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** Short talk and poster presentation
List of Abstracts
Poster Session (Monday, 17 April: 15:20 - 17:50)

Effects of a neonicotinoid pesticide on odour discrimination [board 1]
Androne M, Haase A, Vallortigara G
CIMEC, University of Trento, Italy

Exposure to neonicotinoid pesticides is considered to be one of the main causes of honeybee decline. At sub-lethal doses (close to those utilized in the environment), these chemicals have shown to disrupt a number of basic honeybee behaviours, ranging from navigation to olfactory learning and memory. For what concerns the latter, it is already known that the interference of neonicotinoids with acetylcholine signalling in the mushroom bodies (MBs), the brain centres responsible for multisensory integration and storage of complex memories, plays a key role. However, we hypothesise that some of the memory impairments described before could arise from an incorrect stimulus encoding. We have, thus, studied the effects of neonicotinoids upstream of the MBs, on the honeybee antennal lobes (ALs).

For the first time, the effects of a common neonicotinoid on the AL output neurons, the projection neurons (PNs), were tested in vivo, through 2-photon microscopy calcium imaging. The method allows visualizing neural activity within the cells with high spatial and temporal resolution, in the form of fluorescence changes over time. We analysed the responses of single glomeruli to single odours, and the overall functionality of the AL throughout the treatment.

Acute neonicotinoid treatment was demonstrated to affect the calcium response, in at least some of the glomeruli, by decreasing its amplitude. This effect was partially reversible. Discrimination was shown to be strongly impaired as well, as the representations of different odours in the AL space were not separate anymore after the treatment. These findings support our hypothesis that some of the impairments in olfactory memory, which have been described for the honeybee, may also be due a decline in discrimination abilities.

Single-cell resolution circuit mapping with temporal-focused excitation of soma-targeted channelrhodopsin [board 2]
Max Planck Florida Institute for Neuroscience, USA

Optogenetics using channelrhodopsin (ChR) and its variants has revolutionized the study of long range neural connections, but conventional optical stimulation paradigms likely excite cells above and below the cell of interest and thus limit their use in mapping local neural circuits at single-cell resolution. Here we utilize temporal focusing of two-photon excitation to generate a disk-like field of stimulation in acute brain slices, simultaneously activating multiple ChR molecules in a single focal plane and reducing off-target activation of cells above or below the neuron of interest. Moreover, we have further reduced the potential for off-target effects by spatially restricting ChR expression to the neuronal soma and proximal dendrites. Under these conditions we elicit action potentials only when stimulating the soma of ChR expressing cells; the lack of current responses to stimulating fibers of passage or at axially displaced locations highlights the utility of the technique for fine mapping. We also combine stimulation with simultaneous imaging of neuronal activity using the calcium indicator GCaMP6s to verify action potential generation in response to temporal focusing activation of putative presynaptic cells. Our method allows robust and precise activation of neurons in brain slices for the construction of functional synaptic connectivity maps with single-cell resolution without loss of information about local connections in the region of the dendritic arbor of the recorded neuron or inadvertent activation of axons. We also apply this approach to red-shifted opsin variants such as C1V1 and ReaChR and in mapping of cortex and amygdala in animal models of psychiatric disease.

CLARITY - Visualising the intact brain in 3D [board 3]
Joakim Bastrup & Peter H. Larsen
Synaptic Transmission, in vitro, H. Lundbeck A/S, Denmark

Obtaining accurate biologically detailed information from neural tissue has been a formidable challenge for decades. CLARITY technology for the first time makes it possible to study the molecular, cellular and complex connectivity components of the brain in complete structural context in an optically transparent brain. This poster builds on the current foundation of the existing CLARITY platform with a particular focus on further elucidation of genetic and induced animal models of schizophrenia (i.e. 22q11, 15q13 and phencyclidine (PCP) induced). One of the prevailing hypotheses in schizophrenia is a dysfunction of particular cortical parvalbumin positive (PV+) interneurons. This poster describes: 1) an optimized electrophoretic tissue clearing chamber (ETC chamber), 2) investigation of antibody staining and penetration depth, 3) PV+ interneuron density in prefrontal cortex of 22q11, 15q13 and PCP induced mice models.
Cortical bases of temporal asymmetries in auditory perception [board 4]
Deneux T, Kempf A, Daret A, Ponsot E and Bathellier B
UNIC, CNRS, France

Sound recognition relies not only on spectral but also on temporal cues. This important fact is well demonstrated by the divergent percepts produced by many natural sounds and their time-reversed counterpart, even for short sounds (e.g. single tone from a piano). To address the coding principles underlying such profound but poorly understood asymmetries in auditory perception, we recorded large samples of GCAMP6-labelled auditory cortex neurons in awake mice while playing sounds ramping up or down in intensity. To do so, we used wide-field resonant scanning two-photon calcium imaging, obtaining up to ~1000 automatically segmented neurons monitored in parallel in a 1x1mm field of view. First, we strikingly observed that, up-ramping sounds triggered more activity across the cortical population than down-ramping sounds although both sounds have globally the same physical intensity. However, this asymmetry in cortical saliency was well reflected in the respective perceptual saliencies of the sounds observed in behaving mice. It also matched perceptual asymmetries previously described in humans.

To understand the computations underlying these asymmetries, we extracted in a model-free manner the main temporal patterns of neuronal responses during up- and down-ramps. The analysis revealed that auditory cortex implements a complex map of yet undescribed, spatially clustered neuronal ensembles which detect specific combinations of temporal modulation (e.g. rising or decaying envelope) and intensity features. Imaging of large 3D volumes of auditory cortex indicated that these ensembles are organized in columns. Finally, comparing different models, we show that the observed asymmetric features must result from nonlinearities nested in multiple processing layers. Unlike standard receptive field models of auditory cortex function, such multilayer architectures are able to build divergent representations with asymmetric global saliency for sounds that have identical spectral contents but different temporal structures. This provides an interesting computational mechanism for the divergent percepts we experience in this case.

Common drive not so common: Decoupling of timescales in spinal motor activity reveal large premotor network [board 5]
Radosevic, M, H. Linden, A. Willumsen, P.C. Petersen, R. W. Berg
Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark

During patterned motor activity, such as locomotion and scratching, both the spinal interneurons (INs) and motoneurons (MNs) receive rhythmic synaptic drive, known as common drive. The neuronal circuitry responsible for this common drive is unknown, and it is believed to originate from a relatively small circuit of neurons referred to as a central pattern generator (CPG). The CPG-rhythm arriving at the receiver cells consists of two parts: 1) A fast fluctuation in membrane potential due to individual synaptic potentials (fast timescale) and 2) a slower oscillation due to intensity of input (slow timescale). If two neurons were to receive input from same CPG network, the activity should correlate on both fast and slow timescales, i.e. individual synaptic input, as well as on slow timescale. In contrast, if the activity is correlated only on the slow timescale, and not on the fast timescale, the CPG circuit is likely large with convergent input on the receiver neurons, i.e. large premotor network projecting to relatively few INs and MNs. Here we use this distinction to infer information about the premotor network in the turtle spinal cord. We use intracellular recordings in pairs of either MNs or local INs receiving common drive, and correlate their slow versus fast activities. We find that the fast activity is remarkably uncorrelated across the population of INs and MNs suggesting that the premotor network is much larger than previously thought and has a dominance of convergent connections, i.e. large premotor network projecting to relatively few INs and MNs.

The development and microcircuitry of parvalbumin positive interneurons in layer II of the rat medial entorhinal cortex [board 6]
Berggaard, N, Bjerke, I. E, Paulsen, A. E. B, Hoang, L, Skogaker, N. E. T, van der Want, J.
Institute for Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Norway

Spatial representation in the brain arises from a complex interplay between different functionally defined neurons. Among these neurons are grid cells, which have a characteristic regular hexagonal firing pattern that spans the entire environment that an animal explores. Most of these neurons can be found within layer II (LII) of the medial entorhinal cortex (MEC), which contains principal cell types with either a stellate or a pyramidial morphology. Although it has not yet been established, recent research indicates that grid cells may comprise both stellate and pyramidal neurons, which are involved in rather different inhibitory morphologies (Domnisoru et al, 2013). Inhibitory interneurons that can be labeled with anti-parvalbumin (PV) are heavily involved in the formation of grid fields, as they both directly innervate and receive most of their input from grid cells. Little is however known about how microcircuits involving PV interneurons in LII are organized, how cell type specific excitatory and inhibitory connectivity is arranged and how this relates to microcircuits involving stellate and pyramidial neurons.

In this project we are using a combination of light and electron microscopy to investigate PV positive interneurons within LII of the MEC in adult rats, as well as at postnatal day 10 and 15 (before and after eye-opening). We have scanned vibratome sections to look at the general morphology, size and extent of clustering of PV cells between the different age groups. Confocal microscopy will be done on fluorescent PV interneurons to compare the amounts of labeling throughout development. Transmission electron microscopy was used on ultrathin sections to investigate the morphology and arrangement of connections between labeled and unlabeled cells, and serial block-face imaging and volume reconstruction of the microcircuitry with the Amira software will be performed on PV labeled neurons and terminal profiles.
Purines released from astrocytes inhibit excitatory synaptic transmission in the ventral horn of the spinal cord [board 7]

Carlsen EM, Perrier J-F.
University of Copenhagen, Denmark

Spinal neuronal networks are essential for motor function. They continuously adapt their activity to the internal state of the organism and to the environment. This plasticity can be provided by different neuromodulators. These substances are usually thought of being released by dedicated neurons. However, in other networks from the central nervous system, synaptic transmission is also modulated by transmitters released from astrocytes. The star-shaped glial cell responds to neurotransmitters by releasing gliotransmitters, which in turn modulate synaptic transmission. We investigated if astrocytes present in the ventral horn of the spinal cord modulate synaptic transmission. We evoked synaptic inputs in ventral horn neurons recorded in a slice preparation from the spinal cord of neonatal mice. Neurons responded to electrical stimulation by monosynaptic EPSCs. We used mice expressing eGFP under the glial fibrillary acidic protein promoter to identify astrocytes. Chelating Ca2+ with BAPTA in a single neighbouring astrocyte increased the amplitude of synaptic currents. In contrast, when we selectively stimulated astrocytes by activating PAR-1 receptors with the peptide TFLLR, the amplitude of EPSCs evoked by a paired stimulation protocol was reduced. The paired-pulse ratio was increased, suggesting an inhibition occurring at the presynaptic side of synapses. In the presence of blockers for extracellular ectonucleotidases, TFLLR did not induce presynaptic inhibition. Puffing adenosine reproduced the effect of TFLLR and blocking adenosine A1 receptors prevented it. Altogether our results show that ventral horn astrocytes are responsible for a tonic and a phasic inhibition of excitatory synaptic transmission by releasing ATP, which gets converted into adenosine that binds to inhibitory presynaptic A1 receptors.

Interneurons enhance selectivity in auditory cortex [board 8]

Christensen RK, Nakamura N, Barkat TR
Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark

Interactions between excitatory and inhibitory neurons form the basis of neural processing in the mammalian brain. In sensory systems cortical interneurons are involved in fine-tuning selectivity and timing of responses.

We used light induced activation and silencing of subclasses of inhibitory neurons to study their role in shaping neural representation of sound features in the mouse auditory cortex. Pure frequency tones were presented to awake, head restrained mouse, and neuronal responses were recorded with extracellular electrodes, while parvalbumin (PV) or somatostatin (SOM) interneuron activity were perturbed with light stimulation.

Our results suggest that subtractive gain modulation provided by interneurons onto pyramidal neurons contribute to sharper frequency selectivity in the mouse primary auditory cortex. Next we examined if the frequency preference recorded in cortical neurons also have relevance in sound perception. In a Go/NoGo task we tested the mouse ability to discriminate pure frequency tones, while activating PV or SOM neurons with light. Our results show that adult mice can distinguish frequencies down to 0.2 octaves. The effect of PV/SOM activity manipulation on this discrimination threshold are evaluated.

Manipulating and Imaging neural networks using life-long lasting attenuated Rabies [board 9]

Ciabatti E, Morgese F, Tripodi M.
Department of Neurobiology, MRC - Laboratory of Molecular Biology, United Kingdom

Understanding how neurons are functionally and structurally organized in neuronal networks is key to understanding how they integrate information and generate specific behaviours.

The structure of a neural circuit can be addressed using chemical or viral based tracing techniques and among them Rabies virus based methods. G-deleted Rabies (ΔG-Rabies) provided a way to map, selectively, first-order pre-synaptic partners (Wickersham IR, 2007). Despite the transformative role of ΔG-Rabies based approaches in the anatomical investigation of neuronal circuits, in its current configuration it cannot be used to perform long-term tracing experiments or to follow circuit rewiring. This is due to its inherent cytotoxicity, which leads to cell death within 2 weeks.

The deletion of proteins other than G from the Rabies genome generates a virus unable to complete its life cycle. Thus, we decided to affect the viral proteins stability by fusing them to a degradation domain through a cleavable linker. We screened every viral protein for the capacity of switching down the viral infection and the possibility of reactivating the virus after the protease cleavage of the degradation domain.

We found one candidate virus that has reduced viral protein production, cytotoxicity and that responds to the degradation domain removal in vitro. This virus was able to infect neurons in vivo and activate genes trough CRE mediated recombination, and then disappear from the infected cells.
Thus, we generated a conditional ΔG-Rabies virus that transcriptionally switches off following the infection while leaving permanent genetic access to the mapped neural elements with an unaltered cellular physiology for the entire life of the animal. This opens new possibilities in linking network structure to relevant behaviours in long-term experiments. It represents the first tool to investigate circuit activity in vivo (using genetically encoded calcium indicators and optogenetic actuators) and network reshaping at the whole network level.

Functional Organization of Thalamic Input to the Mouse Auditory Cortex [board 10]
Dahmen JC, Vasquez-Lopez SA, Weissenberger Y, Keating P, King AJ
Department of Physiology, Anatomy and Genetics, University of Oxford, UK

Tonotopy, the sequential spatial representation of sound frequencies, is a fundamental feature of the auditory system. It has its origin in the organisation of the cochlear receptor surface and is maintained through several subcortical processing stages up to the auditory cortex. However, two-photon imaging of cortical cell populations has suggested that the tonotopic organisation in the mouse auditory cortex is weak. Most of the subcortical input to the cortex comes from the medial geniculate body (MGB) of the thalamus, which consists of a primary, tonotopically organised, nucleus in its ventral division (MGBv) and two non-primary nuclei in its medial and dorsal divisions.

We functionally characterised the input from the MGB to the auditory cortex of C57BL/6 mice by recording calcium transients in individual axonal boutons of corticothalamic neurons expressing GCaMP6m. Thalamic input to the auditory cortex arrives mainly into lower layer 3 and layer 4 (L3/4) but we also observed dense populations of acoustically-responsive thalamic axons in layer 1 (L1). We found that the thalamic input was surprisingly heterogeneous. Neighbouring boutons often exhibited differences in best frequency of several octaves, which may explain the lack of fine-scale tonotopic organization seen among populations of cortical cell bodies. This heterogeneity was slightly more pronounced in L1 than in L3/4 populations.

Despite the high degree of heterogeneity a tonotopic organization of the thalamic input became apparent but only on a larger scale, in populations of boutons spanning several hundred micrometres. Surprisingly, tonotopy was not only observed in L3/4 but also in L1. This suggested that a substantial fraction of the thalamic input to L1 may originate in the tonotopically ordered MGBv which was previously thought to be dominated by input from the non-lemniscal nuclei of the MGB. The presence of MGBv-originating axons in L1 was subsequently confirmed using a MGBv-selective mouse line (Sert-cre).

Cognitive Impairment in the Aging Brain: Effects of IGF-1 Treatment on Synaptic Transmission [board 11]
Albert Orock, Sreemathi Logan, Nikolett Szarka, Anna Csiszar, William Sonntag, Ferenc Deak
Department of Neuroscience, University of Oklahoma Health Sciences Center, USA

Rapidly rising number of patients with cognitive impairment and dementia in the aging population of our country is a major challenge for the health care system. Vascular cognitive impairment, Alzheimer’s and other neurodegenerative diseases affect millions of elderly patients in the USA. According to recent results of the dementia research field a key event in the pathomechanism of dementia is the disruption of synaptic connections among neurons. Synapses are the structural elements for information processing, neuronal communication in the brain and essential for learning and memory as well as other cognitive processes. The core mechanism for transmitter release from synaptic vesicles requires the SNARE (SNAP Receptor) complex. Three proteins form the synaptic SNARE complex in the brain: SNAP-25, syntaxin1 and synaptobrevin. Using the knock-out mouse strains as a novel model of dementia and fluorescence imaging functional assays we have found that the levels of SNARE proteins significantly correlate with synaptic release.

IGF-1 is an important neurotrophic hormone and emerging data indicate that expression of Insulin-like Growth Factor (IGF)-1 decreases with age. Deficiency of this hormone influences cognitive decline in the elderly. Although IGF-1 has a complex role in brain function, we hypothesized direct synaptic effects of IGF-1 and tested its role in memory improvement. We present novel data on synaptic mechanisms of learning and memory IGF-1 treatment acquired using electrophysiological and fluorescence imaging functional assays. Based on these new results on the effects of IGF-1 on synaptic communication, we propose the IGF-1/PI3K/Akt pathway as a possible therapeutic target and a novel approach to improve cognitive function for the elderly.

CRH acts anxiolytic by modulating dopamine release through a subset of GABAergic long-range projection neurons [board 12]
Stress Neurobiology and Neurogenetics Department, Max Planck Institute of Psychiatry, Germany

Dysregulated corticotropin-releasing hormone (CRH) circuits play an important role in stress-related disorders including depression and anxiety. While the role of CRH as an indispensable initiator of the HPA axis is well defined, we are just starting to comprehend the function of extrahypothalamic CRH with regards to emotionality and behavioural responses to stress. In this respect, we could recently show that anxiety-related behaviour is modulated by an imbalance between CRH receptor type 1 (CRHR1)-controlled anxiogenic glutamatergic and anxiolytic dopaminergic circuits. However, the identity of CRH-releasing neurons and sites of CRH action that modulate anxiolytic behavioural
responses have not been fully established yet. Using neurochemical and genetic tools we revealed that CRH is primarily expressed in GABAergic neurons, which exhibited distinct morphologies depending on the brain region. Anterograde tracing studies of forebrain limbic CRH neurons revealed GABAergic long-range projecting axons, which innervated distant brain regions including the ventral tegmental area (VTA), which harbours the majority of CRHR1-expressing dopaminergic neurons. We found that deletion of CRH from these GABAergic long-range projection neurons enhanced anxiety and fear memory expression, implicating that this specific CRH circuit is required under physiological conditions to maintain a positive emotional state. In addition, these animals displayed reduced baseline dopamine release in the PFC, suggesting that a subset of CRH-expressing GABAergic projection neurons in the limbic forebrain target CRHR1 on dopaminergic neurons to modulate emotional behaviour by regulating dopaminergic neurotransmission. In conclusion, our results uncover a previously unidentified anxiety-suppressing CRH circuit which regulates DA release to ultimately modulate emotional behaviour.

**Effect of running on spatial integration in inhibitory neurons of mouse visual cortex [board 13]**

Dipoppa M, Ranson A, Krumin M, Pachitariu M, Carandini M, Harris KD

University College London, United Kingdom

The multiple types of cortical GABAergic interneurons are likely to have distinct computational roles. In visual cortex interneurons expressing somatostatin (Sst) have been implicated in suppressing Pyramidal (Pyr) neuron’s responses to large stimuli. In contrast interneurons expressing vasoactive intestinal peptide (Vip) have been implicated in disinhibiting Pyr neurons during running via the inhibition of Sst neurons. Finally interneurons expressing parvalbumin (Pvalb) have been implicated in maintaining an excitatory-inhibitory balance with Pyr neurons. It remains unclear under which conditions these properties can be reproduced.

We measured the responses to visual gratings of Sst, Vip, Pvalb, and Pyr neurons in layer 2/3 of mouse V1, while animals were either stationary or running. When the grating was absent, running increased neural activity in Vip and Sst neurons and had a mixed effect in Pvalb neurons, depending on depth. In contrast in the presence of drifting gratings running increased visual responses in all cell classes. Hence both in the presence and absence visual stimuli, these results do not conform to a running-induced disinhibitory circuit model of Vip and Sst cells. The increase of visual responses with running was stronger for large stimuli in Sst neurons but was stronger for small stimuli in Vip neurons. In particular, in the stationary conditions, these results are not consistent with Sst suppressing Pyr neuron’s responses to large stimuli.

We introduced a classification method based on neural activity statistics, permitting classification of GABAergic and Pyr neurons. Pvalb cells showed strong correlation with excitatory neurons, Vip cells showed milder correlation, and Sst cells showed no correlation. These results provide further evidence that specific classes of interneurons have distinct computational roles. However these roles are more complex than previously suggested and depend on the condition considered.

**Neuronal circuits for multifunctional wing motor control in Drosophila [board 14]**

O’Sullivan A, Ellendersen BE, von Philipsborn AC.

DANDRITE, Aahus University, Denmark

Drosophila uses its wings in various behaviours, e.g. for locomotion during flight and for generating acoustic communication signals (male courtship song) during reproduction. Wing movements are executed by interplay of the flight power musculature, which generates wing lift force and the flight control musculature, which adjusts wing kinematics. There are a 16 pairs of flight control muscles, each innervated by a single motor neuron. The function of most muscles and their motor neurons during flight and other wing behaviours is not well understood.

With the bipartite expression system Split GAL4, we have gained genetic access to control muscle motor neurons as well as candidate upstream interneurons. Based on 3D image registration and reconstruction, we are assembling an anatomical atlas of the wing neuropil in the ventral nerve cord. We address the function of distinct wing control neurons during different wing behaviours with neuronal silencing and optogenetic activation experiments, comparing flight and the rhythmically patterned male courtship song. Our experiments indicate that the circuits generating and fine-tuning song and flight patterns have shared as well as distinct components and are organized in a multifunctional network. Most, but not all motor neurons are required for aspects of flight as well as song. In contrast, a set of sex specific interneurons expressing the male specific transcription factor Fruitless regulates wing motor patterns during song, but not flight.

Ongoing efforts are aimed at establishing calcium imaging of wing control muscles during courtship singing compared to flight, as readout for motor neuron firing patterns. We are investigating connectivity of wing control neuronal circuits. Basic principles of how the nervous system initiates and maintains distinct and mutually exclusive motor patterns employing the same neuronal components during different behavioural contexts are expected to be of general relevance for circuit neuroscience.
Two-photon patterned stimulation of channelrhodopsin-2 in the intact mouse brain [board 15]
Angelo Forli, Claudio Moretti, Serena Bovetti and Tommaso Fellin
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External sensory stimuli generate complex spatial and temporal patterns of electrical activity in neural networks. To causally test the importance of the spatiotemporal structure of these activity patterns in encoding information about the external stimulus, we need a method that allows perturbing the activity of many neurons with cellular resolution in the intact brain. To this aim, we combined two-photon patterned illumination with photostimulation experiments to perturb the spiking activity of individual neurons expressing the excitatory opsin channelrhodopsin-2 (ChR2) in anesthetized mice. Patterned illumination was obtained using a liquid crystal spatial light modulator which was coupled with a conventional laser scanning two-photon microscope. In simultaneous photostimulation and two-photon-guided juxtasomal recordings from ChR2-positive cells in cortical layer II/III, we observed reliable increase in the spiking frequency of recorded neurons upon patterned illumination with extended oval shapes. The increase in the spike frequency and the latency to the first spike were increasing and decreasing functions of light intensity, respectively. Moving the oval shape in the lateral or axial directions with respect to the recorded neuron rapidly decreased the photostimulation effect, with spatial constants of few tens of micrometers. We are currently investigating the effect of illumination with different shapes on the spiking activity of individual neurons and we are currently characterizing these different stimulation protocols in terms of efficiency and spatial resolution. By perturbing the activity of neural circuits with near cellular resolution, this technique has the potential to represent a unique experimental tool to probe how spatiotemporal patterns of neural activation encode information.

Dynamic regulation of cerebral DNA repair genes by psychological stress [board 16]
Psychiatric Centre Copenhagen, Institute of Clinical Medicine, Denmark

Neuronal genotoxic insults from oxidative stress constitute a putative molecular link between stresssand depression on the one hand, and cognitive dysfunction and dementia risk on the other. Oxidative modifications to DNA are repaired by specific enzymes; a process that plays a critical role for maintaining genomic integrity. The aim of the present study was to characterize the pattern of cerebral DNA repair/renzyme regulation after stress through the quantification of a targeted range of gene products involved in different types of DNA repair. 72 male Sprague–Dawley rats were subjected to either restraint stress (6 h/day) or daily handling (controls), and sacrificed after 1, 7 or 21 stress sessions. The mRNA expression of seven genes (Ogg1, Ape1, Ung1, Neil1, Xrccl, Ercc1, Nudt1) involved in the repair of oxidatively damaged DNA was determined by quantitative real time polymerase chain reaction in the prefrontal cortex (PFC) and hippocampus (HC). DNA repair gene expression in PFC exhibited a general trend towards an induction after acute stress and a decrease after subchronic exposure compared to control animals. After chronic stress, a normalization towards control levels was observed. A similar pattern was seen in HC, but with overall smaller effects and without the induction after acute stress. Nuclear DNA damage from oxidation as measured by the comet assay was unaffected by stress in both regions. We conclude that psychological stress have a dynamic influence on brain DNA repair gene expression; however, since we were unable to identify concurrent changes in DNA damage from oxidation, the down-stream consequences of this regulation, if any, remains unclear.

Identification of Draxin as an extracellular Netrin Modulator in neural circuit development [board 17]
Gao X., Maischein H-M., Soellner C.
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Axon guidance cues are crucial signals for neurons to build complex neuronal networks during early developmental stages. The Netrin family comprises canonical secreted axon guidance cues important for nervous system wiring in invertebrates and vertebrates. Distinct Netrins and Netrin receptors have been identified to carry out the guidance function, however, no direct Netrin modulator has been described yet. We used AVEXIS (AVidity-based Extracellular Interaction Screen), a protein-protein interaction screen assay, to identify new binding partners for zebrafish (Danio rerio) Netrins. We found several Netrin family members bind to another secreted guidance protein. Furthermore, we were able to show that these novel interactions are conserved for the orthologous human proteins. In addition, the identified Netrin binding partner is able to outcompete Netrin receptors for the binding to Netrins in a biochemical competition assay. The binding of Netrin receptors to Netrin is reduced in the presence of the novel binding partner, hinting towards a potential inhibitor function in the Netrin signalling network. By generating truncations and deletions, we narrowed down the interaction interface to the third EGF domain of Netrin and to a highly conserved 22 amino acid region in its binding partner. Finally, to test whether the interaction is able to occur in vivo, individual and combined misexpression experiments of both fluorophore tagged binding partners were performed in early zebrafish embryos. Our results showed that zebrafish Netrin was able to re-locate its binding partner to highly concentrated membrane associated densities, indicating that the interaction can occur in vivo. Together, these findings have allowed us to propose and test a model in which the new Netrin binding partner functions as a secreted Netrin signalling modulator in neural circuit development.
Dissection of frequency decomposed network activity pattern in zebrafish larva using high temporal-spatial calcium imaging [board 18]
Mostafa Ghannad-Rezaie, Fatih M. Yanik
ETH Zürich, Switzerland

Dissection of the underline neuronal networks involving in cognitive function of the brain has been long thought to be the key to understand the high level processing power of the brain. The brain wide synchronous oscillation pattern are shown to be connect to the data flow and structural hierarchy. Yet to understand the full cognitive process, the broad interconnection among brain wide networks ideally required a methodology to compile the electrophysiological activity in the whole brain with milliseconds of resolution. Traditional methods such as fMRI and electrophysiology are lacking sufficient spatial or temporal resolution to resolve the functionally activity of the whole brain.

Zebrafish larva due to the small dimensions and optical transparency is now considered the model of choice for study of the whole brain function. Recent advances in calcium imaging techniques enable rapid non-invasive full brain single cell resolution imaging. With unprecedented spatial and temporal resolution, calcium imaging can reveal the underlying electrophysiological activity pattern of the brain in the network level. With recent genomics editing tools such as CRISPR system each sub-systems in the brain can be probed individually. High resolution structural and functional information can be used for dissection of subnetwork communication in the brain.

To fully exploit the wealth of zebrafish electrophysiological data, novel approaches are required for comprehensive quantitative assessment of network connection. These approaches must be high throughput to accommodate studies of large numbers of zebrafish brain structure variants. We have developed calcium imaging system based on selective plane illumination microscopy capable of scanning the whole brain under 50ms with single cell resolution. The data is registered into a detailed anatomical atlas of zebrafish brain. The signal then decomposed in known brain wave form bands.

Large-scale Cortical Dynamics During a Texture Discrimination Task [board 19]
Gilad A., Chen J., Helmchen F
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The mammalian brain integrates behaviourally relevant sensory information by recruiting large parts of the neocortex to enable precise perception, apt decisions and adequate actions. The large-scale interactions and the distinct roles of the various neocortical regions, namely frontal motor-related areas and posterior sensory-related regions, remain poorly understood. Here, we aim to characterize how behaviour-related activity is integrated throughout large parts of the cortex. Head-restrained transgenic mice expressing GCaMP6f in layer 2/3 were trained on a ‘go/no-go’ S1-dependent texture discrimination task. On ‘go’ trials mice were required to lick for a water reward (‘hit’) when presented with a panel of coarse sandpaper (P100); in ‘no-go’ trials they were supposed to withhold licking (‘correct rejection’) when presented with a smoother sandpaper (P1200). In addition mice were required to delay their licking response on ‘go’ trials, enabling temporal segregation between “sensation” and “action”. During the task, we simultaneously imaged large parts of the cortex, including frontal and posterior areas. We find that during sensation (i.e. texture touch) several behaviour-related areas display stimulus-evoked activity (e.g. S1, S2, M1 and M2). Interestingly stimulus-evoked activity is higher on ‘go’ trials compared to ‘no-go’ trials. During the delay period, frontal areas, especially M2, remained high in ‘go’ trials whereas posterior sensory areas displayed lower activity. M2 encodes both motor-related movements, e.g. whisking, and delay related activity implying its role as a centre for integration. These results highlight the importance of wide-field imaging to reveal the complex large-scale cortical dynamics underlying behaviour.

Role of Vasoactive Intestinal Polypeptide (VIP) interneurons in perceptual learning deficits of Fragile X (Fmr1-/−) mice [board 20]
Anubhuti Goel, Steve Cohen, Kaela Cohen, Carlos Portera Cailliau
Neurology Department, University of California, Los Angeles, USA

In addition to circuit hyperexcitability several symptoms of autism are related to the inability to cope with sudden changes in the environment that affects learning and task performance, which can potentially be attributed to inflexibility in adapting to rapid changes in cortical state.

In mice, during active behaviours (e.g., locomotion, whisking, responding to visual stimuli and learning) vasoactive intestinal polypeptide (VIP) cell activity increases, somatostatin (Sst) cell activity decreases, and pyramidal cell activity increases. This VIP-Sst circuit integrates cholinergic inputs from the basal forebrain and is thus an important dynamic regulator of sensory responses and plasticity that demand attention. Our previous data shows that hyperexcitability in Fmr1−/− mice is brain state-dependent and recent studies show that functional output of VIP cells is modified “online”, hence, we predict that abnormal VIP neuron function impairs sensory perceptual learning because of exaggerated stimulus-evoked responses and impaired attention in Fragile X Syndrome (FXS).

Head-restrained mice were thus trained to discriminate between two drifting visual gratings presented at orthogonal orientations; e.g., 45° vs. 135°. We find that while WT and Fmr1−/− mice learn the task, Fmr1−/− mice take 2 days longer on average to reach a discrimination threshold (d’) > 2. With increased task difficulty (reduction in the angle between the 2 orientations or lowered contrast of the gratings), WT mice continued to perform well, whereas discrimination by Fmr1−/− mice was impaired. To examine the underlying network dynamics we will record simultaneously from pyramidal and VIP neurons expressing GCaMP6s using in vivo two-photon calcium imaging in mice performing the visual
discrimination task (in the presence or absence of a distracting sensory stimulus). Further optogenetic silencing of VIP cells by expressing archaerhodopsin, should restore the faulty circuit dynamics and rescue the behavioural performance of Fmr1-/- mice.

Circuit properties contributing to angular integration in Drosophila [board 21]
Jonathan Green, Atsuko Adachi, Gaby Maimon
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While navigating their environment, many animals keep track of their position and orientation over time, even without positional landmarks. Neurons that track these variables have been identified in mammals, yet the neural mechanisms that give rise to their properties remain largely unknown. It has recently been shown that a set of cells in the central complex of Drosophila melanogaster collectively track the fly’s orientation, both in the presence of a visual landmark and in the dark. Here, we describe a second fundamental cell class in the fly’s orientation circuit, using two-photon imaging of head-fixed flies walking on an air-cushioned ball. By expressing GCaMP6, a genetically-encoded calcium indicator, in either cell type, the fly’s orientation can be read out by the position of 2-3 peaks of activity along the protocerebral bridge, a central complex structure that consists of a linear array of 18 glomeruli. Whereas both cell types carry an orientation signal, they differ in two ways. First, by imaging both cell types simultaneously using GCaMP6f and RGECO1a, we find that their peaks of activity are offset with respect to each other in the protocerebral bridge. Second, dynamic modulations in the [Ca2+] signal of the second cell type map to the strength of each turn, unlike the first cell type, suggesting that the second type informs the circuit on how much the peaks of activity should move. We are currently working on perturbations to highlight the different computational roles of these two cell classes. These results, along with recent anatomical work, begin to delineate a circuit for how flies integrate their own movements to update a quantitative estimate of their heading while walking.

Deep brain imaging of amygdala fear circuits in freely moving animals [short talk]
Jan Gründemann, Benjamin Grewe, Sabine Krabbe, Tingjia Lu, Kenta Hagihara, Mark Schnitzer, Andreas Lüthi
Friedrich Miescher Institute for Biomedical Research, Switzerland

Learning and memory shape our daily life, social interactions and mental well-being. Mapping large-scale network activity on identified neuronal circuits during memory formation and retrieval will be essential to understand the neurophysiological and pathophysiological basis of behaviour. Auditory Pavlovian fear conditioning is a well-established associative learning paradigm that highly depends on amygdala function. We use intersectional viral tools and a deep brain imaging approach, which combines gradient-index (GRIN) lens in vivo imaging with an ultra-light (2 g) head-mountable miniature microscope, to elucidate how defined neuronal populations in the amygdala encode anxiety states and acquired fear behaviours in a projection pathway-specific manner.

Super-resolution STED imaging of optical vortices in a high-NA speckle pattern [short talk & board 22]
Pascucci M., Tessier G., Emiliani V., Guillou M.
University Paris Descartes, France

Scalar wavefields passing through random media generate random speckle patterns. These patterns contain hot spots but also true zeros of intensity, which draw lines in three-dimensional space. These zeros of intensities are surrounded by spiral phase patterns and are thus called optical vortices due to the circulation of the optical current around the nodal line. For optical wavefields of high numerical apertures, the contribution of all three components of the field must be taken into account. In particular, the axial field cannot be neglected. In STimulated Emission Depletion (STED) microscopy for instance, resolution is improved by stimulating fluorescence with a donut-shaped beam exhibiting an optical vortex at its dark centre. This beam must be circularly polarized in order to cancel the axial component of the field at the centre of the donut. Using the opposite “wrong” circular polarization yields a significant axial component resulting in deexciting fluorophores located at the donut centre.

Here we study optical vortices in a high-NA speckle pattern thanks to super-resolution STED microscopy. To do so, we first photo-bleach a uniform sample of fluorophores embedded in a polymer matrix with a high-NA speckle illumination pattern having right-handed circular polarization. Then we image the remaining fluorescence by STED microscopy. The same protocol is repeated for left-handed polarization. Results demonstrate sub-diffraction confinement of fluorescence in the sample and sub-diffraction imaging of positively and negatively charged vortices in the speckle pattern. These results are discussed in the context of imaging through scattering media where speckle patterns consistently occur under coherent illumination.

Spinal motoneurons are driven by reciprocal excitation and inhibition during rhythmic motor behaviour [board 23]
Robertas Guzulaitis & Jorn Hounsgaard
University of Copenhagen, Denmark
Central pattern generators (CPGs) are networks of neurons that generate rhythmic motor output in the absence of sensory feedback. It is believed that alternating excitatory and inhibitory input to motoneurons gives rise to rhythmic motor output. This dogma was challenged when the balanced excitation and inhibition was observed in turtle spinal motoneurons during scratching (Berg et al., 2007). Patterns of excitation and inhibition in this study were estimated from total conductance under an assumption that conductance measured during scratching is purely synaptic. Here we demonstrate that turtle motoneurons show strong outward rectification in supra-threshold Vm range. Neglecting outward rectification in a previous study led to overestimation of inhibition and the level of balanced excitation and inhibition. In addition we use whole-cell patch clamp technique to measured patterns of inhibitory and excitatory input directly. We found that motoneurons receive alternating inhibitory and excitatory synaptic input. Our findings justify reciprocal excitation and inhibition in motoneurons during rhythmic motor behaviour.

Seeing is believing- visualizing the molecular mechanisms of dendritic spine initiation [board 24]
Minerva Institute for Medical Research, Finland

The formation and functionality of neuronal synapses requires precise morphogenesis of dendritic spines. However, it is not known how spines are initiated. Here, we identify the Inverse-BAR protein MIM/MTSS1 as a nucleator of dendritic spines. MIM accumulated to future spine initiation sites in a PIP2-dependent manner and deformed the plasma membrane outwards into a proto-protrusion via its I-BAR domain. Unexpectedly, the initial protrusion formation did not involve actin polymerization. However, PIP2-dependent activation of Arp2/3-mediated actin assembly was subsequently required to elongate the protrusion. Consistently, while over-expression of MIM potentiated spine formation through its membrane-bending activity, MIM deficiency led to decreased spine density accompanied by morphological abnormalities of spines. Moreover, MIM deficient mice displayed altered glutamatergic synaptic transmission and compatible behavioural defects. Collectively, our data identifies an essential morphogenetic pathway, which initiates spine protrusions by coupling phosphoinositide signalling, membrane bending and actin assembly together to ensure proper synaptogenesis.

Probing cortical circuit function with neuroprosthetic learning [short talk & board 25]
Prsa M, Galinanes G, Huber D
Department of Basic Neurosciences, University of Geneva, Switzerland
Neuroprosthetic learning, like the acquisition of motor skills, depends on sensory feedback. Current brain-machine interfaces (BMI) rely on natural senses to guide neuroprosthetic actions. Direct cortical activation, however, could substitute for lost sensations and offer a more flexible and faster feedback channel. We therefore tested whether natural cues can be replaced with cortical stimulation when learning a neuroprosthetic skill. We developed an optical BMI where motor cortex neurons were monitored with two-photon calcium imaging and a single neuron was conditioned to increase its firing rate to gain rewards, while feedback was continuously provided by optogenetic stimulation of cells in the somatosensory cortex. This artificial feedback signal was both necessary and sufficient for correct task performance and maintained the learned behaviour. Population imaging of the neighbouring layer 2/3 neurons revealed that the learning related changes were confined to the conditioned neuron. Our findings indicate that the brain is capable of attributing behavioural significance to fabricated neuronal communication channels that bypass both sensory and motor peripheries.

Wide-field imaging of genetically encoded calcium indicators during visual and audio-visual behaviour in mice [short talk & board 26]
Jacobs E, Okun M, Burgess CP, Steinmetz N, Shimaoka D, Carandini M, Harris K.
University College London, United Kingdom
Different behavioural states are associated with different brain states, but how these brain states influence sensory processing during behaviour remains an active area of research. We addressed this question by recording neural population activity using wide-field imaging of genetically encoded calcium indicators during visual and audio-visual behavioural tasks. We used a triple-transgenic strategy to express GCamp6f selectively in excitatory neurons and imaged their activity across visual, auditory and posterior somatosensory cortex. We trained mice to perform head-fixed visual and audio-visual two-alternative forced choice tasks and acquired a four second baseline in each trial in order to assess the brain state before stimulus onset. The stimuli consisted of Gabor gratings in different parts of the visual field, or the same stimuli accompanied by low or high...
Neuronal calcium changes during alpha-synuclein aggregation

P.H. Jensen, C. Betzer, L.B. Lassen, L. Reimer, J.P. Andersen
Biomedicine, Dandrite, Aarhus University, Denmark

Alpha-synuclein (AS) is a presynaptic protein causally linked to Parkinson’s disease, where it changes its native state into pathogenic aggregated forms. We have in cell and primary hippocampal neuron models demonstrated AS aggregation is accompanied by a decrease in cytosolic calcium. This is due to a direct activation of the ER Ca-pump SERCA by aggregated AS. Pharmacological inhibition of SERCA normalize the reduced Ca and protects the in vitro models and AS transgenic C. elegans against AS dependent degeneration.

We are currently planning a larger research program investigating the role of AS aggregate dependent activation of SERCA and accompanying effects on ER, mitochondria, nuclear Ca levels and their effects on neuronal functionality in terms of gene expression, electrophysiological properties, connectivity and survival. The studies will be conducted in cells, transgenic mouse brain slices and in vivo recordings on live animals. This will require an expansion of our functional imaging methodology. I expect participation in the Brain conference can help us get an optimal start for our research program.

Regional brain volumes, diffusivity, and metabolite changes after electroconvulsive therapy for severe depression


Objective: To investigate the role of hippocampal plasticity in the antidepressant effect of electroconvulsive therapy (ECT).

Method: We used magnetic resonance (MR) imaging including diffusion tensor imaging (DTI) and proton MR spectroscopy (1HMRS) to investigate hippocampal volume, diffusivity, and metabolite changes in 19 patients receiving ECT for severe depression. Other regions of interest included the amygdala, dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex, and hypothalamus. Patients received a 3T MR scan before ECT (TP1), 1 week (TP2), and 4 weeks (TP3) after ECT.

Results: Hippocampal and amygdala volume increased significantly at TP2 and continued to be increased at TP3. DLPFC exhibited a transient volume reduction at TP2. DTI revealed a reduced anisotropy and diffusivity of the hippocampus at TP2. We found no significant post-ECT changes in brain metabolite concentrations, and we were unable to identify a spectral signature at 1.30 ppm previously suggested to reflect neurogenesis induced by ECT. None of the brain imaging measures correlated to the clinical response.

Conclusion: Our findings show that ECT causes a remodelling of brain structures involved in affective regulation, but due to their lack of correlation with the antidepressant effect, this remodelling does not appear to be directly underlying the antidepressant action of ECT.

Cervical neurons shape locomotor activity

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Long direct reticulospinal projections have shown to be critical for the activation of the lumbar locomotor network. However, much less is known about the functional connectivity of the cervical propriospinal input during overground locomotion. Here, we first prove that the cervical neurons constitute an excitatory pathway to locomotor areas of the lumbar spinal cord. Further, we found that cervical spondylotic myelopathy (CSM), the commonest form of spinal cord injury, selectively disrupts the cervical propriospinal input, and not the reticulospinal, to the lumbar locomotor network. This finding was associated with specific loss of glutamatergic neurons in the rhythmogenic segments of lumbar cord and subsequent decrease in cadence, speed and mobility.
The loss of speed and cadence over time with intact reticulospinal tracts and early disruption of the cervical excitatory monosynaptic input onto excitatory lumbar neurons early in CSM indicate an important role of specific cervico–lumbar pathway in locomotion. To prove this, we initially ablated the lumbar projecting cervical neurons in naïve mice. Gait analysis during overground locomotion demonstrated that ablation of these neurons resulted in progressively decreased speed and cadence compared to controls mirroring the locomotion deficits seen in severe human and mice CSM. In addition stereological analysis demonstrated a decreased number of neurons in the lumbar enlargement of the AAV- DTA treated animals compared to controls.

To further confirm the role of lumbar projecting cervical neurons in initiating and maintaining locomotion, we next selectively silenced the lumbar-projecting cervical neurons using TeLC. Overtime, TeLC treated animals demonstrated decreased speed, cadence and overall mobility compared to controls. This phenotype was characterized by a significant increase in the inactive time and decrease in the mobile time compared to control mice indicative of a perturbed ability to initiate and maintain mobility. TeLC-mediated silencing closely reproduced the locomotor phenotype of CSM and DTA treated animals.

**Chronic sub-anesthetic ketamine stimulates formation of cortical dendritic spines in awake mice imaged longitudinally in the Mobile HomeCage [board 30]**

Khiroug, L; Kolikova, J; Marshall, P; Toptunov, D; Pryazhnikov, E

Neurotar Oy, Finland

**Aims**

Ketamine has attracted significant attention as a drug of abuse and as a rapidly-acting antidepressant. However, the synaptic alterations during repeated ketamine administration remain unknown. We studied the effects of chronic ketamine injections on spine formation and elimination in awake, behaving mice.

**Methods**

Young adult (2-3 m.o.) female mice expressing YFP in cortical layer 5 pyramidal neurons were head-fixed in the Mobile HomeCage device enabling microscopic imaging in the cortex of awake behaving mice. Ketamine (10mg/kg) or vehicle (PBS) was injected intraperitoneally and spines were imaged at 0, 6, 24, 72 and 120h, with ketamine/vehicle treatment at 1, 25, 49, 73 and 97h. Apical dendrites of layer 5 neurons in primary somatosensory cortex were imaged in layers 1 and 2 in 1um z-steps, up to 8 stacks per animal. Between 150 and 300 spines per animal were analysed.

**Results**

Ketamine doubled the spine formation rate at 120h as compared to the control PBS-treated group. Despite a trend to decrease the rate of spine elimination, this effect of ketamine was not significant. Overall, the control group displayed a net loss of spines, which was reversed by ketamine. The mobile/stable spine fraction ratio was unaffected by ketamine treatment.

**Conclusions**

Chronic 5-day once-daily treatment with ketamine strongly stimulates spine formation in mouse primary somatosensory cortex, reversing the net loss of spines observed in control animals.

**Serotonin 1B receptors elicit anxiolytic effects by gating specific inputs to prefrontal cortex [board 31]**

Kjaerby, C; Athilingam, J; Robinson, S.E. and Sohal, V.S.

Center for Basic and Translational Neuroscience, University of Copenhagen, Denmark

Serotonin plays major roles in mood and anxiety, and most commonly prescribed drugs for depression and anxiety disorders, as well as many antipsychotics, act via serotonergic mechanisms. The prefrontal cortex (PFC) is central to mood, anxiety, and psychotic disorders, and drugs like selective serotonin reuptake inhibitors (SSRIs), and atypical antipsychotics are believed to act, at least in part, by modulating serotoninergic mechanisms in the PFC. Nevertheless, we still do not fully understand the actions of serotonin in the PFC. More importantly, there is no direct evidence linking any prefrontal actions of serotonin to anxiety-related behaviours.

Here we describe a fundamentally new way in which serotonin can regulate PFC. Whereas most previous studies have focused on how serotonin acts via postsynaptic receptors to increase or decrease the excitability of specific neurons, we show that serotonin can act presynaptically through 5-HT1B receptors to suppress long-range inputs to layer 5 of the PFC. Moreover this suppression is input-specific: serotonin suppresses callosal and hippocampal inputs, but not those from MD thalamus.

We go on to show that activating 5-HT1B receptors in the PFC elicits anxiolytic effects and suppresses prefrontal theta power in vivo. We then "optogenetically instantiated" these effects in behaving mice, by optogenetically suppressing callosal or hippocampal inputs to PFC. We find that these manipulations elicit anxiolytic effects in behaving mice. Interestingly, nonspecific suppression of the entire PFC, or of thalamic inputs fails to elicit similar anxiolytic effects. Together, these results define a novel mechanism through which 5-HT potently modulates prefrontal regulation of anxiety-related behaviour by gating specific inputs and forms of activity within the prefrontal cortex.
The mice traversed a corridor and reported the position of a grating by either turning right or turning left at the end of it.

To address these questions we used 2 related to decision or to navigation, or a mixture of these and other signals. Transformations and decision making in the posterior parietal cortex (PPC) are intertwined with different sensory and motor areas. In primates it is believed to be involved in cognitive operations such as coordinate transformations and decision-making. In rat PPC, some experiments revealed decision signals, but others revealed signals related to navigation. Does rodent PPC carry signals related to decision or to navigation, or a mixture of these and other signals?

To address these questions we used 2-photon calcium imaging to record population of neurons in PPC of mice that performed a contrast discrimination task in a virtual T-maze. The mice traversed a corridor and reported the position of a grating by either turning right or turning left at the end of it.

**Gamma oscillations mediate top-down control of feeding**

M. Carus Cadavieco, M. Gorbati, S. van der Veldt, N. Denisova, F. Ramm, F. Bender, K. Deisseroth, A. Ponomarenko, T. Korotkova

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2. Howard Hughes Medical Institute, USA
3. Department of Bioengineering
4. Department of Psychiatry and Behavioural Sciences, Stanford University, USA

How the brain initiates, maintains and coordinates innate behaviours is largely unknown. Lateral hypothalamus (LH) is crucial for the regulation of innate behaviours, including food intake and sleep-wake cycle. We have recently shown that LH GABA cells control arousal (Herrera et al., Nature Neuroscience, 2015) whereas theta-rhythmic input onto LH cells regulates locomotion (Bender et al., Nature Communications, 2015). Yet little is known about the regulation of LH by top-down inputs from cognitive control regions. Combining optogenetics and chemogenetics (DREADDs) with multisite electrophysiological recordings in behaving mice, we report that gamma oscillations coordinate signalling between medial prefrontal cortex (mPFC), lateral septum (LS) and LH. We show that gamma-rhythmic signalling 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 behaviour and improves performance in a food-rewarded learning task. Overall we show that mPFC-LS-LH gamma signalling regulates feeding behaviour by dynamic reorganization of functional cell groups in hypothalamus.

**Investigating neural dynamics in the zebrafish habenula**

Krishnan S, Cheng R-K, Jesuthasan S.

NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore

The behaviour of an animal depends on the context, reward value of sensory stimuli and current brain state. These factors lead to the selection of a functional network that enables specific behaviours, via the release of appropriate neuromodulators. How the optimal set of neuromodulators is selected is unknown. To address this question in a vertebrate, we use a series of optical techniques, including activity imaging, single neuron ablation and optogenetics, and work with intact larval zebrafish. The evolutionarily conserved habenula complex, located in the diencephalon, receives input from sensory systems and basal ganglia, and projects to the raphe, VTA and locus coeruleus, making it candidate for a selector of brain mode. We have found that the habenula responds to variety of rewarding and aversive sensory stimuli, differentiates novel stimuli, exhibits experience dependant modulation, responds to change in stimulus states and has large amounts of spontaneous activity making it computationally and technically interesting to study.

Specifically, high-speed, three dimensional calcium imaging, using a two-photon resonance scanner, revealed reproducible activity patterns to light versus dark in the zebrafish habenula. Discrete pulses of light in an otherwise dark environment produced both excitatory, inhibitory, tonic and sustained responses to both light ON and OFF. PCA revealed two distinct attractor networks and a phase transition that indicated the switch between light and dark. Since zebrafish larvae choose to stay in light over the dark, and cells responding to dark also responded to electric shock, this hints at an unique context based circuitry. To characterise the current state of the habenula network, an essential parameter in understanding information transformation here, it will be necessary to record voltage. To do this at single cell resolution, we are currently testing several new genetically encoded voltage indicators as well as new imaging modalities like SPIM and wide-field two photon.

**On representation of space and choice in mouse parietal cortex during virtual navigation**

Krumin M, Harris KD, Carandini M.

University College London, United Kingdom

Posterior parietal cortex (PPC) is interconnected with different sensory and motor areas. In primates it is believed to be involved in cognitive operations such as coordinate transformations and decision-making. In rat PPC, some experiments revealed decision signals, but others revealed signals related to navigation. Does rodent PPC carry signals related to decision or to navigation, or a mixture of these and other signals?

To address these questions we tested 2-photon calcium imaging to record population of neurons in PPC of mice that performed a contrast discrimination task in a virtual T-maze. The mice traversed a corridor and reported the position of a gratings by either turning right or turning left at the end of it.
The activity of PPC neurons was strongly modulated by the mouse’s virtual position along the main corridor (z) and virtual head direction (θ). Many of the cells had localized ‘space fields’ in these coordinates, and this z-θ model gave a prediction of each neuron’s activity with explained variance of up to 89% (21±16% for all active cells). These space fields could occur at any location in z-θ coordinates, and indeed the neurons recorded in a single session typically tiled the T-maze. Neural activity was also modulated by other sensory or behavioural parameters, e.g. the visual stimulus, choice, or the motor actions of the mouse. However, for the majority of the cells the space fields accounted for the largest fraction of the neural responses.

These results suggest that mouse PPC can play a key role in visually guided behaviour. Although this region might play other roles in other task conditions, the large fraction of variance that could be predicted by spatial variables alone suggests that our mice have adopted a strategy in which PPC is involved in spatial computations, rather than choice representation.

**Turtle visual cortex studied by combining wide-field calcium imaging and electrophysiology [board 35]**

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Department of Neural Systems, Max Planck Institute for Brain Research, Germany

Reptilian cerebral cortex contains 3 layers. Pyramidal cells are found only in layer 2, which is thought to be equivalent to layers 5/6 of mammalian isocortex. Turtle cortex offers experimental advantages: it is resistant to anoxia and operates at room temperature. Cortical slabs can be cut in orientations that minimize lesions and keep inter-neuronal connections intact. The entire brain can even be extracted and kept in vitro for several days.

Processing of visual information in turtle cerebral cortex is not understood, and clear single-neuron receptive field properties have yet to be discovered there. We show network analysis of activity in the visual cortex of turtles (Trachemys scripta/Chrysemys picta) using a combination of electrophysiology and calcium imaging. Advanced image processing enables calcium imaging in wide-field mode and thus the fast monitoring of large fields of view in combination with electrophysiological methods. Sensitivity is sufficient to resolve sub-threshold signals in cell bodies and calcium signals elicited by single action potentials can be detected even in dendrites. Using AM-dyes, hundreds of neurons can be stained and monitored. Depth penetration sufficient to image cortical layer 2 from the ventricular surface in an ex vivo preparation (>100μm) is obtained.

By combining imaging with patch-clamp recordings of one or a few interneurons, the distributed and coordinated effects of single interneurons on tonically active neurons can be analysed.

By combining imaging with multi-electrode array (MEA) recordings, electrical signals from hundreds to thousands of spiking neurons can be observed. After spike sorting (i.e. the assignment of the electrical signals to discrete neurons) electrical activity can be correlated with calcium signals enabling a broad ensemble view of stimulus-evoked and spontaneous activity in this system.

1 Aboitz, F; Zamorano, F, Front Neuroanat 2013, 7:38.
2 Fournier, J; Christian, M; Laurent, G, Current Opin Neurobiol 2014, 31:119

**Brain on the run: Whole brain calcium imaging in freely swimming larval zebrafish [short talk]**

Dalhyung Kim, Wenchao Gu, Jennifer Li, Drew Robson
Rowland Institute at Harvard, USA

Cellular resolution calcium imaging typically requires an animal to be tethered under a microscope, but that significantly restricts the range of behaviours that can be studied. To expand the behavioural repertoire amenable to imaging, we have developed a microscope system that enables whole brain calcium imaging in freely swimming larval zebrafish. We use high speed infrared imaging to track the target animal while it moves freely in a 60 mm circular arena, roughly 15 times the body length of the animal. Based on the predicted trajectory of the brain, we apply optimal control theory to a motorized stage system to cancel brain motion in three dimensions. This motion cancellation system overcomes the immense technical challenges posed by this animal, including a peak acceleration of 20 m/s², similar to the acceleration of a Formula One race car. We have combined this system with structured illumination fluorescence microscopy to stably image the entire brain of a freely swimming larval zebrafish for over an hour. This work opens the door to comprehensive neural recording and stimulation during natural vertebrate behaviour.

**Concurrent fast imaging and optogenetic inhibition in the intact mammalian brain [board 36]**

Moretti C, Bovetti S, Zucca S, Dal Maschio M, Bonifazi P, Fellin T
Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, Italy

Combining fast functional imaging with optogenetics would provide a powerful approach to dissect neural circuit function. However, performing high-speed imaging and optogenetic perturbation simultaneously has been difficult to establish in the mammalian brain, because of the poor signal-to-noise ratio that characterizes fast functional imaging and because of potential cross-talk between the different wavelengths that are used for imaging and for optogenetic stimulation. Here, we developed a technique based on
patterned two-photon illumination to perform high-speed scanless imaging of GCaMP6 signals during single-photon optogenetic inhibition of Archaerhodopsin. Combining imaging and electrophysiological recordings in the intact mouse brain, we first showed that single and short bursts of action potentials in pyramidal cortical neurons could be detected with millisecond precision and higher signal-to-noise ratio in the scanless configuration compared to the raster scanning approach. We then demonstrated that our optical system removed the artifacts in the fluorescence detection that were induced by single-photon optogenetic illumination and that prevented concurrent imaging and optogenetic perturbation in the laser scanning configuration. As proof of concept, we applied our technique to investigate the role of parvalbumin-positive interneurons in the regulation of spontaneous network dynamics. Optogenetic inhibition of parvalbumin-positive cells significantly increased correlation of calcium signals in layer 2/3 neurons expressing GCaMP6. By allowing the readout of network responses to the optogenetic perturbation with unprecedented spatiotemporal resolution, concurrent fast imaging and optogenetic manipulation will likely represent a fundamental tool to understand the function of mammalian neural networks.

Whole-brain calcium imaging with cellular resolution in freely behaving Caenorhabditis elegans [board 37]
Jeffrey P. Nguyen, Frederick B. Shipley, Ashley N. Linder, George S. Plummer, Mochi Liu, Sagar U. Setru, Joshua W. Shaeowitz, and Andrew M. Leifer
Lewis Sigler Institute, Princeton University, USA

The ability to acquire large-scale recordings of neuronal activity in awake and unrestrained animals is needed to provide new insights into how populations of neurons generate animal behaviour. Acquiring this data, however, is challenging because it is difficult to track and image individual neurons as an animal deforms its posture and moves many body lengths. Here, we present an instrument capable of recording intracellular calcium transients from the majority of neurons in the head of a freely behaving Caenorhabditis elegans with cellular resolution while simultaneously recording the animal’s position, posture, and locomotion. 3D volumetric fluorescent images of neurons expressing the calcium indicator GCaMP6s are recorded at 6 head-volumes/s using spinning disk confocal microscopy. At the same time, we record low magnification images of the animal to measure the animal’s behaviour and track its head as it moves. We develop a time independent neuronal matching algorithm that uses non-rigid point set registration and machine learning to correctly match neurons across time. The algorithm performs favorably when compared to human annotated neural tracking. This method allows us to observe calcium transients from up to 140 neurons for over 8 minutes and correlate the animal’s neural activity with its behaviour.

Sleep stage-specific regulation of cortical excitation and inhibition [board 38]
Niels Niethard, Masashi Hasegawa, Takahide Itokazu, Carlos N. Oyanedel, Jan Born, Takashi R. Sato
Institute for Medical Psychology and Behavioural Neurobiology, University of Tübingen, Germany

Sleep is characterized by unique patterns of cortical activity alternating between the stages of slow wave sleep (SWS) and rapid-eye movement (REM) sleep. How these patterns relate to the balanced activity of excitatory pyramidal cells and inhibitory interneurons in cortical circuits is unknown. We investigated cortical network activity during wakefulness, SWS, and REM sleep globally and locally using in vivo calcium imaging in mice. Wide-field imaging revealed a reduction in neural activity during SWS, compared with wakefulness and, unexpectedly, a further profound reduction in activity during REM sleep. Two-photon imaging on local circuits showed that this suppression was associated with reduced activity of pyramidal cells, accompanied by activation of PV+ interneurons, but not SOM+ interneurons. PV+ interneurons most active during wakefulness were also most active during REM sleep. Our results reveal a sleep-stage specific regulation of the cortical excitation/inhibition balance, with PV+ interneurons conveying maximum inhibition during REM sleep, which might help shape memories in these networks.

Associative plasticity in the auditory cortex with single-cell resolution using two-photon microscopy and a chronic cranial window [board 39]
Zelenka O, Novak O, Syka J
Institute of Experimental Medicine, ASCR, Czech Republic

The sensory cortex is able to modify its function based on preceding experience in order to optimize processing of behaviourally relevant stimuli. In the auditory cortex, tonotopic reorganization follows various types of learning. Fear conditioning leads to expansion of the regions representing the conditioned stimulus. However, the extent of this plasticity as described by classical electrophysiological approaches has been unclear at the level of populations of neurons. We used two-photon calcium imaging in vivo to elucidate functional plasticity in the auditory cortex with single-cell resolution. In mice with an implanted chronic cranial window and neurons expressing an ultrasensitive calcium indicator GCaMP6 in the auditory cortex, we measured the coding properties of exactly reidentified neurons before and after fear conditioning. In different subsets of neurons, both shifts towards the CS+ and CS- were present. The shift direction was inversely dependent on the neuron’s best frequency before fear conditioning. Neurons with low initial best frequencies retuned upwards and vice versa. In some neurons no tuning shifts were observed, even when they were intermingled with the retuned neurons. This evidence contradicts the view of the simple expansion of CS+ tuned regions. We also observed a broadening of receptive fields after fear conditioning. Behaviourally, these changes were accompanied by selective spatial attention towards the conditioned stimuli. The adaptive purpose of these learning-induced physiological and behavioural changes can possibly be explained as an information processing optimization, improving the ability to discriminate
between threatening (CS+) and non-threatening (CS-) stimuli. The orienting responses towards CS+ were more often followed by escape behaviour, instead of conventional freezing reactions. The probability of the escape behaviour was inversely dependent on footshock intensity.

A large field-of-view two-photon microscope (mesoscope) with subcellular resolution for in vivo imaging [board 40]

Sofroniew, N. ¹, Flickinger, D. ², King, J. ², Svoboda, K. ¹
¹ Janelia Research Campus, Ashburn VA 20147, USA
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Poster Session (Tuesday, 18 April: 10:20 - 12:00)

Encoding of food intake and arousal by GABAergic neurons in the lateral hypothalamus [board 41]

Lukas Oesch, Carolina Gutierrez Herrera, Ivan Bozic, Antoine Adamantidis
Zentrum für experimentelle Neurologie (ZEN), University of Bern, Switzerland

In mammals, the sleep-wake cycle and feeding behaviours are very conserved processes engaging a broad range of brain regions. The hypothalamus has a key role in the integration and regulation of these two behaviours as it receives information from intra- and extra hypothalamic networks. Here we investigate how GABAergic cells in the lateral hypothalamus (LHGABA) modulate food intake and arousal.

We selectively manipulated neuronal activity by targeting expression of channel-rhodopsin 2 (ChR2) to LHGABA cells in the lateral hypothalamus from tg(vgat)::cre freely-moving mice. State-dependent optogenetic stimulation of LHGABA cells through a chronically implanted optic fiber at 1, 20 Hz for 10 s or 1 s continuous illumination during NREM sleep elicited rapid arousal. Interestingly, all these stimulation, but 1 Hz, initiated food intake when the animal was awake, as well as immediately after arousal. In a semi-chronic, state-invariant protocol (10 s at 20 Hz every minute over 1 h) both food consumption and the number of feeding events were higher in ChR2 compared to control animals.

In an effort to track the activity of the stimulated cells during sleep and food intake, we monitored calcium transients in GCaMP6s-expressing LHGABA neurons using an integrated miniature fluorescence microscope through a gradient refractive index lens. Consistent with our optogenetic results, we found that the activity of GCaMP6s-expressing LHGABA neurons strongly correlate with food intake behaviour. Over the time course of the feeding event the calcium transients decayed back to the level observed for locomotion (i.e., active wakefulness). Furthermore, during NREM sleep and immobility the calcium transients were lower than during movements and active behaviours.

These findings indicate that LHGABA cells control both sleep and metabolic function. Further research will investigate possible subsets of LHGABA neurons and their respective thresholds.

Sensory context and state-dependent modulation of inhibitory circuit activity in mouse V1 [board 42]

Pakan JMP, Lowe SC, Dylda E, Keenink SW, Coutts CA and Rochefort NL. Centre for Integrative Physiology, University of Edinburgh, United Kingdom

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 is mediated through inhibitory neurons resulting in the disinhibition of pyramidal neurons during locomotion. We tested this model using two-photon calcium imaging to record the activity of three distinct non-overlapping populations of inhibitory neurons (expressing either parvalbumin [PV], somatostatin [SST] or vasointestinal peptide [VIP]) in layers 2/3 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 hypothesis of pyramidal neuron disinhibition through SST neurons.

In addition, we found that responses of inhibitory neurons to locomotion strongly differed in darkness and during visual stimulation, revealing a context-dependent cell-type specific response to locomotion 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 according to the sensory and behavioural context of the animal. We propose a new model of how locomotion modulates neuronal activity in V1, highlighting the dynamic nature of interneuron function that strongly depends on the sensory and behavioural context of the animal.
Understanding the pathophysiological role of a novel SLC25 family protein in modeled neurological disease

Papastefanaki F, Segkla K, Charalambous M, Terzenidou M, Douni E, Matsas R
Neurobiology Department, Hellenic Pasteur Institute, Greece

Dominant optic atrophy (DOA) and axonal peripheral neuropathy Charcot-Marie-Tooth type 2 (CMT2) are heterogeneous neurodegenerative disorders mainly caused by mutations in nuclear genes encoding mitochondrial proteins. Mutations in a nuclear gene encoding a novel member of the mitochondrial SLC25 (solute carrier) protein family with unknown function, have been recently identified in patients with both DOA and CMT2. Following forward genetics through random mutagenesis in mice, a nonsense point mutation was identified in this gene that introduces a premature stop codon causing severe recessive neurological disease characterized by ataxia, tonic-clonic seizures, reduced muscle strength, growth retardation and early lethality. Histopathological analysis revealed that the mutant mice exhibit disorganization of ganglion cell axons extending from the retina to the optic nerve, indicative of optic atrophy. In line with the ataxic phenotype, morphological analysis demonstrated a smaller cerebellum with pronounced abnormalities, including: a) a narrower molecular layer with impaired dendritic arborisation of Purkinje cells (PCs); b) reduced vesicular distribution of parallel fiber-PC synapses and climbing fiber-PC synapses as testified by immunoreactivity of the vesicular glutamate transporters vGLUT1 and vGLUT2; c) reduction in the PC calcium-binding protein calbindin. Given the muscle atrophy of mutant mice, also observed in CMT2 patients, we investigated the neuromuscular junctions in the mutant’s diaphragm. We noted an increase in the number, but also in the density of endplates, resulting in tighter endplate distribution along the muscle. Further several abnormalities were observed in endplate innervation, including poly-innervation, increased axonal arborisation, axon terminal protrusions extending beyond the endplate boundaries and bifurcation of single axons into two endplates, none of which were noted in wild-type mice, and are indicative of defects in synapse elimination during neuromuscular junction development. Our analysis shows that the mutant mice phenocopy the hallmarks of a complex human disease and constitute a useful tool for elucidating its pathogenesis.

Neuronal Activity Analysis Tool

Juan Pablo Prada Salcedo, Robert Blum and Thomas Dandekar
Bioinformatics Department, University of Würzburg, Germany

Neuronal activity detection is a key component in the study of several phenomena in the brain, for example signalling cascades occurring in the neurons or network interactions among neuron clusters. This neuronal activity can now be visualized with the help of calcium imaging. The analysis of the videos resulting from calcium imaging is an expensive process in terms of time and money. The tool presented on this paper does an automatic analysis of the videos, informing accurately where and when the activity occurred. It also has the option to automatically detect a general change in the activity tendency, which can be useful on studies of activity enhancement or inhibition. It relies on ImageJ and R (WMTSA package). After equalizing the videos, signals are extracted and pixel intensities calculated then, using Wavelet Transform, peaks are detected and filtered by occurrence resulting from calcium imaging is an expensive process in terms of time and money. The tool presented on this paper does an automatic analysis of the videos, informing accurately where and when the activity occurred. It also has the option to automatically detect a general change in the activity tendency, which can be useful on studies of activity enhancement or inhibition. It relies on ImageJ and R (WMTSA package). After equalizing the videos, signals are extracted and pixel intensities calculated then, using Wavelet Transform, peaks are detected and filtered by occurrence.

Rescue of impaired reward learning in mouse model of fragile X syndrome

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Department of Neurophysiology, Nencki Institute of Experimental Biology, PAS, Poland

Loss-of-function mutations in the fragile X mental retardation protein (FMRP) result in fragile X syndrome (FXS), which is the most wide-spread single-gene cause of autism. FXS is a trinucleotide repeat disorder in which a CGG element located within the 5’ untranslated region of FMR1 gene expands and becomes hypermethylated. In case of the complete silencing of FMR1 gene male patients have an average IQ of 40 and cope with severe cognitive impairments and excluding learning disabilities.

In our studies we exploited a mouse model of FXS (Fmr1 knockouts) mimicking above-described phenotype in humans. As FMRP is a major local-translation suppressor its lack leads to overexpression of many synaptic plasticity proteins. Among those matrix metalloproteinase-9 (MMP-9) is a crucial player in reward-motivated learning, specifically its proper level in central amygdala (CA) is required for mice’ ability to discriminate between highly-motivating reward and neutral stimuli.

Using fully automated behavioural testing (IntelliCage system), which enabled us to investigate characteristics that can be observed only in a continuous, long-lasting study, we showed that Fmr1 knockouts are unable to efficiently perform such discrimination tasks. However, downregulation of heightened MMP-9 level in central amygdala was sufficient to fully rescue this impairment. This effect was obtained by a local injection of nanoparticles gradually releasing selective MMP-9 inhibitor (TIMP-1). It is noteworthy, that nanoparticles are able to cross brain-blood barrier and thus the implemented paradigm holds promise of obtaining clinically relevant solutions for the most severely disabled FSX patient.
Impact of intracellular α-synuclein aggregation on subcellular Ca2+ homeostasis and Ca2+ dependent response [board 46]
Reimer L, Jensen PH.
Department of Biomedicine/DANDRITE, Aarhus University, Denmark

The small unfolded presynaptic protein α-synuclein (AS) is involved in maintenance of the neurotransmitter release machinery. However, AS play a less fortunate role, in familial and sporadic Parkinsons Disease (PD) and other neurodegenerative disorders characterized by the presence of AS-containing intracellular inclusions in selective neuronal and glial populations. Ca2+-dyshomeostasis has previously been found to play a significant role in PD, while recent cell experiments, suggest that elevated levels of intracellular free Ca2+ can cause a significant increase in the proportion of cells showing AS aggregates.

In line with this, our preliminary data supports a link between Ca2+-dyshregulation and AS mediated toxicity. i) intracellular aggregation of AS was found to cause a biphasic cellular Ca2+ response. Neutralization of this response by sarco/endoplasmic reticulum Ca2+-ATPase (SERCA)-inhibitor, CPA, reverted the cytosolic Ca2+-increase and cellular degeneration. ii) we also identified SERCA as a target for aggregated AS by a proteomic screen, where AS aggregates were found to activate the pump. iii) this study identified several other AS-interacting proteins, that are either being regulated by Ca2+ or act as cellular Ca2+-regulators themselves, including pumps, channels, molecular scaffolds and enzymes.

Hence we hypothesize early stage AS aggregates to initiate the degenerative process by binding and deregulating decisive i) calcium regulatory molecules like SERCA and VDAC1 and ii) tethering molecules that position Ca2+-storage organelles in functional apposition. Using a core of live-cell Ca2+-imaging techniques such as Fura-2 and GCaMP at specific subcellular sites in different cell-lines and primary cultures we will characterize critical molecules involved in Ca2+-dyshomeostasis upon AS aggregation. Furthermore, using optogenetics, at milli second time scales on cell-lines, primary cultures and in vivo, we will study the restoration of subcellular Ca2+-levels post depolarization under stressful AS-aggregation conditions and attempt to rescue potential Ca2+-dyshomeostasis.

CLARITY-compatible lipophilic dyes for electrode marking and neuronal tracing [board 47]
Kristian H. R. Jensen, Rune W. Berg
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Fluorescent lipophilic dyes, as DiI, stain cellular membranes and are applied extensively in retrograde/anterograde labelling of neurones, and marking the position of extracellular electrodes after electrophysiology.

Convenient histological clearing techniques, as CLARITY, enable immunostaining and imaging in large volumes for 3D-reconstruction. However, such clearing works by removing lipids and, as an unintended consequence, also lipophilic dyes.

To remedy this wash-out, the molecular structure of the dye can be altered to adhere to both membranes and proteins so the dye remains in the tissue after lipid-clearing.

Nevertheless, the capacity of such modified dyes to remain in tissue has not yet been tested. Here, we test dyes with molecular modifications that make them aldehyde-fixable to proteins. We use three dyes: CM-DiI, SP-DiI and FM 1-43FX, which are DiI-analogues, modified to be CLARITY-compatible candidates. We use the challenging adult and myelin-rich spinal cord tissue, which requires prolonged lipid-clearing, of rats, mice and turtles. All three dyes remained in the tissue after lipid-clearing, but CM-DiI had the sharpest and FM 1-43FX had the strongest fluorescent signal.

Brain on the run: Whole brain calcium imaging in freely swimming larval zebrafish [short talk]
Rowland Institute at Harvard, USA
Dalhyung Kim, Wenchao Gu, Jennifer Li, Drew Robson

Cellular resolution calcium imaging typically requires an animal to be tethered under a microscope, but that significantly restricts the range of behaviours that can be studied. To expand the behavioural repertoire amenable to imaging, we have developed a microscope system that enables whole brain calcium imaging in freely swimming larval zebrafish.

We use high speed infrared imaging to track the target animal while it moves freely in a 60 mm circular arena, roughly 15 times the body length of the animal. Based on the predicted trajectory of the brain, we apply optimal control theory to a motorized stage system to cancel brain motion in three dimensions. This motion cancellation system overcomes the immense technical challenges posed by this animal, including a peak acceleration of 20 m/s^2, similar to the acceleration of a Formula One race car. We have combined this system with structured illumination fluorescence microscopy to stably image the entire brain of a freely swimming larval zebrafish for over an hour. This work opens the door to comprehensive neural recording and stimulation during natural vertebrate behaviour.
Rewiring of spinal respiratory neural network via cervical glutamatergic interneurons preserves respiratory function in progressive cervical spinal cord injury [board 48]

K. Satkunendrarajah, S.K. Karadimas, M. Khazaeei, Patricia Samson, M.G. Fehlings
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Cervical spinal cord injury (SCI) disrupts the spinal respiratory neural circuitry leading to the loss of diaphragmatic and intercostal muscle function. While acute traumatic SCI leads to significant ventilatory impairment, cervical spondylotic myelopathy (CSM), in which the cervical spinal cord is progressively injured over time, results in milder respiratory dysfunction despite significant disruption of the cervical respiratory neural network.

Using a unique mouse model of CSM, we found a significant loss of phrenic motoneurons (PMNs) that innervate the main inspiratory muscle, yet these animals do not exhibit severe respiratory insufficiency similar to what is observed in human CSM patients. Progressively increased Vglut2 positive boutons on preserved PMNs indicates that despite the significant loss in the number of PMNs, respiratory motor output is maintained via compensatory and progressive increases in glutamatergic input onto preserved PMNs. Moreover, the number of prephrenic cervical interneurons labeled with PRV-152 was significantly increased compared to sham animals suggesting that the increased glutamatergic presynaptic inputs directly arise from prephrenic cervical interneurons in CSM. To further confirm this we injected a PRV-152 into the diaphragm of CSM and sham Vglut2::cre; tdTomato mice. We observed significantly increased tdTomato+/GFP+ cells in the cervical spinal cord of CSM mice compared to sham mice. Next we set out to decipher if the increase in the connectivity between prephrenic cervical glutamatergic interneurons and PMNs under CSM are the strengthening of connectivity that existed early during development. Hence, we injected RbΔG-GFP and AAV-RG into Vglut2::cre; tdTomato mouse pups at postnatal day 4 (P4).

In conclusion, this study provides novel insights into the alterations in spinal respiratory networks that occur in the setting of CSM and a greater understanding of the neural control of breathing. Specifically, we show that chronic and progressive nature of this disease drives the respiratory neural network to early developmental stages.

In vivo and ex vivo multiphoton imaging of brain circuits involved in Alzheimer’s Disease [board 49]
Bioengineering Department, Imperial College London, United Kingdom

One of the primary motivations behind the development of new approaches to image and manipulate cortical circuits is to advance our understanding, and ultimately ability to treat, brain disorders. Alzheimer’s Disease, a neurodegenerative disorder which, due to aging demographics, affects an increasing fraction of the population, is a priority in this respect. In this poster we describe a new multi-pronged project to image changes in the spatiotemporal dynamics of cortical and hippocampal circuits during age in the APP23 mouse model of Alzheimer’s Disease. Using whole brain ex vivo two photon tomography, we image the development of amyloid plaques throughout the mouse brain. Using two photon imaging of an in vitro brain slice preparation, we image spatiotemporal dynamics in large neural populations in hippocampus and neocortex, characterising changes in information flow during spontaneous activity between APP23 mice and age-matched controls. Using in vivo two photon imaging of neocortical neural ensembles labelled with Cal-520 AM, we examine changes in cortical circuit dynamics as a function of local amyloid density. We aim to develop sensitive markers for changes in information processing in hippocampus and neocortex that can be used to validate therapeutic strategies.

Sleep Changes in a Rat Prenatal Stress Model of Depression [board 50]
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Major depression is one of the most frequently occurring mental health disorders, but is characterized by diverse symptomatology. Sleep disturbances, however, are commonplace in depressive patients. These alterations include increased duration of Rapid Eye Movement Sleep (REMS) and increased sleep fragmentation. Stressful life events during the second trimester of human pregnancy increase the risk of depression in the offspring. Similarly, rodents exposed to prenatal stress (PNS) during gestation express depression-like behavioural changes.

Accordingly, we investigated sleep changes in a rat PNS model of depression, to elucidate whether these are similar to those seen in clinical depression.

Pregnant Sprague-Dawley rats were submitted to repeated variable stress during gestational days 13-21. The young adult offspring were surgically implanted with electrodes for subsequent electroencephalographic determination of sleep-wakefulness state. As traumatic episodes can trigger episodes of clinical depression, we also investigated effects of an acute stressor during the recording period.
PNS animals (n=21) had an 82% increase in amount of REMS (11.6±1.4% vs 6.3±0.9%; p<0.05) during the first hours of the dark phase, compared to controls (n=24). Interestingly, this was due to a larger number of REMS bouts (16±2 vs 10±2; p<0.05), rather than altered bout lengths. After acute stressor-exposure, control animals had 68% more REMS after lights-off, compared to the day before (p<0.05; n=21-24). The PNS-related increase in REMS after lights-off (p<0.05), was also seen after acute stress (43%), but to a lesser extent than on the baseline day. REMS rebound thus seems blunted in PNS animals. PNS alters sleep-wakefulness behaviour under baseline conditions and after acute stress. This underscores the value of the PNS model for addressing scientific questions regarding core symptoms of depression.

Probing the retinal circuit with computer generated holography and two photon calcium imaging [board 51]
Spampinato G. L., Ronzitti E., Papagiakoumou E., Khabou H., Dalkara D., Picaud S, Marre O., Emiliani V.
The Vision Institute, France

A major purpose of neuroscience is to understand how a neural circuit generates complex activity patterns in response to sensory stimuli. While recent works have made possible to access the full connectome of a neural circuit, there is still a gap between its detailed anatomical reconstruction, and the functional characterization of its neural responses. To bridge this gap, we need to understand how the perturbation of each neuron will influence the activity of the circuit, and reconstruct the complete functional connectivity diagram. Here we have developed an all optical method to measure systematically the functional connectivity between different neural layers, and applied it to the retina. A key component of retinal processing is the information transfer from the intermediate bipolar cell layer - which integrates the photoreceptor responses - to the ganglion cell layer - the output of the retina.
We expressed Channelrhodopsin2 in ON bipolar cells and GCaMP6s in the ganglion cells of wild type mice. We then performed 2 photon imaging of dozens of ganglion cells, while we used 2 photon computer generated holography focused in the bipolar layer to stimulate single cells.
Thanks to this 3D probing of the retinal circuit, we could measure the impact of single bipolar cell stimulation on the ganglion cells. We were able to reconstruct the projective fields of each bipolar cell, i.e. the ensemble of ganglion cells that responded to their stimulation. Finally, our method also allowed us to stimulate several bipolar cells simultaneously to measure the impact of complex stimulation patterns on the ganglion cell layer.
This method paves the way towards complete functional connectomics of the retina, and could potentially be applied to any layered structure in the brain.

Super resolution microscopy in brain tissue of the fruit fly reveals protein organization of synapses [board 52]
Spühler IA, Conley GM, Sprecher SG, Scheffold F
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Visualizing fine details of synapses using optical microscopy remains a major technical challenge. Super resolution microscopy opens the possibility to image and study features of synapses beyond the diffraction limit. With direct stochastic optical reconstruction microscopy, dSTORM, we imaged the localization pattern of pre- and postsynaptic proteins in the brain tissue of the fruit fly, Drosophila melanogaster. Imaging structures in tissue is demanding due to light scattering and increased background signal. We took advantage of the genetic tools available in Drosophila and imaged fluorescently tagged synaptic proteins expressed in a small number of olfactory projection neurons and Kenyon cells within the calyx of the mushroom body, a distinct brain region involved in associative memory formation. Applying dSTORM on brain sections allowed us to localize synaptic proteins and to quantify nanoscale features. We strongly believe that nanoscopic characterization of synapses involved in associative learning can lead to a better understanding of synaptic plasticity and memory formation.

High temporal and spatial resolution optogenetic via opsins engineering and sculpted light illumination [board 53]
Shemesh O, Tanese D, Zampini V, Linghu C, Ronzitti E, Papagiakoumou E, Boyden E, Emiliani V.
CNRS-University Paris Descartes, France

Targeting different regions of neurons for optogenetic control may enable the analysis of how specific subcellular compartments -axons, dendrites, and cell bodies- contribute to neural computation. Ideally such targeting would involve joint engineering of optical as well as molecular technologies, to enable as detailed control as possible of subcellular events.
We are screening different opsins for optimal performances (high light sensitivity, fast kinetics) under two-photon illumination and different trafficking sequences that may enable subcellular regions to be targeted for optogenetics expression.
We use holographic photostimulation and temporal focusing to address these subcellular regions with micrometer spatial resolution and millisecond temporal resolution.
Our goal is to eventually arrive at a toolbox that will enable arbitrary sections of cells to be depolarized or hyperpolarized, at arbitrary points in a 3-d brain circuits in a living mammal.

**Functionally distinct parallel circuits in the hippocampus** [board 54]
Annapporani Udhayachandran, Pico Caroni
Friedrich Miescher Institute, Switzerland

The hippocampal formation is one of the primary structures involved in episodic memory. The hippocampus consists of three main subdivisions interconnected through feed forward excitatory connectivity: the dentate gyrus (DG), Cornu Ammonis 3 (CA3) and Cornu Ammonis 1 (CA1). There are three or more principal neuron subpopulations in the hippocampus of which subpopulations Lsi1 and Lsi2 are revealed by mGFP expression in Thy1-mGFPpreporter lines (Deguchi et al, 2011). Together, Lsi1 and Lsi2 constitute about 50% of total hippocampal principal neuron populations, and account for the earlier half of neurogenesis. Notably, Lsi1and Lsi2 principal neurons are genetically matched and preferentially interconnected (30-94% selectivity) with neurons of the same subpopulation across hippocampal subdivisions: DG-CA3 (mossy fiber input), CA3-CA3 (recurrent collaterals), CA3-CA1 (Schaffer collaterals) (Deguchi et al, 2011), thereby establishing partially separate parallel circuits in the hippocampus. This selective connectivity is achieved by matched windows of neurogenesis and synaptogenesis. In this study, I investigated the involvement of hippocampal principal neuron subpopulations in learning and memory by analysing plasticity marker expression after learning in all principal neurons vs Lsi1 or Lsi2 principal neurons. We find that Lsi1 pyramidal cells specifically express cFos upon incremental learning whereas Lsi2 pyramidal cells specifically express cFos upon definite learning. Short-term plasticity as revealed by pERK accumulation was initially detected in both subpopulations, but only one of them was recruited for long-term memory consolidation. Interfering with the development of preferential Lsi1 connectivity led to an abnormal Lsi1 c-fos plasticity upon fear learning, and to generalized fear learning.

**Real time imaging of clot formation and the study of astrocyte injury during prolonged ischemia** [board 55]
Valentino M, Zammit R, Zammit C, Muscat R
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Astrocytes are the predominant glial-cell type of the neurovascular unit but little is known about their functional impact during ischemia. We describe the use of a photothrombotic technique employing a tightly focused green laser to optically excite the circulating photosensitizer rose bengal whilst visualization in real time clot formation and astrocyte injury. This method ensures that instances of spontaneous clot dissolution and recanalization do not precipitate and confirms that a stable clot is formed and maintained throughout the observation period. By using this technique we could follow in real-time clot formation using high-speed intravital two-photon microscopy via a SIM scanner. To block tissue autofluorescence and reflected green light from the green laser, the light path was blocked by a 420-465nm filter that only allowed the blue emission of the intravenous injected cascade blue dextran that is used to label the plasma. Unlike other methods this technique allows real-time imaging of clot formation and permits the visualization of the green clot thus formed after intravenous loading of rhodamine 6G by switching to the green channel. Two photon laser scanning fluorescence microscopy permitted the observation of changes in blood flow, blood redistribution after clot formation, platelet aggregation and the loss of integrity of neighbouring astrocytes through a cranial window in GFP-GFAP-expressing mice after targeted occlusion. Contrary to what has been published so far regarding the resilience of astrocytes to ischemic injury, by using this technique, we demonstrate here that the time-dependent damage of astrocytes differs between different brain regions, and that different subclasses of astrocytes also exist within the same brain region exhibiting differential vulnerabilities to injury. Since neuronal death is seen as a consequence of the failure of astrocytes to support the metabolic demand of neurons, efforts designed to protect the astrocytes may constitute an alternative strategy for neuroprotection.

**Axonal calcium imaging in freely moving C. elegans nematodes** [board 56]
Institute of Biochemistry and Buchmann Institute for Molecular Life Sciences, Johann Wolfgang Goethe-University Frankfurt, Germany

To correlate natural behaviour with fast neuronal activity fluctuations in a non-invasive manner is one of the great challenges in neurobiology. Sensitive genetically encoded calcium indicators (GECIs), targeted to single neurons of interest, now facilitate robust quantitative monitoring of calcium dynamics. Recent developments combining calcium imaging in the whole brain of Caenorhabditis elegans nematodes, using nuclear localized GECIs, and simultaneous behavioural tracking of freely moving animals is currently unraveling the neuronal ensembles regulating worm locomotion. [Schrödel et al. 2013, Nature Methods vol. 10] However, as calcium transients along axons are known to differ in spatiotemporal kinetics from nuclear transients, a microscopic setup for axonal calcium imaging in freely moving worms has been optimized in our lab. Our system allows fast-automated tracking of a fluorescent marker [Faumont et al. 2011, PLoS ONE 6 (9)] while maintaining a broad field of view with sufficient spatial resolution for both somal and
axonal calcium imaging. This approach enables measuring the spatiotemporal extent of axonal calcium dynamics as well as synchronously monitoring accurately timed locomotory behaviour.

Optogenetic stimulation of single neurons furthermore revealed their thus far unknown roles in the control of worm locomotion. Simultaneous quantitative behavioural tracking analysis demonstrated their involvement in for instance the fine-structure of body bending or overall velocity. This reverse-optogenetics approach thus enables us to identify interesting candidate interneurons and link their functionality to the regulation of downstream motoneurons mediating locomotion. Measuring the spontaneous activity of these interneurons in freely moving worms with our innovative automated calcium imaging setup is used to validate findings from optogenetic experiments and study the natural function in more detail. Correlation of the spatiotemporal calcium dynamics along an axon with behavioural parameters like speed, body angles, length or forward-backward transitions permits an in depth analysis of higher order neuronal regulation of natural locomotion in C. elegans.

Understanding the mechanisms that regulate the migration of midbrain dopaminergic neurons in the developing brain [board 57]
Ankita Ravi vaswani, Jan-Hendrik Spille, Martin Schwarz, Wolfgang Hübner, Ulrich Kubitscheck, and Sandra Blaess
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Midbrain dopaminergic neurons (MbDNs) are involved in the regulation of voluntary movement, reward behaviour 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 behaviour and axonal outgrowth are not understood. We are investigating the role of the Reelin signalling pathway in these processes. Reelin is an extracellular matrix protein that regulates neuronal migration in the cortex and other brain areas. Disabled 1 (Dab1) is a key regulator of Reelin signalling. 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 the densely clustered MbDNs 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. We will present the imaging techniques and data analysis methods that we have established in time-lapse imaging of MbDNs and in 3D visualization of the developing dopaminergic system.

Dynamical organization of spinal interneuron population activity [board 58]
Vestergaard M, Lindén H, Willumsen A, Berg RW
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The spinal cord generates motor behaviours through the orchestrated activity of interneurons that project to the motoneurons innervating the muscles. Despite decades of research the extent and principal organization of the spinal neural networks that generate motor program activity is still unknown.

In this study, we measure the activity of up to hundreds of spinal neurons simultaneously using extracellular electrodes during fictive scratching behaviour in the turtle. We also record the nerve output from the spinal network that in the intact animal would control the muscles directly. This preparation allows us to measure the spinal activity for extended periods of time without any pharmacological intervention.

We characterize the spatiotemporal organization of neuronal population activity during behaviour. Preliminary analysis reveals a rich diversity in the interneuron activity, with a majority of cells showing rhythmic modulation of their firing. We observe distributed phase preferences among the neurons in relation to bursts in the nerve output. The variability in firing is large across trials.

Our data suggest that the rhythmic motor programs are widely distributed across the different segments in the spinal cord. While we cannot rule out that smaller hub-like central pattern generators control the rest of the spinal network, our data points towards an alternate explanation in which the rhythmic motor activity arises as a network phenomenon without a central core. We employ a computational model of a recurrently connected neural network of excitatory and inhibitory rate-neurons to further substantiate this idea.
Preferential inhibition of tonically over phasically activated NMDA receptors by neurosteroid analogues [board 59]
Vyklicky V., Smejkalova T., Krausova B., Vales K., Nekardova M., Chodounska H., Stastna E., Balik A., Horak M., Vyklicky L
Cellular Neurophysiology Department, Institute of Physiology CAS, Czech Republic

Postsynaptic N-methyl-D-aspartate receptors (NMDARs) phasically activated by presynaptically released glutamate are critical for synaptic transmission and plasticity. However, under pathological conditions, excessive activation of NMDARs by tonically increased ambient glutamate contributes to excitotoxicity associated with various acute and chronic neurological disorders. Here, using heterologously expressed GluN1/GluN2A and GluN1/GluN2B receptors, and rat autaptic hippocampal microisland cultures, we show that pregnanolone sulfate inhibits NMDAR currents induced by a prolonged glutamate application with a higher potency than the NMDA component of EPSCs. For synthetic pregnanolone derivatives substituted with a carboxylic acid moiety at the end of an aliphatic chain of varying length and attached to the steroid skeleton at C3, the difference in potency between tonic and phasic inhibition increased with the length of the residue. The steroid with the longest substituent, pregnanolone hemipimelate, had no effect on phasically activated receptors while inhibiting tonically activated receptors. In behavioural tests pregnanolone hemipimelate showed neuroprotective activity without psychomimetic symptoms. These results provide insight into the influence of steroids on neuronal function and stress their potential use in the development of novel therapeutics with neuroprotective action.

Focal areas of neuropathogenesis in an indolent animal model of 4-repeat tauopathy [board 60]
Westaway, D., Eskandari-Sedighi, G., Yang, J., and Daude, N.
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Background:
Mammalian prion strains are defined in situations where host genetic background is invariant; they can be conceived in physico-chemical terms where the precursor protein can mis-fold to distinct biophysical forms or derivatives. Each derivative can then have a unique ability to make copies of itself, to move with in the CNS ("spreading") and to cause damage to synapses or to axons or to the neuronal cell body. A similar overarching concept is thought to apply to the microtubule-associated tau protein encoded by the MAPT gene, but in neither case do we know the origin of the earliest misfolded species.

Preliminary Data
While scrutinizing an indolent transgenic model of Tauopathy (Murakami et al, Am J Pathol 2006) based on an exon 10 mutation we were unable to correct a marked heterogeneity in neuropathology by breeding the original mouse transgenic founders into three different inbred mouse strain backgrounds (C57BL6, 129SvEv and FVB/N). This heterogeneity was marked in all three backgrounds not only by a diversity of the cell types accumulating hyperphosphorylated Tau (neurons, glia and oligodendocytes) but also in the age of onset of tau deposition, the neuroanatomical location of Tau deposition and the chemical resilience of the misfolded Tau species.

Objective
The overall objectives of our studies are to visualize the earliest origins of misfolded tau species and to understand their evolving chemical signatures as they disseminate transynaptically (or by other means) through the aging brain. Exposure to state-of-the-art imaging techniques at this conference will facilitate this path of discovery.

Imaging of concerted activity of neuronal circuit elements labelled by trans-synaptic viral tracing [short talk]
Yonehara K., Farrow K., Ghanem A., Hillier D., Balint K., Teixeira M., Jüttner J., Noda M., Neve RL., Conzelmann KK., Roska B.

Inferring the direction of image motion is a fundamental component of visual computation and essential for visually guided behaviour. In the retina, the direction of image motion is computed in four cardinal directions, but it is not known at which circuit location along the flow of visual information the cardinal direction selectivity first appears. We recorded the concerted activity of the neuronal circuit elements of single direction-selective (DS) retinal ganglion cells at subcellular resolution by combining GCaMP3-functionalized transsynaptic viral tracing and two-photon imaging. While the visually evoked activity of the dendritic segments of the DS cells were direction selective, direction-selective activity was absent in the axon terminals of bipolar cells. Furthermore, the glutamate input to DS cells, recorded using a genetically encoded glutamate sensor, also lacked direction selectivity. Therefore, the first stage in which extraction of a cardinal motion direction occurs is the dendrites of DS cells.
Correlative microscopy is an umbrella term for combining instrumentation of light and electron microscopy for deeper insights in various research fields. The Delphi is an all-in-one solution for correlative light and electron microscopy (CLEM). It is an integrated tabletop scanning electron microscope (SEM) including an inverted fluorescence microscope. This integration enables scientists to do correlative microscopy without the challenges typically associated with CLEM:

- The Region of Interest (ROI) does not need to be retrieved, as working with the Delphi offers staying in the same ROI switching from LM to EM and vice versa.
- The sample does not need to be transferred from one microscope to the other, saving sample quality as well as time.
- Correlation accuracy is fully automated and highly precise.

The Delphi is the world’s first fully integrated CLEM solution that enables fast correlative microscopy with unique overlay precision. This aims for a huge field of applications, as up to four fluorescent colors can be observed and put into context with electron microscopy images. In life science research whole cells, thin cells or tissue sections may be observed, and applications are diverse, including hematology, food science and geology.

As the system is extremely easy to use for both light- and electron-microscopy, even inexperienced users are easily able to operate the system. Due to the automated overlay they can acquire and interpret data quickly. This approach also simplifies sample preparation as there is no need for adding fiducial markers to the sample.

Electrical signalling in dendritic spines: theory and measurements [board 62]
Popovic M., Carnevale N., Zecevic D.
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Thousands of dendritic spines on individual neurons process information and mediate plasticity by generating electrical input signals using a sophisticated assembly of transmitter receptors and voltage-sensitive ion channel molecules. Our understanding, however, of the electrical behaviour of spines is limited because it has never been possible to record input signals from these structures with adequate sensitivity and spatiotemporal resolution. On the conceptual level, a key question that has not been answered is whether the hypothetical electrical isolation of synapses on spine heads caused by a narrow spine neck is responsible for specific functions, which are not supported by synapses on dendrites. Here we used multicompartmental modelling based on experimentally determined dendritic diameters to estimate the attenuation ratio AR= EPSPspine/ EPSPdendrite for spines at different dendritic locations. The theory predicts that the AR was close to unity in most parts of the dendritic tree. Additionally, because our model shows relatively low spine neck resistance and comparatively high and variable dendritic impedance, the spatial pattern of EPSPspine amplitudes follows the classical spatial distribution of local dendritic input impedance. To advance the results from modelling to experimental measurements, we developed a technique based on an electrochromic voltage-sensitive dye which can be thought of as a transmembrane optical voltmeter with a linear scale capable of monitoring directly and simultaneously electrical signals from individual spines and parent dendrites. In agreement with modelling predictions, the measurements demonstrated that synapses on spines are not electrically isolated by the spine neck to a significant extent. Electrically, they behave as if they are located directly on dendrites. Our data agree with and help explain new published evidence based on calcium measurements which showed a clear lack of correlation between spine neck lengths and the EPSP rise time and associated Ca2+ signal in the spine head.

Associative plasticity in the auditory cortex with single-cell resolution using two-photon microscopy and a chronic cranial window [board 63]
Zelenka O., Novak O., Syka J
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The sensory cortex is able to modify its function based on preceding experience in order to optimize processing of behaviourally relevant stimuli. In the auditory cortex, tonotopic reorganization follows various types of learning. Fear conditioning leads to expansion of the regions representing the conditioned stimulus. However, the extent of this plasticity as described by classical electrophysiological approaches has been unclear at the level of populations of neurons. We used two-photon calcium imaging in vivo to elucidate functional plasticity in the auditory cortex with single-cell resolution.

In mice with an implanted chronic cranial window and neurons expressing an ultrasensitive calcium indicator GCaMP6 in the auditory cortex, we measured the coding properties of exactly reidentified neurons before and after fear conditioning. In different subsets of neurons, both shifts towards the CS+ and CS− were present. The shift direction was inversely dependent on the neuron’s best frequency before fear conditioning. Neurons with low initial best frequencies retuned upwards and vice versa. In some neurons no tuning shifts were observed, even when they were intermingled with the retuned neurons. This evidence contradicts the view of the simple expansion of CS+ tuned regions. We also observed a broadening of receptive fields after fear conditioning. Behaviourally, these changes were accompanied by selective spatial attention towards the conditioned stimuli. The adaptive purpose of these learning-induced physiological and behavioural changes can possibly be explained as an information processing optimization, improving the ability to discriminate
between threatening (CS+) and non-threatening (CS-) stimuli. The orienting responses towards CS+ were more often followed by escape behaviour, instead of conventional freezing reactions. The probability of the escape behaviour was inversely dependent on footshock intensity.

**Parallel processing of spatiotemporal frequencies in the mouse visual cortex** [board 64]

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In mammals, visual signals are processed through hierarchically organized visual cortical areas. How local neurons in different brain areas respond to visual inputs has been studied extensively. However, how the representations of the spatiotemporal component are transformed between brain areas is poorly understood.

Here, we used the mouse as model system to address that question. The mouse visual cortex consists of a primary area (V1) and retinotopically organized higher visual areas (HVAs). Recent studies have shown that mouse HVAs are functionally specialized: distinct areas respond to different spatial and temporal frequencies. Moreover, distinct HVAs receive differential inputs from V1, which in general functionally match the recipient areas. However, the functional properties of V1 populations projecting to distinct HVAs and how the cortical representations of the spatiotemporal frequency components are transformed remain unclear.

We therefore seek to determine whether distinct V1 populations encode different spatiotemporal frequencies of the visual scenes, and if so, do distinct populations project to distinct HVAs for subsequent visual processing. We used a rich set of stimuli to probe the diversity of responses of V1 and HVA neurons. By clustering response patterns from large populations of neurons, we identified groups of neurons with distinct response characteristics, suggesting they may form distinct functional cell classes. These clusters appeared to be differentially distributed across visual areas. Retrograde tracing was used to identify specific V1-to-HVA pathways. V1 neurons projecting to the same HVA had stereotyped functional profiles, contrasting with the high diversity in the population of V1 neurons. These results suggest parallel processing strategy is applied in the mammalian brain for the processing of distinct spatiotemporal components of complex visual scenes.