulia L.B. Spampinato, Elric Esposito, Baptiste Lefebvre, Stephane Deny, Christophe Gardella, Marcel Stimberg, Florian Jetter, Guenther Zeck, Serge Picaud, Jens Duebel
- [T27-1D](#) Replication of Riehle et al (1997) by an Open Source Implementation of the Unitary Events Analysis Method
Vahid Rostami, Junji Ito, Sonja Grün
- [T27-2D](#) Robust threshold estimation based on without near threshold measurements
Achim Schilling, Patrick Krauss, Claus Metzner, Konstantin Tziridis, Holger Schulze

- [T27-3D](#) The use of Click chemistry for quantitative analysis of protein palmitoylation
Tatiana Kuznetsova, Alexander Dityatev, Patricia M.-J. Lievens
- [T27-4D](#) Tracer electrophoresis through the nerve sheath for neuroanatomical and functional labeling of neural pathways
Berthold HEDWIG, Matthew D. Isaacson
- [T27-5D](#) Transcranial functional ultrasound imaging in freely-moving awake mice and anesthetized young rats without contrast agent through the intact skull
Zsolt Lenkei, Elodie Tiran, Jeremy Ferrier, Thomas Deffieux, Jean-Luc Gennisson, Sophie Pezet, Mickael Tanter
- [T27-6D](#) Transcriptome and Neuropeptidome analysis of *Carausius morosus*
Sander Liessem, Susanne Neupert, Lapo Ragionieri, Ansgar Büschges, Reinhard Predel

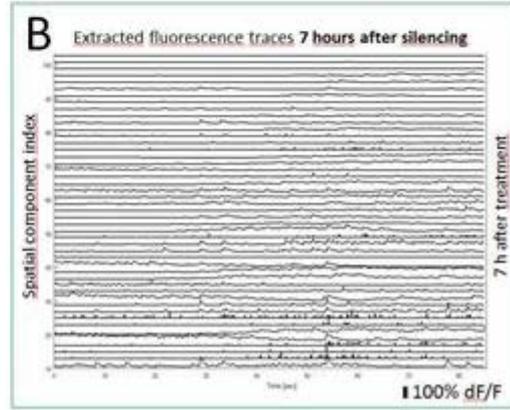
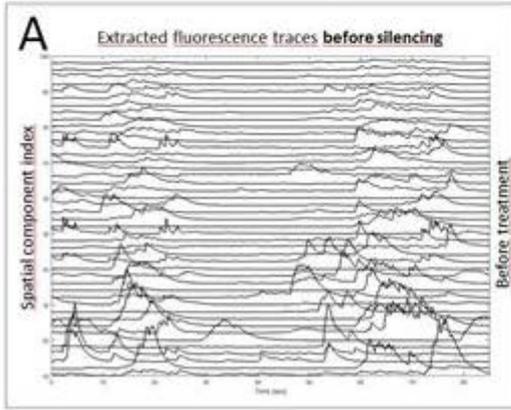
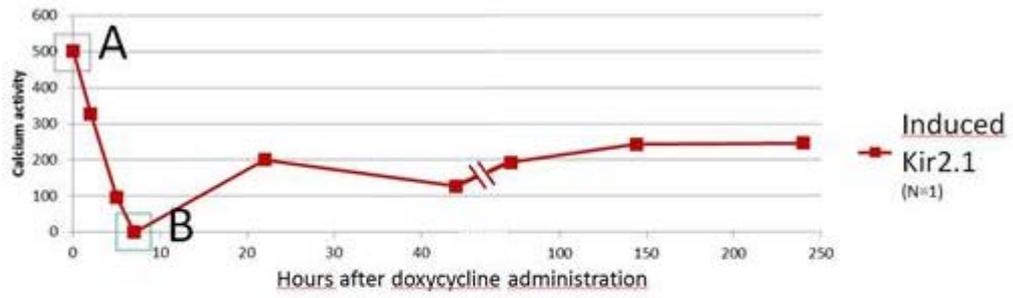
A genetically encoded system with high spatiotemporal resolution for modification of neuronal network activity patterns in vivo

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The patterns of neuronal network activity are fundamental to brain function. To correlate changes in network activity with genetic manipulation of neurons, we established a method that allows targeted transgene expression in a defined set of previously identified neurons. The goal is that these neurons can be analyzed before, during, and after induced gene expression to precisely correlate phenotypic changes to the genetic manipulation of neuronal excitability. To achieve transgene expression with high spatio-temporal control in living mice, we developed an optimized version of the inducible Tetracycline (TetOn) system with substantially reduced background expression. For acute genetic manipulation of neuronal excitability, we chose the inward rectifying potassium channel Kir2.1 which cell-autonomously silences neurons. In vitro, we found that Kir2.1 induction led to a significant reduction of neuronal activity within three hours. Two-photon microscopy in cortices of living mice indicated that injection of doxycycline and induction of Kir2.1 reduced neuronal activity within hours as visualized by a co-expressed GCamP6 calcium indicator. The goal is to induce transgene expression of Kir2.1 in a subset of cells to investigate the consequences on neuronal network homeostasis of larger ensembles. By imaging of the network patterns and morphology before and after Kir2.1 expression, the phenotypic homeostatic network changes can be directly correlated to the changes in gene expression. In particular, with this flexible high-resolution approach, the dynamics of homeostasis can be captured in much detail including potentially very early events which was previously not possible.

Imaging activity in motor cortex of awake mice



A new approach for ratiometric calcium imaging of intact microglia *in vivo*

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Microglia are the major immune cells of the central nervous system. Upon an immune challenge these cells undergo an activation process, which is characterized by morphological alterations, proliferation, production and release of cytokines, and phagocytosis. *In vitro* studies suggest that many of these effector functions rely on transient or sustained alterations of intracellular Ca^{2+} levels. However, it turned out to be challenging to study microglial Ca^{2+} signaling in the intact brain *in vivo*.

Here, we introduce a novel approach enabling *in vivo* measurement of spontaneous and evoked Ca^{2+} signals as well as the basal intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in microglia. Our approach is based on a new microRNA-regulated viral vector enabling the expression of a ratiometric Ca^{2+} indicator Twitch-2B in microglia (Akerblom et al., 2013; Thestrup et al., 2014). MicroRNA is a small non-coding RNA involved in post-transcriptional modification of gene expression, especially in gene silencing. Expression of microRNA leads to degradation of the specific transgene messenger RNA. As microglia are the only cell type in the brain lacking microRNA-9 expression, we used a microRNA-9-regulated vector for selective labeling of these cells. We stereotactically injected the new lentiviral construct enabling microRNA-9 regulated expression of Twitch-2B into the somatosensory cortex of young wild type mice. Microglia up to the depth of 270 μm could be visualized using this approach and Ca^{2+} signaling was measured in chronic as well as acute preparations. The new technique enabled us to study spontaneous as well as evoked Ca^{2+} activity of microglial cells in the intact brain. Consistent with previous data, microglial cells were rather silent at rest but responded with large Ca^{2+} signals to stimulation of purinergic receptors or local damage of surrounding neurons. Because of the use of a ratiometric indicator, our *in vivo* Ca^{2+} measurements were not sensitive to small tissue movements, focus drifts or slight bleaching of the dye. Moreover, the use of Twitch-2B for the first time allowed characterization of basal levels of $[\text{Ca}^{2+}]_i$ in the cytosol of microglial cells *in vivo*. We estimated basal levels of $[\text{Ca}^{2+}]_i$ under *in vivo* conditions as well as in acute brain slices and in primary microglial cell culture. These experiments showed that in reduced preparations microglial Ca^{2+} levels were significantly increased compared to *in vivo*, suggesting that microglia respond to tissue injury with a sustained increase of basal Ca^{2+} levels. These data identify the basal level of $[\text{Ca}^{2+}]_i$ as a versatile microglial activation marker, which is highly sensitive to the cell's environment.

Akerblom M, Sachdeva R, Quintino L, Wettergren EE, Chapman KZ, Manfre G, Lindvall O, Lundberg C, Jakobsson J (2013) Visualization and genetic modification of resident brain microglia using lentiviral vectors regulated by microRNA-9. *Nat Commun* 4:1770.

Thestrup T, Litzlbauer J, Bartholomäus I, Mues M, Russo L, Dana H, Kovalchuk Y, Liang Y, Kalamakis G, Laukat Y, Becker S, Witte G, Geiger A, Allen T, Rome LC, Chen TW, Kim DS, Garaschuk O, Griesinger C, Griesbeck O (2014) Optimized ratiometric calcium sensors for functional in vivo imaging of neurons and T lymphocytes. *Nat Methods* 11:175-182.

A novel high-throughput, low-cost ethological screening device using a visual stimulus system running on a Raspberry Pi

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Many neurophysiological, -ethological, psychophysical and cognitive studies require a reliable visual stimulus generator. Dedicated specialised hardware that delivers frame-by-frame precision is usually priced about 8000 EUR (e.g. Cambridge ViSaGe Mk II¹). Software solutions running on gaming PCs can be open source and free of charge (e.g. VisionEgg²), but still need a complete computer system to run on and minor programming skills to operate.

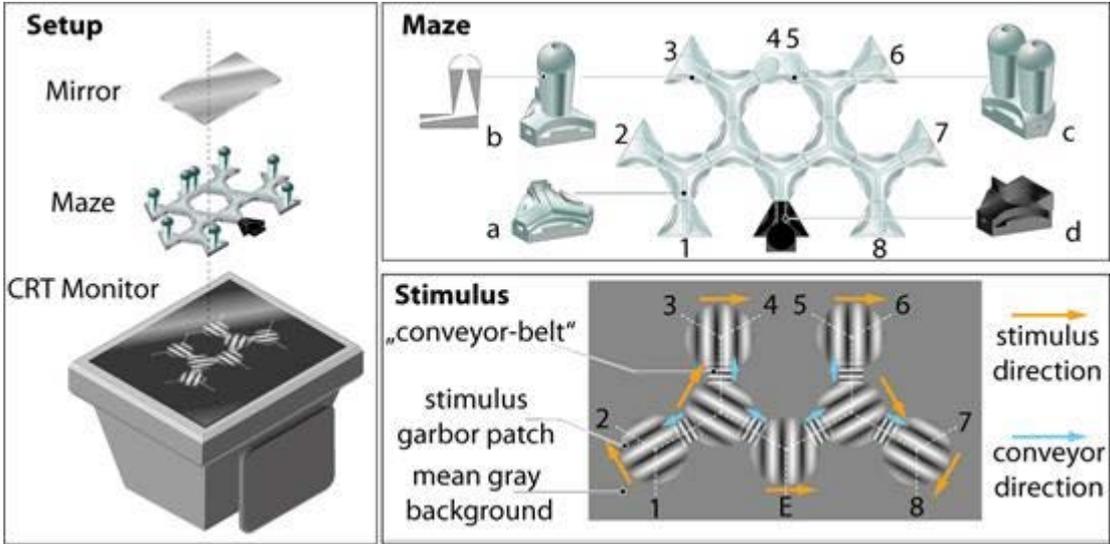
We present here a stimulus system (IONS) based on the Raspberry Pi platform (hardware cheaper than 40 €). IONS comes with a large library of the most commonly used visual stimuli in, such as 2D gratings, spinning drums, gabor patches, moving bars and can also play predesigned movies. It furthermore responds to digital inputs via the built-in GPIO pins of the Raspberry Pi and outputs its refresh rate and stimulus presentation over the same pins. IONS is build as a network solution so that the experimenter can administrate all stimuli through a web browser.

We demonstrate IONS capabilities in a new high-throughput low-cost behavioural assay with *Drosophila* mutant and wildtype flies walking in an optomotoric maze. Based on a design published by van Swinderen et al.³, this maze is assembled from identical Y-elements and has a deterministic tree layout with eight exits (see figure). Reaching a certain exit follows from a specific sequence of accordant (+) and opponent (-) turns relative to the stimulus direction, representing a total number of (+) choices. The frequencies of (+) and (-) choices in a large number of flies can easily be determined by counting the animals which reach each of the exits. The modular anatomy of the maze allows the experimenter to build many different optomotoric tests.

¹<http://www.crs ltd.com/tools-for-vision-science/visual-stimulation/visage/#npm>

²Straw, Andrew D. 2008. "Vision Egg: An Open-Source Library for Realtime Visual Stimulus Generation." *Frontiers in Neuroinformatics* 2 (4). Frontiers: 4. doi:10.3389/neuro.11.004.2008.

³van Swinderen, Bruno, Amber McCartney, Sarah Kauffman, Kris Flores, Kunal Agrawal, Jenée Wagner, and Angelique Paulk. 2009. "Shared Visual Attention and Memory Systems in the *Drosophila* Brain." Edited by Hiromu Tanimoto. *PLoS ONE* 4 (6). Public Library of Science: e5989. doi:10.1371/journal.pone.0005989.



Assessing cortical cellular composition and volume changes by longitudinal in vivo imaging of cell nuclei

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Introduction:

In various diseases affecting the nervous system, morphometric MRI studies have revealed local gray matter volume (GMV) alterations in brains of patients and rodent models. The cellular mechanisms accounting for these macroscopic changes remain largely unknown. To study microscopic correlates for these findings, we aimed to develop an approach suited to follow rearrangements in the 3-dimensional cytoarchitecture of whole cortical areas in the mouse brain *in vivo*. For this purpose, we applied two-photon laser scanning microscopy (2PLSM) through a chronic cranial window to repetitively image large cortical volumes of living mice that ubiquitously express a transgenically encoded EGFP fused to Histone H2B (Histone-GFP), allowing us to detect the cell nuclei of all cell types. Monitoring the nucleus count enables us to determine cell loss or proliferation, whereas a change in layer thickness or mean distances between nuclei indicates a shift in local cortical volume. Additionally, we exploit characteristic nucleus features of different cortical cell types such as nuclear shape, size and histone-distribution in order to classify single nuclei as belonging either to a neuron, glia or vasculature and therewith quantify the abundance of these cell types. Here, we show first results that indicate the feasibility of in vivo imaging of Histone-GFP to assess cellular composition and volume changes in the mouse cortex.

Methods:

After implantation of a chronic cranial window, cortical volumes of anesthetized mice are scanned with 2PLSM. Different reporter mouse lines expressing Histone-GFP and a red fluorescent reporter protein in either neuronal, endothelial, astrocytic, oligodendrocytic or microglial cells were used to identify nuclei of the distinct cell types. Identified nuclei were segmented manually and measurements describing shape, volume and histone signal distribution were used to train an automated classifier in the Classifier Learner App implemented in MATLAB. Different programs and algorithms were tested on our data for automated image segmentation.

Results:

Cortical volumes of ~ 2 mm³ with detected signal up to a depth of 750 µm can be scanned repetitively, showing nuclei of all cell populations in the cortex. Nuclei of neurons, astrocytes, oligodendrocytes and endothelial cells were identified in the reporter mouse lines and analyzed manually. A trained classifier predicted the correct nucleus class with an accuracy of 92,7%. A Convolutional Neuronal Network which was trained with ground truth labels to automatically segment our data showed most promising results compared to other available tools for automated segmentation.

Conclusion:

Our results suggest the high potential of in vivo 2PLSM of Histone-GFP to find cellular correlates for GMV changes and their dynamics associated with different diseases in longitudinal mouse studies.

Coating of fluorescent PLGA-Dil nanoparticles with poloxamer 188 leads to enhanced duration and intensity of the fluorescence signal in rat retinal endothelium

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Background

As shown previously, coating of the poly (lactic-co-glycolic acid) nanoparticles (PLGA NPs) with poloxamer 188 enables their penetration across the blood-brain barrier (BBB) upon intravenous administration. In this experiment, the unique in vivo Confocal Neuroimaging (ICON) technique was utilized for real time monitoring of the biodistribution of the fluorescent PLGA NPs in the rat retina.

Methods

For visualization in vivo, the poly (lactic-co-glycolic acid) nanoparticles (PLGA NPs) were labeled with the fluorescent dye 1,1'-dioctadecyl-3,3,3'- tetramethylindocarbocyanine perchlorate (Dil). The PLGA-Dil NPs were prepared by a high-pressure emulsification-solvent evaporation method and had an average diameter of 140 ± 1.5 nm, PDI 0.175 ± 0.002 , and a zeta-potential of -13.2 ± 0.4 mV. For coating the nanoparticles were incubated in a 1% aqueous solution of poloxamer 188 for 30 minutes immediately before the injection. Real time monitoring of the biodistribution of the fluorescent NPs in the rat retina was performed using ICON technique. The Lister-Hood male rats were anaesthetised, and the PLGA-Dil NPs uncoated and coated with poloxamer 188 were injected intravenously into the tail vein. The animals were fixed on the microscope stage and the retina was imaged via the eye using the confocal laser scanning microscope (LSM 5 Pascal, Carl Zeiss Jena) before and after injection of the NPs. The fluorescence intensity was quantified with software Image J (<http://imagej.net/Fiji>).

Results

The retina is a part of the CNS and can be used as a BBB model. The ICON results showed that the fluorescence signal of the uncoated PLGA-Dil NPs was detectable as a fluorescent lining of the blood vessels in the retina from 1 min to 120 min post injection. At the late time point the fluorescent lining of the vessels was weak but still clearly visible. In the case of the poloxamer-coated PLGA-Dil NPs, a similar temporal/spatial pattern of the fluorescent signal was observed; however, the signal intensity was 2.5-fold higher as compared to the uncoated PLGA NPs, which could be an indication of their more effective uptake into the endothelial cells.

Conclusion

The results of the real-time microscopical study using the ICON technique demonstrated that coating with poloxamer 188 enhances the interaction of the PLGA-Dil NPs with the endothelial cells of the retinal blood vessels, which is in agreement with the previous observations and it might provide a vehicle that makes longer lasting drug release possible.

Data organization made easy: Safe and efficient data management for neuroscience

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Management of scientific data, including consistent organization, annotation and storage of data, is a challenging task. Accessing and managing data from multiple workplaces while keeping it in sync, backed up, and easily accessible from within or outside the lab is even more demanding. To minimize the time and effort scientists have to spend on these tasks, we here present the GIN (G-Node Infrastructure) services [1], a free data management system designed for comprehensive and reproducible management of scientific data. It keeps track of changes to the contents and organization of the files and provides secure remote access to the data. More specifically, once a directory has been put under GIN control, the contents will be synced to a dedicated GIN server. With proper authorization, data can be accessed and changed from remote clients, making it easy to work from multiple workplaces while keeping all data at hand and in sync. Data can be managed from web and file browsers as well as through a command line interface, which enables integrating data management and access into the data acquisition and analysis procedures. The system handles any kinds of directory structures and file types, and tracks all changes, using Git [2] tracking mechanisms. This supports reproducible data workflows and in particular keeps previous versions accessible when datasets are updated. The service furthermore makes it straightforward to share any data within a lab or with off-site collaborators and to work on it in parallel.

A special feature of the GIN services is support for the NIX (Neuroscience information exchange) data format [3]. This file format is specifically designed to store recorded data, analysis results and annotations (metadata) in a single file, supporting the concept that all information about an experiment is kept in one place. The contents of NIX files managed by a GIN repository are indexed, providing fast search for specific datasets or data analysis.

NIX files can be created and accessed by a variety of programming languages, including Python [4], Matlab [5] and Java [6], and through the Neo package [7] to specifically support working with electrophysiological data.

Comprehensive organization and reproducible management of scientific data is challenging, in particular with complex experiments and heterogeneous data. The GIN services in combination with the NIX format enable to streamline the lab data workflows and reduce the efforts involved in sharing data with collaborators or the community.

Support

Supported by BMBF (Grant 01GQ1302)

References

[1] <https://gin.g-node.org/>

[2] <https://git-scm.com/>

[3] <https://github.com/G-Node/nix>

[4] <https://github.com/G-Node/nixpy>

[5] <https://github.com/G-Node/nix-mx>

[6] <https://github.com/G-Node/nix-java>

[7] <https://github.com/G-Node/python-neo-nixio>

Evaluation of brain pharmacokinetic properties in awake animals: from rodents to non-human primates

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Of the clinical candidates that enter phase 1, approximately one out of ten is approved by FDA. This low success rate is very concerning for drug developers, regulators and patients. Unacceptable pharmacokinetic properties is in part the reason for discontinuation of clinical development. An additional hurdle comes in play for neurological and psychiatric indications: the blood brain barrier. Clinical candidates need to be selected on the property to reach the target, and therefore, adequate free brain concentrations need to be reached that can induce the desired pharmacodynamic effects. This can be directly measured in awake animals by using Metaquant microdialysis in various species.

In the present study, we compared the absolute brain concentrations as well as CSF concentrations of dextroamphetamine and its pharmacodynamics effect in rats and non-humans primates (NHPs).

Wistar rats and cynomolgus monkeys received dextroamphetamine. The concentration of dextroamphetamine was measured bilaterally in the prefrontal cortex (PFC) and striatum (Str) up to 6 hours post dosing. A separate group of animals receiving the same dose had an indwelling catheter surgically placed into the cisterna magna to support cerebrospinal fluid (CSF) sampling for up to 4 hours post-dosing. Compound concentrations in plasma were measured via venous sampling.

In monkeys, ECF and CSF dextroamphetamine concentrations were similar to each other and to unbound plasma drug concentrations across the time course and brain regions, supporting rapid equilibration of compound throughout the monkey CNS. In rats, ECF and CSF concentrations were similar, but appeared to be 1.5 to 2 times greater than plasma unbound concentrations over the sample time course. Equilibration was also achieved by the first sample time point (30 minutes). These results suggest facile movement of dextroamphetamine across the blood-brain barrier of both species, possibly also the choroid plexus, and rapid equilibration within the CNS. Observation of a net uptake of dextroamphetamine into rat CNS, but not monkeys, may indicate an important species difference in the absorption of this drug into the brain.

This study confirms that pharmacokinetic and pharmacodynamics properties may differ between species. Furthermore, it indicates that cross species evaluation is valuable to select clinical candidates and their predictive efficacy dose.

Efficient isolation of viable primary neural cells from adult murine brain tissue based on a novel automated tissue dissociation protocol

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Tissue dissociation and preparation of single-cell suspensions with high cell viability and a minimum of cell debris are prerequisites for reliable cellular analysis, cell culture, and cell separation. As dissociation of adult brain requires sophisticated mechanical and enzymatic treatment to successfully disaggregate the tightly connected neural cells, cell analysis is often restricted to embryonic or neonatal rodent tissue. We have set up technologies for dissociation of neonatal brain by combining automated mechanical dissociation using the gentleMACS™ Octo Dissociator with an optimized enzymatic treatment. To extend the analyses to adult neural cells we have further optimized the method by including a novel protocol for removal of debris and erythrocytes, which is crucial for effective cell isolation and culture.

The standardized process allows fast and reproducible dissociation of adult murine brain tissue and was optimized to increase the number of viable cells. Protocols for the magnetic isolation (MACS® Technology) of astrocytes, oligodendrocytes, neurons, microglia, and endothelial cells to high purities were also established and cultivation conditions were optimized to successfully cultivate adult neural cell populations. Furthermore, highly purified astrocytes were subjected to single-cell mRNA sequencing analysis in order to characterize neonatal and adult astrocyte diversity. In summary, we present a novel standardized technology to generate highly purified and viable adult neural cells that extends the analysis from neonatal to adult murine brain tissue and facilitates sophisticated cellular and molecular analyses.

Fast Imaging And Probabilistic Reconstruction of Light-Evoked Activity in the Mouse Retina

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Two-photon imaging, in concert with calcium and glutamate sensitive fluorescent indicators, allows us to record the activity of neural structures in the mouse retina, ranging from large ganglion cell populations to fine neurites such as bipolar cell axon terminals or ganglion cell dendrites, to a relatively high spatiotemporal precision. This approach has been used with considerable success to characterize the functional complexity of the mouse retina [1, 2]. However, scanning speeds are typically slow and limited to one focal plane. In addition, two-photon measurements show complex spatiotemporal interdependencies induced by measurement noise, the kinetics of the fluorescent indicators, and the intrinsic variability of the system itself. Each of these makes it more difficult to robustly infer the “true” neural activity.

Here, we address these challenges using a combination of innovative sampling strategies, Bayesian non-parametric regression and bootstrap statistical estimation, comprising a complete pipeline incorporating data acquisition, processing and analysis. We use radial scan configurations to image patches of light-evoked activity in the mouse retina, which can sample fields at 30Hz with a comparable spatial resolution to a 7.5Hz linear scan. These configurations permit fast sampling trajectories on conventional galvanometric mirror systems with minimal mechanical error. In contrast to cases in the literature (e.g. [3]), we do not seek to sparsely sample from cell bodies in the recording field, but rather to densely sample a uniform lattice and reconstruct the whole imaging plane.

The sample lattice is projected onto an isotropic latent space through a non-linear mapping [4] to which a Gaussian random field is fitted, providing a probabilistic approximation of the underlying activity. Importantly, this procedure does not assume binning of activity measurements in time, as is conventionally done in many imaging systems. The fitted distribution can be sampled from to bootstrap inference of statistical properties such as confidence intervals [5]. This is particularly valuable for estimates of properties which are sensitive to noise, such as partial correlation matrices with many variables of interest, or which are time-dependent. We are currently extending our method to allow scanning of multiple imaging planes simultaneously, allowing dense recordings from e.g. the entire axonal terminal system of a bipolar cell.

References

- [1] Baden, T., Berens, P., Franke, K., Román Rosón, M., Bethge, M., & Euler, T. (2016). The Functional Diversity Of Retinal Ganglion Cells In The Mouse. *Nature*, 529(7586), 345–50.
- [2] Euler, T., Haverkamp, S., Schubert, T., & Baden, T. (2014). Retinal Bipolar Cells: Elementary Building

Blocks of Vision. *Nature Reviews Neuroscience*, 15(8), 507–519.

[3] Kummer, M., Kirmse, K., Zhang, C., Haueisen, J., Witte, O. W., & Holthoff, K. (2016). Column-like Ca^{2+} Clusters in the Mouse Neonatal Neocortex Revealed by Three-Dimensional Two-Photon Ca^{2+} Imaging In Vivo. *NeuroImage*, 138, 64–75

[4] Sampson, P., & Guttorp, P. (1992). Nonparametric Estimation of Nonstationary Spatial Covariance Structure. *Journal of the American Statistical Association*, 87(417), 108–119.

[5] Kirk, P. D. W., & Stumpf, M. P. H. (2009). Gaussian Process Regression Bootstrapping: Exploring the Effects of Uncertainty in Time Course Data. *Bioinformatics*, 25(10), 1300–1306.

Functional characterization of new, flexible multi-contact silicon probes for chronic intra-cortical recording and stimulation

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Here we describe a new multi-contact silicon probe design, its fabrication process and results from in vitro testing and in vivo applications. The new design allows for chronic implantation of a floating probe with minimized mechanical coupling to the skull. It consists of a 60 mm long and 10 μm thick highly flexible polyimide based ribbon cable including 16 electrode sites attached to a few mm short silicon shaft on wafer level. The final electrode was 30 μm thick, and at its upper end 130 μm wide. The ribbon cable kept this width until 3 mm after the electrodes end where its width and the width of the conducting paths increased. The connector region at the end of the cable and the conducting paths was stabilized by an underlying silicon rectangle on which the conducting paths terminated in connector pads. Layouts with different spacing of individual electrodes including one tetrode configuration and different shaft lengths grant optimal electrode arrangement for a plethora of applications. To increase the signal-to-noise ratio and enable long-term electrical stimulation, the gold electrodes were coated with the electrical conductive polymer PEDOT:PSS using a galvanostatic electropolymerization process. In vitro electrical impedance spectroscopy measurements approved the functionality of these micro electrodes and confirmed a reduction in electrical impedance due to the use of a PEDOT:PSS coating. Long-term in vitro tests of PEDOT coated electrodes approved long-term stability of these electrodes for more than 7 weeks of continuous electrical stimulation with more than 4.2 billion bipolar current pulses in total. Stimulated and unstimulated electrodes showed no signs of degradation after more than 10 months of immersion in phosphate buffered saline solution at 37C.

Chronic implantation into rat primary motor and primary visual cortex and subsequent electrophysiological measurements yielded successful recordings of single- and multi-unit activity along with the local field potentials for more than 12 weeks. In vivo electrical impedance spectroscopy supported the results of the in vitro functional tests. After an initial increase of electrical impedance in the course of the first week after implantation the average impedance of the electrodes settled at about 1 M Ω for the following weeks. Initial tests of delivering single bipolar current pulses through the electrodes show that the electrodes are stable for charge densities of up to 2.5mC/cm².

This research was supported by funding from the DFG (SPP 1665).

Impact of the insertion speed of the recording probe on the quality of neural recordings in acute experiments

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The application of electrophysiological recording techniques led to numerous major discoveries in the field of neuroscience. A large fraction of these discoveries has been made by investigating the extracellularly recorded spiking activity of multiple single neurons. Thanks to the fast development of multichannel electrophysiological devices, we are now able to record the activity of tens to hundreds of neurons simultaneously. Although the main reason behind the growing number of simultaneously recorded neurons is the continuous increase in the number of contact sites found on recording probes, other factors might significantly contribute to it as well. The surgical techniques and implantation procedure used, the methods and algorithms applied during spike sorting are just a few examples which may influence the final number of single units available for further examinations. In this pilot study, we investigated whether the insertion speed of the recording probe has an impact on the quality of the recorded neural activity. Four different speeds were used during the experiments (0.002 mm/s, 0.02 mm/s, 0.1 mm/s, 1mm/s). A 128-channel passive silicon probe with closely spaced contact sites has been inserted multiple times into the infragranular layers of the primary somatosensory cortex of ketamine-xylazine anesthetized rats ($n = 3$) with the aid of a motorized stereotaxic micromanipulator. Each penetration has been carried out with a different speed chosen quasi-randomly from the four speed values. Two penetrations have been made in each hemisphere, and the recording probe was cleaned before the third penetration. The dura mater was carefully removed over each of the implantation sites. After each implantation, wideband brain electrical activity (0.1-7500 Hz) was recorded at 20 kHz sampling rate and with 16-bit resolution for 45 minutes. The recording started right after the recording probe reached the appropriate cortical depth. To assess the recording quality and to compare it between different speeds, we estimated the number of separable single units using spike sorting. Another indicators of data quality were the signal-to-noise ratio (SNR) and the peak-to-peak amplitude of mean action potential waveforms of sorted single units. We also examined the rate of electrode drift by evaluating the spatiotemporal change of action potential waveforms along the contact sites of the recording probe. Our results show that by inserting the recording probe with slower speeds, both the number of separable single units and the SNR tend to be higher compared to faster implantation speeds. The number of separable single units did not change significantly during the 45 minutes. However, the average SNR improved over time when faster implantation speeds were used. In contrast, the SNR measured at the slowest speed decreased slightly over time, but was higher in most cases even after this decrease than the SNR level calculated at faster implantation speeds. Our preliminary findings suggest that using slower implantation speeds ($\sim 1 \mu\text{m/s}$) during acute experiments might result in neural recordings of higher quality, especially in the frequency range of spiking activity (500-5000 Hz). This research was supported by the Hungarian Brain Research Program (Grant No. KTIA_13_NAP-A-IV/1-4,6) and the EU FP7 Grant No. 600925 (NeuroSeeker).

KCC2 dependent steady state chloride levels in mouse layer 2/3 cortical neurons *in vivo*.

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Neuronal intracellular Cl⁻ is a key factor governing a wide range of processes such as neuronal inhibition, resting membrane potential dynamics, intracellular pH or cell volume. Currently, there is no fully validated *in vivo* study of neuronal intracellular steady state Cl⁻ levels or the factors affecting it. Thus, in this work we implemented the genetically encoded ratiometric Cl⁻ indicator Superclomeleon (SCLM) to produce *in vivo* optical estimations of steady state intracellular Cl⁻ levels from layer 2/3 cortex neurons in adult mice using 2-photon microscopy. Due to the known pH sensitivity of SCLM we implemented Superecliptic pHluorin as a ratiometric sensor to gain insight into the intracellular steady state pH of layer 2/3 cortex mouse neurons *in vivo*. To study the factors affecting neuronal steady state intracellular Cl⁻ *in vivo*, we deleted the cation-Cl⁻ co-transporter KCC2 in single identified layer 2/3 cortex neurons finding a significant change in the intracellular neuronal steady state Cl⁻ level. Altogether, this report represents to the best of our knowledge the first *in vivo* evidence of the relevance of KCC2 for producing low intracellular Cl⁻ levels in adult mouse neurons. Additionally, this is the first attempt to generate validated *in vivo* ratiometric optical estimations of mammalian neuronal steady state intracellular Cl⁻ levels and pH, providing clarifying insights into the strengths and caveats of this approach which will aid to pave the way towards establishing routine *in vivo* optical recordings of intracellular Cl⁻ or pH.

CRISPR-Cas9 Lipid Nanoparticles as an Efficient Delivery Tool in Primary Neural Cultures_Proof of Concept

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Advances in the gene editing arena, specifically with CRISPR-Cas9, has pushed the demand for efficiently delivering payloads even further. Of the tools available, developments in the field of lipid nanoparticles (LNPs) has allowed for the reliable and efficient delivery of CRISPR components, both in research and clinical settings. Here, we bridge that gap by describing the development of an LNP delivery system for CRISPR components, robustly manufactured with clinical-grade materials using microfluidic technology at scales for screening applications, in vitro experiments and research in animals. We describe the use of lipid-based nanoparticles for highly efficient encapsulation and delivery of payloads, such as siRNA, mRNA and plasmid. In this proof of concept, we show that representative small RNAs, mRNAs and plasmids can be successfully delivered to primary neurons. LNPs manufactured to encapsulate various nucleic acids can do so with high efficiency, encapsulating more than 95% of the payload, minimizing payload loss. Transfection efficiency of the LNPs is >95%, quantified using a fluorescent dye. The biological endpoint assays used to determine the accessibility of the payloads delivered varies for siRNA, mRNA and plasmid. Using doses of 1g per mL of media, we achieved >90% knockdown with siRNA delivery, >90% of the primary neurons are GFP+ with GFP mRNA delivery and >60% of the primary neurons are GFP+ with GFP plasmid delivery. The LNPs are well tolerated, such that 5x the required doses have no observable cytotoxicity. We show that the LNPs can also be used to deliver payloads into various regions of the animal brain. The localized injections into the cortex and the striatum are well tolerated and have extensive distribution. These validation studies provide suitable insights in establishing strategies for efficiently delivering CRISPR components into primary cultures and into the animal. The use of LNPs can be extrapolated to CRISPR components with a simple change in payload.

Manipulation of Neurons with Precisely Controlled Illumination in Space and Time Using Two-Photon Lasers and Spatial Light Modulators

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Manipulation of cells with light has become a powerful and widely used tool in neuroscience and other areas of research. For this approach to reach its full potential, two conditions need to be met: the precise spatial and temporal control of light delivery inside the sample, and the availability of light-controllable molecules that can modify crucial cellular and neuronal functions. We present a novel holographic illumination system that can be coupled to existing microscopes of various brands (including two-photon microscopes) which provides high resolution control for photo-manipulation experiments. The system is optimized for two-photon excitation using femto-second lasers to improve axial confinement of the excitation patterns as well as tissue penetration. Four dimensional light patterns (x,y,z,t) can be defined by the user based on fluorescence images of the sample. It should be noted that the system can also be used for single-photon excitation using continuous lasers. In this case, however, axial confinement depends primarily on the numerical aperture of the objective in use. In addition to a technical characterization of the system we present biological applications of the illumination module using various types of light-sensitive molecules, including caged compounds and newly developed photolabile groups introduced into DNA strands, photo-switchable ligands of neuronal receptors and molecules used in optogenetics.

Low cost open source hardware and software in behavioral and electrophysiological experiments: Arduino & Raspberry Pi

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Behavioral science can be accelerated greatly by the use of digitally controlled devices capable of measuring relevant parameters and, if necessary, interact with the experiment. Those machines can be bought and they can do things in an implemented way. However, they are neither cheap nor as adaptive as they should be. The focus of this poster is to demonstrate how to overcome the rather small barriers in order to develop their own machines. This process teaches electronics and programming in an iterative and playful way. Recently, open hardware projects like the Arduino or the Raspberry Pi draw enormous attention and their communities are full of helpful enthusiasts sharing code and hardware plans. Coding and building elements can be fused together to fit the experimenters needs. We as scientists can benefit from large numbers of projects that are documented for beginners, mostly by tutorial videos including component lists and the necessary code. Here we present a number of simple suggestions how to build yourself helpful devices for your behavioral and neurophysiological experiments.

Multi-scale detection of rate changes in spike trains with weak dependencies

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Dynamic changes in the firing rates of neuronal discharges can be important signals for information processing as well as problematic for statistical analyses that are sensitive to rate changes. Therefore, we propose a multiple filter test (MFT) that tests the null hypothesis of constant rate and an algorithm that estimates the change points in the rate. Most importantly, our method incorporates practically relevant issues: (1) an unknown number of rate changes can occur (2) on multiple time scales, (3) other process parameters such as the variance of inter spike intervals are unknown, and (4) processes can show a high variety of patterns and distributions, including also serial dependencies.

Our MFT uses a filtered derivative process with multiple filters that converges weakly to a parameter free limit process that can be used to obtain the rejection threshold for the test. By specifically estimating serial dependencies in the test statistic, we show that the MFT can be applied to a variety of empirical firing patterns, including positive and negative serial correlations as well as tonic and bursty firing. We apply the newly proposed method to an empirical data set of spike trains with serial correlations and use simulations to show improved performance against methods that assume independence. For positive correlations, our new MFT is necessary to reduce the number of false positives, which can be highly enhanced when falsely assuming independence. For the frequent case of negative correlations, the new MFT shows an improved detection probability of change points and thus, a higher potential of signal extraction from noisy spike trains.

Supported by the German Federal Ministry of Education and Research (BMBF, Funding number: 01ZX1404B) and by the Priority Program 1665 of the German Research Foundation.

odML-tables

Providing a graphical interface for odML based metadata management

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Experimental observations are an essential part of scientific research and are used to validate or reject a scientific hypothesis. In the scientific approach, observations are typically quantified and data are recorded for subsequent analysis. These primary data are always accompanied by information about their origin and the circumstances of their recording. Such information is typically called metadata and includes a variety of information, such as, the date of the recording, a seemingly unimportant change in measurement settings or the expectation of the experimenter (open trial vs. blind trial experiments). Metadata are crucial for performing reproducible data analysis and are essential for the interpretation of the results. They also enable queries to answer scientific questions that researchers did not previously consider (e.g. transversal studies) and are one of the main components for implementing replicable and reproducible research [1]. In addition a comprehensive metadata collection facilitates the communication between members of a project and therefore saves valuable time and effort. In neuroscience, and in particular experimental neurophysiology, the development of approaches to metadata management is an ongoing effort [2]. A promising metadata framework in this field is odML (open metadata Markup Language) [3]. This XML-based language is designed to represent complex metadata collections as hierarchically organized key-value pairs.

In practice however, embedding metadata based on the odML framework into workflows for sharing data in concrete use cases of experimental and theoretical groups revealed that generating the structure of an odML document, and later filling it with metadata from the respective sources, involved extensive programming experience [2]. In addition there are always metadata, which require manual entry during or after the experiment. The lack of software support for certain processing steps and editing capabilities in these use cases effectively prevented our experimental partners from using odML to capture metadata into one coherent collection. To address this shortcoming, we developed odML-tables, a software solution that bridges the gap between hierarchical odML and a tabular representation of metadata that is convenient for editing.

odML-tables is an open-source software tool implemented in Python, which offers a graphical user interface (GUI) [4,5]. The main features of odML-tables are:

- Generation of a template (tabular) structure facilitating the initial design of an odML structure
- Conversion between odML files and tabular formats (.xls, .csv) in order to enable manual entry and modifications using spreadsheet software (e.g., Microsoft Excel, LibreOffice)
- Filtering metadata by defined search criteria to simplify access to parts of a complex odML structure

- Merging of multiple odML files
- Generation of a comparison table of similar entries within an odML file

We show how odML-tables can be applied in a sustainable workflow for metadata management and illustrate the practical usage of odML-tables from structuring available metadata to daily enrichment of the metadata collection (cf. also [1,2]).

Acknowledgements: Helmholtz portfolio theme SMHB, EU Grant 720270 (HBP), DFG Priority Program SPP 1665 (Grants DE 2175/2-1 and GR 1753/4-2).

References:

1. Denker, M., & Grün, S. (2016). Designing workflows for the reproducible Analysis of Electrophysiological Data. In: Brain Inspired Computing, eds: Katrin Amunts, Lucio Grandinetti, Thomas Lippert, Nicolai Petkov. *Lecture Notes in Computer Science*, Springer. (in press)
2. Zehl, L., Jaillet, F., Stoewer, A., Grewe, J., Sobolev, A., Wachtler, T., Brochier, T., Riehle, A., Denker, M., & Grün, S. Handling Metadata in a Neurophysiology Laboratory. *Frontiers in Neuroinformatics*, 10, 26.
3. Grewe, J., Wachtler, T., & Benda, J. (2011). A Bottom-up Approach to Data Annotation in Neurophysiology. *Frontiers in Neuroinformatics*, 5, 16.
4. python-odmltables on PyPi: <https://pypi.python.org/pypi/python-odmltables/>
5. python-odmltables on GitHub: <https://github.com/INM-6/python-odmltables>

Optical activation of neurons through two-photon excitation of gold nanoparticles

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Optically controlling neuronal activity is a widely used and powerful method in neuroscience. However, currently used tools still suffer from limitations and drawbacks that need to be overcome in order to achieve non-invasive, non-toxic, and readily usable methods. Recently, it has been shown that neuronal cultures can be activated through optical activation of gold nanoparticles (Au NPs) with visible light (Carvalho-de-Souza *et al.*, 2015: Photosensitivity of Neurons Enabled by Cell-Targeted Gold Nanoparticles. Neuron). In this case, the generation of plasmons – a light-interaction phenomenon in metal NPs allowing for channeling of the absorbed excitation energy – causes the neuron to be photothermally stimulated indirectly through the NPs. However, for practical purposes using light in the visible regime limits the use of this technique in life tissue, as the penetration depth of these wavelengths is rather small. In contrast, using fs-pulsed near infrared lasers employed in two-photon microscopy allows for deep tissue excitation. Here we demonstrate highly effective, repeatable, and relatively easily implementable evoking of cell activity by two-photon excitation of Au NPs in acute brain slices and *in vivo*.

First, we established whole-cell patch-clamp recordings of layer 5 cortical neurons in acute brain slices. NPs were immobilized by adhering them onto the neuronal membrane. This was achieved by tethering streptavidin-functionalized NPs onto the membrane through application of concanavalin A (conA)-biotin complex or incubation with a NHS-biotin linker. Using a fs-pulsed Ti:Sapphire laser at 1040 nm (5-10 mW on sample) and spiral-shaped scanning-patterns, we were able to reliably achieve action potential firing upon stimulation.

We next proceeded to experiments in the visual cortex of anesthetized mice. Prior to the stimulation procedure, the conA-biotin complex was injected in layer 2 of the visual cortex. Subsequently, a pipette with NPs was inserted in the vicinity of the injection site and NPs were applied. The neuron somata were visualized as shadows through the fluorescent signal of Alexa Fluor 488, enabling the subsequent establishing of targeted loose-seal patch-clamp recordings. Using 40 mW excitation power on sample, we were able to evoke responses, either in the form of short bursts of APs directly following the stimulation or increasing the overall intrinsic firing rate.

Lastly, the NP stimulation experiment was performed on transgenic *Hydra vulgaris* expressing the calcium sensor GCaMP6s in epithelial muscle cells. Here, the NPs were internalized into the hydra by overnight incubation and gastric injection with PEG-coated NPs with a fluorescent tag. Using synchronous two-photon imaging and stimulation, we were able to record an increase in GCaMP fluorescence only when stimulating those cells on which we could localize NPs.

In conclusion, this study demonstrates that NPs are an attractive alternative to currently available optical techniques, especially due to their low toxicity level, photo-stability, and high excitation efficiency. Moreover, they enable great flexibility in their applicability, as all their fundamental properties (i.e. NP

size, shape, and configuration) can be altered to adjust their plasmon resonance, and their surface biochemically modified enabling implementation in or tethering to virtually any type of biological tissue. In this way, they can be custom designed to fit a desired experimental goal.

Quantitative detection of intracellular sodium using FLIM with CoroNa-Green

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Recent work has established that brain activity is accompanied by changes in intracellular Na⁺-concentrations in astrocytes and neurons. These changes are especially prominent in fine processes such as dendrites and spines of excitatory neurons or in perisynaptic processes of astrocytes. While conventional ratiometric and two-photon imaging allows reliable determination of baseline Na⁺ in somata and of absolute Na⁺ changes in processes, it cannot be employed for quantification of baseline Na⁺ in cellular microdomains. Information on this parameter, is, however, required to fully understand Na⁺ homeostasis and signalling. Fluorescence Lifetime Imaging Microscopy (FLIM) enables measurement of ion concentrations independent from fluorescence intensity. In the present study, we tested if the sodium indicator dye CoroNa-Green can be employed for FLIM in primary cell culture and in acute tissue slices of the mouse brain.

The suitability of CoroNa-Green for FLIM was first studied in tsA201 cells (a modified HEK cell line). For calibration, cells were loaded with the membrane-permeable form of the dye and exposed to salines containing different concentrations of Na⁺ (0 – 100 mM). In addition, a cocktail of 3 µM gramicidin (Na⁺ ionophore), 10 µM monensin (Na⁺/H⁺ carrier) and ouabain (Na⁺/K⁺-ATPase blocker) was added to promote rapid exchange and equilibration of intra- and extracellular Na⁺. These experiments revealed that fluorescence lifetime of CoroNa-Green increased with increasing Na⁺ concentrations. Next, CoroNa-Green was loaded into cultured hippocampal neurons and astrocytes and the response to glutamate application was studied. Application of 60 µM glutamate for 1 min caused a transient increase in fluorescence lifetime on CoroNa-Green in both, neurons and astrocytes, indicating an increase in intracellular Na⁺, as described before using conventional, intensity-based imaging. Finally, the same experiment was repeated in acute hippocampal brain slices, where glutamate (1 mM, 1 min) was applied by bath perfusion. Again, a prolongation in fluorescence lifetime in hippocampal CA1 pyramidal neurons was observed, indicative of a glutamate-induced sodium increase, and thereby confirming earlier observations obtained with widefield and two-photon imaging.

Taken together, our study shows that CoroNa-Green is suitable for determination of intracellular Na⁺-concentrations both in cultured cells as well as in situ using FLIM.

Supported by the DFG (Ro2327/8-1) and by BioSoft (International Helmholtz Research School, FZ Jülich).

Fast and accurate spike sorting in vitro and in vivo for up to thousands of electrodes

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Understanding how assemblies of neurons encode information requires recording large populations of cells in the brain. In recent years, multi-electrode arrays and large silicon probes have been developed to record simultaneously from hundreds or thousands of electrodes packed with a high density. However, these new devices challenge the classical way to do spike sorting. Here we developed a new highly automated algorithm to extract spikes from extracellular data, and show that this algorithm reached near optimal performance both in vitro and in vivo. The algorithm is composed of two main steps: 1) a "template-finding" phase to extract the cell templates, i.e. the pattern of activity evoked over many electrodes by the spikes of one neuron ; 2) a "template-matching" phase where the templates were matched to the raw data to find the spike times. The time spent on manual curation did not scale with the number of electrodes. We tested our algorithm with large-scale data from in vitro and in vivo recordings, up to 4225 electrodes, and performed simultaneous extracellular and patch recordings to obtain "ground truth" data, where the solution to the sorting problem is at least partially known. The performance of our algorithm was always close to the best expected performance. We thus provide a general solution to sort spikes from large-scale extracellular recordings.

Replication of Riehle et al (1997) by an Open Source Implementation of the Unitary Events Analysis Method

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The Unitary Events (UE) analysis [1,2] is a statistical method to capture the dynamics of correlation between neuronal activities and to extract significant excess spike synchrony that is beyond chance level given the firing rates. This method has been progressively improved after the first publication to account for typical features of electrophysiological data, such as non-stationary firing rates (in time, across trials) and deviation from Poisson [3], which could cause false positives if not accounted for in the statistical test.

We recently re-implemented the UE method in Python. This implementation is now available in Elephant [4], an open source electrophysiology analysis toolbox which contains various methods for analyzing electrophysiological recordings such as spike trains and LFP.

For validation of our implementation, we decided to – besides having run successfully numerous unit tests that are required for the integration into Elephant – redo the UE analysis results that were published in [5]. This was also an interesting further test if we are able to replicate the results, even if the original raw data, used software tools, documentation, and metadata were not available.

Our goal was to replicate the results of [5], in particular Figures 2 and 4A, because they contained the main findings. The analysis programs used in [5] were not available, nor the documentation of details of the analysis. From one of the coauthors of [5] (SG) we knew that Fig. 2 was analyzed by a UE implementation in Matlab, and we also had some version of the data available. Some analysis parameters (such as bin width, sliding window width, trial type, etc) were provided in [5]. Thus we created the same type of figure as Fig. 2 in [5] using our Python implementation and compared our result to the original one visually in terms of exact positions of spikes, UEs, etc. This first analysis of the same data did not lead to completely identical results although their overall behavior was similar. However, when we changed the trial alignment to a particular task event (RS instead of PS; not mentioned in [5]) the results became identical. The discrepancy arose from the detail that the time difference between experimental events was not always exactly identical in all trials because of hardware features.

For Fig. 4A we did not know which data were used since [5] did not provide this information. After contacting the first author of [5] (AR) we acquired this information and also learned that Fig. 4A was not produced using Matlab but with IDL. This brought up new challenges that we were able to overcome, i.e. to reproduce Fig. 4A, only after further communication with MD, who was another coauthor of [5] and wrote at that time a detailed private report on a comparison of the original implementations in IDL and Matlab.

In summary, we provide an new implementation of the UE method in Python, with which we were able to replicate the main results of [5]. The UE method involves a number of numerical computations and is very sensitive. Therefore this replication provides a strong indication that our Python implementation faithfully implements the UE method. The fact that such a replication is not trivial calls for tools that support reproducibility of data analysis.

[1] Grün et al. (1999) Journal of Neuroscience Methods

[2] Grün et al. (2002a,b) Neural Computation

- [3] Grün (2009) Journal of neurophysiology
- [4] github.com/NeuroEnselble/Elephant/
- [5] Riehle et al. (1997) Science

Robust threshold estimation based on without near threshold measurements

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We present a novel robust and precise method for universal estimation of physiological or behavioral thresholds using examples of hearing threshold estimation based on auditory brainstem responses (ABR), neuronal recordings in the auditory and somatosensory cortex and pre-pulse inhibition (PPI) of the acoustic startle response (ASR). By definition, the threshold represents the smallest possible stimulus strength evoking a response significantly different from the non-stimulus condition. For threshold estimation from physiological or behavioral responses it is common practice to use stimulus intensities that are close to the putative threshold. Trivially, the signal-to-noise ratio (S/N) is worst near the threshold since the intensities of evoked responses are positively correlated with stimulus strength. In other words, thresholds are usually determined from measurements with low S/N, and threshold estimates consequently are prone to errors.

Virtually all psychometric and physiological stimulus-response functions show a sigmoid progression like the logistic function. Here we demonstrate that thresholds may be estimated without performing near threshold measurements if data are fitted to a generalized logistic function which is extended by an additive term representing the measured signal intensity during the non-stimulus condition. We demonstrate that the goodness of fit becomes best if the supporting points are located within the area of the logistic function with the highest gradients, i.e., the dynamic range. We show that threshold estimation based on noisy data is systematically biased: The fewer measurement repetitions are performed the more the threshold is overestimated. To become independent from the number of measurement repetitions and thus also from the absolute level of noise, subsampling with increasing sample size is performed. Subsequently the threshold as a function of sample size is extrapolated to the hypothetical case of an infinite sample size corresponding to the case of zero intensity noise thus allowing for the estimation of the unbiased ("true") threshold.

The method is validated using extensive numerical simulations with artificial test data as well as a broad variety of different experimental data. We conclude that our new approach may be applied to any physiological or behavioral data for threshold estimation.

The use of Click chemistry for quantitative analysis of protein palmitoylation

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S-palmitoylation attracts more and more attention of neuroscientists, since it is the most common and the only reversible posttranslational lipidation of neuronal proteins. The reversibility of this modification allows it to regulate function of proteins in a dynamic way by controlling their membrane binding, lipid raft localization, trafficking, stability and interactions with other proteins. Selection of the adequate S-palmitoylation analysis method is particularly important for quantification of S-acylation turnover dynamics. The conventional technique is the metabolic labeling with radioactive ³H- or ¹²⁵I- palmitate and subsequent detection by fluorography. Despite it is widespread, this approach is hazard and time-consuming. The last decade was marked by the real breakthrough in S-palmitoylation field due to the development of new methods, as acyl-biotin exchange (ABE) and metabolic labeling with a palmitic acid analog followed by click chemistry (MLCC). MLCC that is based on metabolic labeling with bioorthogonal palmitic acid analog, linked post-vivo to the specific tag by click chemistry, is an alternative to radiolabeling. Yet this method was used mainly for global qualitative profiling of S-palmitoylated proteins (palmitome), while quantitative palmitoylation analysis of single protein is usually assessed by radiolabeling. Here, we describe the optimization of methodology applicable for quantification of protein palmitoylation based on MLCC, focusing on palmitoylation of the neural cell adhesion molecule (NCAM) by its acyltransferase DHHC3 and the autopalmitoylation of DHHC3. Briefly, quantification is achieved by the enrichment of palmitoylated proteins, linked to biotin tag during MLCC, by streptavidin-sepharose beads, followed by detection of protein of interest (NCAM or DHHC3) using immunoblotting and normalization to total protein level recovered after MLCC. The procedure is relatively safe and fast, with a possibility to access dynamical changes. The key feature of the method is standardization of the palmitoylation assessment of the chosen protein. It increases the reproducibility of observed palmitoylation changes, minimizing the variations introduced by numerous steps of MLCC reaction and protein enrichment. The optimized protocol allows to detect palmitoylation of several proteins in parallel, one of which could serve as an additional internal control.

Tracer electrophoresis through the nerve sheath for neuroanatomical and functional labeling of neural pathways

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The delivery of fluorescent tracers and calcium indicators into specific neuronal populations can be difficult in non-standard model systems not currently amenable to genetic manipulation. Methods of localized electroporation and pressure-injection of cell-permeable dyes are of great use in these systems, though they are unable to target specific or functionally-related cell types easily. To label neurons in insects not amenable to genetic techniques we delivered electrophoretically polar tracers through the sheath covering the nervous system. In auditory nerves this method simultaneously stained peripheral sensory structures and central axonal projections, it labeled neuron populations through the brain's surface. The same method delivered calcium indicators into central neuropils for in vivo optical imaging of sound-evoked activity, demonstrating a substantial advance for neuroscience in non-model animals.

Transcranial functional ultrasound imaging in freely-moving awake mice and anesthetized young rats without contrast agent through the intact skull

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Ultrasensitive ultrasound Doppler imaging enables dynamic measure of cerebral blood volume with high-sensitivity and has recently led to the development of functional ultrasound (fUS) imaging (Macé et al, 2011). fUS imaging is a powerful tool for measuring brain activation and connectivity (Osmanski et al, 2014) with high spatiotemporal sampling (100 μ m, 1 ms), through the neurovascular coupling. It was recently demonstrated both in anesthetized and in awake and freely-moving rats, allowing to image the brain activity in near natural conditions, without anesthesia effects (Sieu et al, 2015). However, currently either skull surgery or contrast agents are required to overcome skull-induced attenuation of ultrasonic waves.

In this study, we investigated the use of fUS imaging for mice and young rats, both without contrast agents and through the intact skull. First, we show that ultrasensitive Doppler imaging of the entire brain is possible fully non-invasively through the intact skull and skin without any contrast agents both in anesthetized mice, regardless of age, and in anesthetized young rats up to postnatal day 35. Using a motorized probe, we also demonstrate non-invasive transcranial high-resolution 3D Ultrafast Doppler tomography in adult mice. Next, we demonstrate high-quality full-transcranial fUS imaging in awake and freely-moving mice in a minimally invasive setting. For this purpose we magnetically clipped a newly developed ultralight ultrasound probe to a small and flat metal frame that we chronically fixed to the mouse skull. An optimization of the ultrasonic sequence was required to avoid a previously not reported specific muscle-related artifact in awake rodents. This setup allowed us to establish full-depth high-resolution images of the awake mouse brain vasculature and also the detection of the activation of the barrel cortex during whisker stimulation as an illustrating example. These results pave the way for transcranial fUS studies without anesthesia bias as well as in behavioral contexts. It will also enable longitudinal studies of postnatal brain development in rodents, in either anesthetized or awake conditions.

Transcriptome and Neuropeptidome analysis of *Carausius morosus*

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In the past few years, an increasing number of studies focused on the identification of neuropeptides from insects. Neuropeptides represent one of the largest classes of signalling molecules in animals and are involved in a wide range of physiological and behavioural processes such as reproduction, regulation of energy balance, circadian rhythm and locomotion. One of the most thoroughly studied insect species, with respect to locomotion behaviour, is the Indian stick insect *Carausius morosus*. In the stick insect detailed information exists on premotor networks in controlling walking. However, little is known about neuroactive substances such as neuropeptides, which could be involved in the generation and modulation of motor activity. In this study, we investigated the transcriptome of the central nervous system and characterized the neuropeptidome of *C. morosus* as complete as possible. In total, we assembled and identified 52 neuropeptide and neuropeptide-like precursors in *C. morosus* using Trinity (v2.2.0), Bridger (v2014-12-01) and SOAPdenovo (v2.04-r240). Neuropeptide precursor sequences from other arthropods (e.g. Derst *et al.*, 2016) were used as a reference query. Single precursors were found for adipokinetic hormone-1, adipokinetic hormone-2, adipokinetic hormone corazonin-like-peptide, agatoxin-like peptide, allatostatins-A, allatostatin-C, allatostatin-CC, allatotropin, bursicon alpha, bursicon beta, diuretic-hormone-31, CAPA, crustacean cardioactive peptide, CCHamide-1, CCHamide-2, CGP, CNMamide, corazonin, corticotropin-releasing-factor-like-DH46, eclosion-hormone, elevenin, extended FMRFamides, glycoprotein hormone alpha, glycoprotein hormone beta, IDL, IMFamide, inotocin, ion transport peptide 1, ITG, long neuropeptide F-2, myoinhibitory peptides/allatostatins-B, myosuppressin, natalisins, neuroparsin, neuropeptide-like-precursor-1, NVP-like, pigment dispersing factor, proctolin, pyrokinins, RYamides, short neuropeptide F, SIFamide, tachykinin-related peptides, trissin and tryptopyrokinins. Furthermore, two precursors for longNPF-1, calcitonin, insulin-like-peptides, and orckinins were identified.

Using different mass spectrometric approaches such as LC-ESI-LTQ-Orbitrap MS and MALDI-TOF MS, we characterized in total 197 peptides from the precursor sequences. Of these sequences, 139 likely represent novel bioactive neuropeptides, 15 sequences confirmed already known neuropeptides and 43 additional sequences are considered to be precursor peptides.

Interestingly, 6 novel peptide precursors could be identified in our study. Using mass spectrometry, mature products of these precursors were identified, which have not been described from any insect. However, further investigations are necessary to elucidate, whether these molecules indeed activate receptors. Some of the rather common neuropeptide precursors, such as the insect kinin precursor, could neither be found in the transcriptome nor does peptidome data support the presence of products from these precursors. Therefore, we used immunocytochemistry to validate our transcriptome data. Preliminary results show at least weak anti-leucokinin immunoreactivity in ganglia of the ventral nerve cord and brain.

In further studies, electrophysiological experiments in combination with drug applications as well as imaging mass spectrometry will be used to study the neuropeptidergic modulation of motor neurons and the premotor microcircuitry that drives locomotion.

Satellite Symposia

[Sat1](#) 5th Schram Foundation Symposium

[Sat2](#) Integrative Analysis of Olfaction

[Sat3](#) "Brain in a dish" - explant and stem cell models of neurodegenerative diseases

Satellite Symposia

Sat1: 5th Schram Foundation Symposium

[Sat1-2](#) Molecular mechanisms of neurodegeneration caused by defective synaptic vesicle recycling
Ira Milosevic

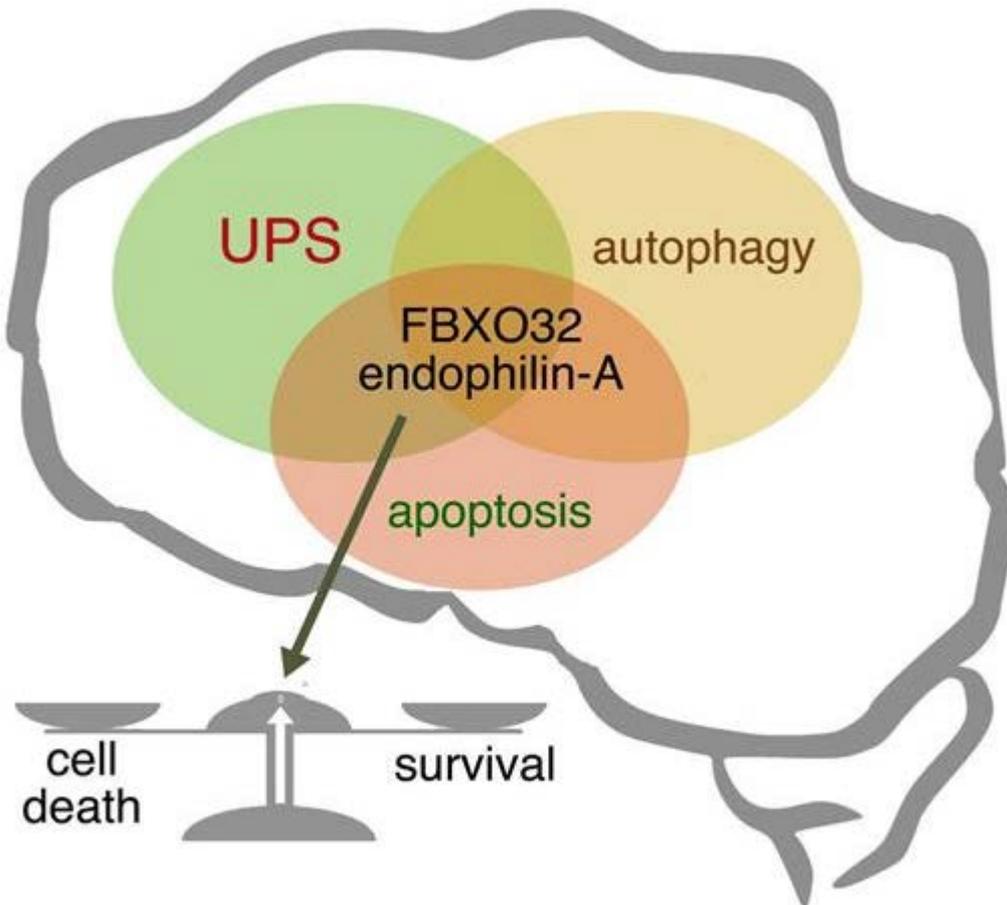
[Sat1-7](#) Predictable or unpredictable threat: what the extended amygdala has to do with it
Hans-Christian Pape

Molecular mechanisms of neurodegeneration caused by defective synaptic vesicle recycling

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Endophilin-A, a well-characterized endocytic adaptor essential for synaptic vesicle recycling, has recently been linked to neurodegeneration. We report here that endophilin-A deficiency results in impaired movement, age-dependent ataxia and neurodegeneration in mice. Transcriptional analysis of endophilin-A mutant mice, complemented by proteomics, highlighted ataxia- and protein homeostasis-related genes, and revealed upregulation of the E3-ubiquitin ligase FBXO32/atrogin-1 and its transcription factor FOXO3A. FBXO32 overexpression triggers apoptosis in cultured cells and neurons, but remarkably, coexpression of endophilin-A rescues it. FBXO32 interacts with all three endophilin-A proteins. Similarly to endophilin-A, FBXO32 tubulates membranes and localizes on clathrin-coated structures. Additionally, FBXO32 and endophilin-A are necessary for autophagosome formation, and both colocalize transiently with autophagosomes. Our results point to a previously unreported role of endophilin-A proteins in autophagy and protein degradation, which are impaired in their absence, potentially contributing to neurodegeneration and ataxia.



Predictable or unpredictable threat: what the extended amygdala has to do with it

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The brain circuits underlying behavioral fear have been extensively studied over the last decades. While the vast majority of experimental studies assess fear as a transient state of apprehension in response to a discrete threat, such phasic states of fear can shift to a 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. The networks of the so-called extended amygdala, involving the central amygdala and the bed nucleus of the stria terminalis (BNST), have been suggested keys to the control of phasic and sustained states of fear, although the underlying synaptic pathways and mechanisms remain poorly understood.

We developed a fear training paradigm involving predictable versus unpredictable pairings of conditioned and unconditioned aversive stimuli allowing to distinguish phasic and sustained fear states in mice. Through the use of genetic mouse lines combined with optogenetic and electrophysiological approaches, we studied synaptic interactions in specific amygdala to BNST pathways, and through local pharmacological intervention in behaving animals we assessed their functional relevance for conditioned fear. More specifically, we identified dedicated synaptic pathways of the extended amygdala involving pathway-specific transmitters and synaptic proteins that are both necessary and sufficient for the shift from phasic to sustained fear response profiles. The results thereby identify the causal role of defined proteins in a distinct brain pathway for the temporal development of a sustained state of anxious apprehension during unpredictability of environmental influences, reminiscent of anxiety symptoms in humans.

Satellite Symposia

Sat2: Integrative Analysis of Olfaction

- [Sat2-1](#) Reception and coding of pheromone signals in insects
Jürgen Krieger, Monika Zielonka, Elisa Badeke, Pablo Pregitzer, Silke Sachse
- [Sat2-3](#) Morphological and Transcriptomic Analysis of a Beetle Chemosensory System Reveals a Gnathal Olfactory Center
Stefan Dippel, Martin Kollmann, Joachim Schachtner, Ernst A Wimmer
- [Sat2-5](#) Odor discrimination learning in *Drosophila*: from behavior to neural circuits
André Fiala, Jonas Barth, David Vasmer, Shubham Dipt, Thomas Riemensperger
- [Sat2-8](#) Behavioral and neuronal mechanisms of olfactory imprinting in zebrafish
Gabriele Gerlach, Daniela Biechl, Kristin Tietje, Iori Namekawa, Rainer Friedrich, Mario Wullmann
- [Sat2-9](#) New insights into the subsystem organization of the mammalian sense of smell
Frank Zufall
- [Sat2-10](#) Rhythmogenesis in the Mouse Accessory Olfactory Bulb
Marc Spehr

Reception and coding of pheromone signals in insects

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Many insects release pheromones to elicit distinct behaviors in conspecifics. In moths, mate finding highly depends on female-released sex pheromones that are detected by the males with high accuracy and sensitivity. Data we have accumulated for the moth *Heliothis virescens* indicate that the sensitive and accurate recognition of pheromone molecules is based on an interplay of pheromone receptors (PRs) in the dendrites of pheromone-responsive olfactory sensory neurons (OSNs), and pheromone-binding proteins (PBPs) in the lymph of hair-like sensilla housing the OSNs. In this process “sensory neuron membrane proteins” (SNMPs), appears also to play a crucial role by operating as co-receptor for docking PBP/pheromone complexes and/or by contributing to the transfer of pheromones to PRs.

Unexpectedly, recent studies have indicated that also the larvae of moth respond to female sex pheromones and suggested a role of pheromone components in food source selection. Moreover, also female moths were shown to “autodetect” sex pheromone components released from conspecifics, which is thought to trigger behavior reducing the competition between females for ecological resources. To elucidate the molecular basis for the larval and female responsiveness to pheromones, we examined their antenna for molecular elements that are involved in pheromone detection by adult males, namely PRs, PBPs and SNMP1. The results indicate that the responses of *H. virescens* larvae and females to distinct sex pheromones components are based on the same molecular machinery as in the antennae of adult males.

Since certain plants odorants that coexist in the natural environment of males inhibit the electrophysiological response of their sex pheromone-specific OSNs and the pheromone-induced activity in the brain, we assessed the molecular targets for this inhibitory effect and found that the pheromone receptors but not PBPs were affected. Moreover, we tested if plant odorants effect pheromone-guided flight behavior in males. Together, the results revealed that pheromone-plant interactions in *H. virescens* might be an effect of stimulation with supra-natural plant odor concentrations, whereas under more natural conditions the olfactory system of the male moth appears to be well adapted to follow the female pheromone plume.

This work was supported by research grants from the Deutsche Forschungsgemeinschaft (SPP 1392, KR1786/4-2 and SA 909/3-2).

Morphological and Transcriptomic Analysis of a Beetle Chemosensory System Reveals a Gnathal Olfactory Center

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The red flour beetle *Tribolium castaneum*, is an emerging insect model organism representing the largest insect order, Coleoptera, which encompasses several serious agricultural and forest pests. Despite the ecological and economic importance of beetles, most insect olfaction studies have so far focused on dipteran, lepidopteran, or hymenopteran systems.

Here, we present the first detailed morphological description of a coleopteran olfactory pathway in combination with genome-wide expression analysis of the relevant gene families involved in chemoreception. Our study revealed that besides the antennae also the mouthparts are highly involved in olfaction and that their respective contribution is processed separately. In *T. castaneum*, olfactory sensory neurons from the mouthparts are projecting to the lobus glomerulatus, a structure so far only being characterized in hemimetabolous insects, as well as to a so far non-described unpaired glomerularly organized olfactory neuropil in the gnathal ganglion, we term gnathal olfactory center. The high number of functional odorant receptor genes expressed in the mouthparts also supports the importance of the maxillary and labial palps in olfaction of this beetle. Moreover, gustatory perception seems equally distributed between antenna and mouthparts, since the amount of expressed gustatory receptors is similar for both organs.

Odor discrimination learning in *Drosophila*: from behavior to neural circuits

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Drosophila represents a favorable model organism to dissect neuronal circuits underlying behavior. We investigate those neuronal structures that mediate associative learning and encode complex associative memories. Fruit flies can learn to avoid an odor temporally paired with an electric shock punishment. The genetic techniques available in this organism allow one to selectively manipulate distinct neuronal populations. Moreover, optical imaging using genetically encoded fluorescence sensors enables one to monitor neuronal activity in response to the trained stimuli. Thereby, those neuronal subsets embedded in a complex brain underlying associative learning can be determined. We have analyzed two aspects of aversive associative olfactory learning in detail.

First, we performed associative conditioning experiments using chemically similar odorants that evoke overlapping neuronal activity in the fly's antennal lobes and highly correlated activity in mushroom body lobes. We compared the animals' performance in discriminating between these odors after subjecting them to one of two types of training: either absolute conditioning, in which only one odor is reinforced, or differential conditioning, in which one odor is reinforced and a second odor is explicitly not reinforced. We show that differential conditioning decreases behavioral generalization of similar odorants in a choice situation. The mushroom body represents a site of convergence between the punitive, reinforcing stimulus and the neuronal representation of the odor stimulus. We show that differential, but not absolute, training causes decorrelation of odor representations in the mushroom body. In conclusion, differential training with similar odors ultimately induces a behaviorally expressed contrast enhancement between the two similar stimuli that facilitates fine discrimination of odors.

Second, we addressed the question whether the neuronal activity of the mushroom body's intrinsic neurons, the Kenyon cells, in coincidence with a punitive stimulus is sufficient to acquire and store an aversive associative odor memory. We experimentally bypassed olfactory sensory input and thermogenetically activated sparse and random ensembles of Kenyon cells directly. We found that if the artificial activation of Kenyon cell ensembles coincides with a salient, aversive stimulus, learning was induced. The animals adjusted their behavior in a subsequent test situation and actively avoided reactivation of these Kenyon cells. Our results show that Kenyon cell activity in coincidence with a salient aversive stimulus can suffice to form an associative memory. Associative short-term memory retrieval is characterized by a closed feedback loop between a behavioral action and the avoidance to re-activate sparse ensembles of Kenyon cells.

Behavioral and neuronal mechanisms of olfactory imprinting in zebrafish

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Olfactory imprinting on environmental, population- and kin-specific cues is a form of life-long memory. Associating with kin can prompt increased growth and reduced aggression. This suggests that selection should favor the capability of recognizing kin. One mechanism to discriminate between kin and non-kin is based on phenotype matching, when an individual learns a template of itself or of its kin and can later use this template to recognize even unfamiliar kin.

We could show that in zebrafish olfactory kin recognition depends on an imprinting process that requires a two-step learning process of olfactory as well as visual cues of kin. If larvae are exposed to visual cues of kin at day 5 and chemical cues of kin at day 6 post fertilization, zebrafish will recognize kin throughout life. Larvae isolated from all contact with conspecifics did not imprint on their own chemical cues; therefore, we see no evidence for kin recognition through self-matching in this species. Surprisingly, exposure to non-kin during the sensitive phase of development did not result in imprinting on cues of unrelated individuals, suggesting a genetic predisposition to kin signals. Through this combined imprinting process larvae can avoid false imprinting on unrelated individuals.

Despite its ecological significance, natural chemicals for olfactory imprinting have not been identified yet. Urine-born chemical imprinting signals are transcribed by genes expressed by polymorphic genes of the immune system - the major histocompatibility complex MHC genes – which are important messengers carrying information about ‘self’ and ‘other’. We show that MHC peptides function as chemical signals for olfactory imprinting in zebrafish. MHC peptides consisting of nine amino acids elicit olfactory imprinting and subsequent kin recognition depending on the MHC genotype of the fish. In vivo calcium imaging shows that some olfactory bulb neurons are highly sensitive to MHC peptides with a detection threshold at 1 pM or lower, indicating that MHC peptides are potent olfactory stimuli. Responses to MHC peptides overlapped spatially with responses to kin odor but not food odor, consistent with the hypothesis that MHC peptides are natural signals for olfactory imprinting.

The zebrafish olfactory epithelium harbors ciliated olfactory sensory neurons (OSNs) expressing OR and TAAR gene family receptors (mammals: main olfactory epithelium) and microvillous OSNs with V1R and V2R gene family receptors (mammals: vomeronasal organ). In addition to these two main OSN types, teleosts exhibit crypt cells, bearing cilia and microvilli and expressing apparently only a single olfactory receptor, the V1R-related ORA4 and kappe neurons possessing only microvilli. To investigate which olfactory sensory neurons (OSN) is/are involved in detection of kin odor we used the activity marker pERK (phosphorylated extracellular signal regulated kinase) and stimulated imprinted and non-imprinted 9 day old zebrafish larvae with kin odor. We provide the first evidence that crypt cells, and likely a subpopulation of microvillous OSNs play a role in detecting a kin odor related signal. In addition, we show a lower activation of olfactory bulb neurons in non-imprinted than in imprinted zebrafish larvae after exposure to kin-odor, especially at the level of the medial dorsal glomerulus 2, the only glomerulus in which crypt cells project their axon.

New insights into the subsystem organization of the mammalian sense of smell

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This lecture will discuss recent advances in the cellular and molecular organization of the mammalian sense of smell.

Rhythmogenesis in the Mouse Accessory Olfactory Bulb

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The accessory olfactory system controls social and sexual behavior. However, key aspects of sensory signaling along the accessory olfactory pathway remain largely unknown. Here, we investigate patterns of spontaneous neuronal activity in mouse accessory olfactory bulb mitral cells, the direct neural link between vomeronasal sensory input and limbic output. Both in vitro and in vivo, we identify a subpopulation of mitral cells that exhibit slow stereotypical rhythmic discharge. In intrinsically rhythmogenic neurons, these periodic activity patterns are maintained in absence of fast synaptic drive. The physiological mechanism underlying mitral cell autorhythmicity involves cyclic activation of three interdependent ionic conductances: subthreshold persistent Na⁺ current, R-type Ca²⁺ current, and Ca²⁺-activated big conductance K⁺ current. Together, the interplay of these distinct conductances triggers infralow intrinsic oscillations with remarkable periodicity, a default output state likely to affect sensory processing in limbic circuits.

Satellite Symposia

Sat3: "Brain in a dish" - explant and stem cell models of neurodegenerative diseases

[Sat3-1](#) Neural stem cells and their niches: focus on the extracellular matrix
Andreas Faissner

Neural stem cells and their niches: focus on the extracellular matrix

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Radial glia represent the major neural stem/progenitor cells (NSPCs) of the developing CNS. In the adult CNS, NSPCs are confined to specialized regions, the stem cells niches. The subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampus have been identified as canonical regions of neurogenesis in the adult CNS. The specialized niche milieu in the CNS is built by astrocytes, endothelia of blood vessels, and neuronal afferents. The niche environment conceals morphogens, cytokines, extracellular matrix (ECM) constituents and neurotransmitters. The ECM consists of glycoproteins, proteoglycans and complex glycan structures that form the matrisome. Increasing evidence points to important functional roles of the ECM during development, plasticity and regeneration of the CNS.

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