

## Dynamic organization of presynaptic calcium channels

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The molecular composition and dynamic organization of synapses between neurons is fundamental for the performance of neuronal networks. Key effector molecules as postsynaptic receptors, transsynaptic adhesion molecules and presynaptic calcium channels have been shown to be flexible within synaptic membranes. Particular the precise arrangement between calcium channels and ready releasable vesicles has been proposed as critical variable to control presynaptic neurotransmitter release. We use single molecule imaging approaches together with optical and electrophysiological readouts to follow calcium channels within the presynaptic membrane and probe how local displacements of channels may alter synaptic transmission and plasticity.

Multiple interactions between calcium channels and scaffold proteins have been identified, particular with the C-terminus of dominant presynaptic calcium channels as P/Q- (Cav2.1) and N-type (Cav2.2) channels. By exploring the splice variants that are expressed within the rat brain we realized that alternative splicing can have a drastic impact on the channel C-terminus of P/Q channels and may be critical for their organization in the presynaptic membrane. Altering the presynaptic expression of so called long and short-C-terminal P/Q-type channels, we identified that indeed their altered scaffold interactions are critical for their function within the synapse. Using optogenetic tools we can directly demonstrate that presynaptic release is different if synapses are dominated by long or short C-terminal splice variants. We hypothesise that alternative splicing, as already shown for adhesion molecules, is an important mechanism to tune the impact of particular neuron populations within local neuronal networks. Currently we investigate whether distinct neuronal populations use exclusive populations of calcium channel splice variants to tune their synaptic properties. In parallel we probe whether synaptic activity does impact in local channel organization.

## Visualizing the spatio-temporal dynamics of endocytosis and recycling in neuronal dendrites

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Endocytosis and recycling in neuronal dendrites play a critical role in synaptic function and plasticity. We have used live cell fluorescence imaging to visualize individual exocytic and endocytic events in cultured hippocampal neurons with high temporal resolution. First, we show that clathrin-coated endocytic zones (EZs) are optically static in mature neurons and that they are slightly enriched towards synapses. A pH exchange protocol [1] reveals that these stable EZs produce endocytic vesicles containing the cargo transferrin receptor (TfR) continuously. These structures are slightly enriched near synapses. Perisynaptic EZs represent preferential sites for the endocytosis of postsynaptic AMPA-type receptors (AMPA-Rs), but not for non-synaptic TfRs. Moreover, the frequency of AMPAR endocytosis events increases after the induction of NMDAR-dependent chemical LTD, but the activity of perisynaptic EZs is not differentially regulated [2]. Second, we recorded exocytosis events of recycling endosomes (REs), also labelled with TfR-SEP, which have been identified as the main source of post-synaptic cargo for the expression of LTP [3]. Moreover, LTP critically depends on the vesicular SNARE VAMP2, which is cleaved by tetanus neurotoxin. However, VAMP2 labels only a small subset of REs. We identify VAMP4 as a prominent marker and the key vesicular SNARE protein that mediates most dendritic RE exocytosis. I will present evidence for the role of VAMP4 in the exocytosis of REs but also in the sorting of AMPARs into a specific reserve vesicular pool that is mobilized upon LTP induction.

### References

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## Dissecting pain circuits using chemogenetics, optogenetics and targeted cell ablation

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Despite intensive research, the precise functional roles played by the different neuronal component in central pain circuits are still lacking. Recently, the development of optogenetic and chemogenetic tools together with the increased availability of genetically modified animals has enabled the manipulation of neuronal subtypes with high specificity. The exact functional nature of each neuronal component can now be tested directly with these new technologies.

We use these techniques to test the functional importance of various neuron types in controlling pain sensitivity through gain-of function and loss-of function experiments. This allows us to identify critical components of pain-related behaviours, and identify the neuronal circuitry in which they are integrated.

## Cortical alterations underlying chronic pain

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The perception of pain is a complex sensory and emotional/affective experience arising from the activity of regions distributed in the brain. However, the involved brain territories are not selective for pain. Thus, how the cortex distinguishes nociception from other aversive and salient stimuli remains elusive. We will discuss how the use of in-vivo imaging techniques and classification methods have allowed us to investigate the principles of nociceptive coding in the anterior cingulate cortex and the alteration in chronic pain states. Moreover, by the use of miniaturised micro-endoscopes in freely moving mice we can now unveil the neuronal correlation between pain and other complex emotions, such as anxiety.

## **Digital Biomarkers for Human Behavior – Current State of the Art and Perspectives**

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Sensor based digital biomarkers are an emerging method to measure patient behavior in the laboratory and in the patient's home. Using machine learning algorithms to interpret multimodal sensor data, activities can be recognized, and this information can be used as an additional diagnostic tool to better understand the pathophysiology of neurological diseases and to optimize patient's care. In the talk we will discuss basic methods and two applications in Alzheimer's disease and Parkinson's disease.

## Electroencephalography: from neuronal firing to brain waves

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In this class, I will give a systematic overview of the correlate between prominent brain oscillation and the underlying generative neuronal activity. I will focus on physiological concepts and mention a few strengths and weaknesses of the methodological arsenal developed over the past decades. I will give a rapid overview of neuronal types and canonical circuits. I will then list the known brain oscillations and how they are organizing the sleep-wake cycle. For each oscillation (alpha, theta, delta, spindles, etc.), I will give the highlights of the current knowledge about their function. I will mention abnormal brain oscillations (e.g. seizures) and future ideas about oscillotherapy. I will conclude with some of the deepest remaining questions on how such an oscillatory organization enables the emergence of an information processing engine: the brain.

## Principles and steps toward tailored deep brain stimulation for movement disorders

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Deep brain stimulation (DBS) is an established treatment for patients in the advanced stage of Parkinson's disease and other movement disorders, and represents one of the most important advances in clinical neuroscience of the past three decades. During the DBS surgery, electrodes are implanted in deep brain structures called basal ganglia, where the delivery of electrical current can improve certain clinical symptoms. The state-of-the-art DBS technology is however limited to the continuous delivery of stimulation, without the option to automatically adjust over time. Note, motor symptoms such as tremor or slowness of movements are not constantly present but fluctuate, and similarly, states of wakefulness or sleep would require adjustments of DBS over time. Thus, continuous DBS inevitably leads to periods where the current delivered does not match the level required, resulting either in over stimulation (causing side effects) or insufficient stimulation (leading to a sub optimal control of symptoms). To alleviate these problems, substantial effort is currently being made to modernize DBS technologies, and one such optimization is the development of closed loop-DBS. This refers to novel stimulation protocols in which the amount of stimulation is adapted in reaction to a feedback signal that can be derived from the brain or from peripheral sensors.

Neurological disorders, such as Parkinson's disease, are brain circuit pathologies associated with characteristic features in brain oscillations that can be measured through implanted electrodes. Such electrophysiological biomarkers could indicate the presence and severity of symptoms and serve as feedback signal to guide neuromodulation in real time. Indeed, the first in-human closed-loop DBS proof of principles studies using specific experimental set-ups demonstrated promising results. Until recently, chronically implanted neurostimulators only allowed to stimulate, but were not capable of brain signal recording. Thanks to the release of new neurostimulators last year, which have both stimulation and brain sensing capabilities, electrophysiological brain biomarkers can now be studied in the chronically implanted patients, bringing us closer to the implementation of closed-loop DBS.

We are about to enter a new era of neuromodulation therapies, where stimulation will be no longer delivered blindly, but rather adjusted based on the moment-to-moment needs of the patients. This talk will provide an overview of the current state-of-the-art DBS for movement disorders, the research on electrophysiological brain biomarkers and the development of tailored closed-loop DBS systems.

## New technological approaches for precision neurorehabilitation

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Cognitive deficits affecting attention, language, memory or executive functions represent a frequent and debilitating consequence of brain injury, neurodegeneration or ageing. Despite the globally high prevalence of neurological disorders, solutions to alleviate cognitive deficits are limited, only modestly efficient and thus largely inadequate. With the rapid advances in information and communication technology (ICT) over the last two decades, novel computerized cognitive neurorehabilitation protocols have emerged. Computerized training programs in general, and serious video games and virtual reality in particular, are believed to enhance neural plasticity and ultimately recovery of cognitive function. Real-time adaptation of difficulty level combined with graphically rich, ecological and immersive environments boosts motivation, learning and affords personalized training. Personalized interventions tailored to the individuals' specific needs and deficits complement existing neuropsychological interventions and provide the foundation for precision neurorehabilitation.



## Sleep, Thalamus and cognition.

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Sleep is “a rapidly reversible state of behavioural quiescence and greatly reduced sensory responsiveness to environmental stimuli”. It is a primary and essential biological need for higher and lower vertebrates’ classes. While the functions of sleep are still a matter of debate, it has been suggested to play a role in memory consolidation, metabolite clearance, anabolism and energy allocation. Interestingly, sleep disturbances and disruption of the neural regulation of the sleep-wake rhythms in human have emerged as early markers of pre-symptomatic phase of several neurological and neuropsychiatric disorders. Yet, the cellular and molecular mechanisms of these associations, related risks and treatment considerations are mostly unclear. Here, I will summarize our findings on non-rapid eye movement (NREM) sleep regulation. Typical NREM sleep electroencephalographic (EEG) activity predominant slow waves (< 1 Hz) associated with delta (1-4 Hz) and spindles (9-16 Hz) macroscopic oscillatory events. These oscillations are thought to be generated by the interactions of thalamo-cortical networks and modulated by subcortical inputs. On this regards, my objective is to review the experimental strategies and technology that we have employed to investigate the role of the hypothalamic-thalamic modulation of state stability/switches. As well as the recent findings on the role of the dorsal and medial central thalamus on state modulation and sleep oscillations, with an emphasis on the contribution of thalamic lesions to sleep and cognition.

Relevant publications.

Herrera, C. G. et al. Hypothalamic feedforward inhibition of thalamocortical network controls arousal and consciousness. *Nat. Neurosci.* 19, 290–298 (2016).

Bandarabadi, M & Herrera CG. et al. A role for spindles in the onset of rapid eye movement sleep. *Nat Commun* 11, 5247–12 (2020).

## Neural dynamics of auditory processing in coma

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The integrity and organization of neuronal responses to external stimuli is indicative of conscious processing and cognition. One neural circuit that is particularly interesting for studying consciousness is that of processing auditory regularities, as it is often preserved even in the absence of conscious perception. Several studies have linked the integrity of this circuit, mainly evaluated by characterizing scalp EEG responses to external stimuli with conscious processing. However, the properties and neural dynamics that support processing of auditory regularities in the absence of consciousness remain under-explored.

In this talk, I will present evidence suggesting that discrimination of auditory regularities is preserved in early coma, irrespective of patients' outcome, and that it deteriorates for patients who do not regain consciousness already within the first few days of coma. Then, I will focus on the spatiotemporal organization of electrophysiological responses to auditory stimuli in coma and will present work quantifying properties of integration and differentiation of neural information and their link to conscious processing and patients' outcome.

## The mesoSPIM initiative - open-source light-sheet microscopes for imaging *large cleared tissue samples*

*Fabian Voigt*

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Over the past decade, tissue clearing methods reached a high level of sophistication and became commonplace in neuroscience. The main advantage of tissue clearing - methods that render tissue samples such as whole mouse brains transparent while keeping anatomical structures and fluorescent labeling intact - is that they allow high-throughput imaging of large tissue samples without the need for time-consuming serial sectioning and subsequent image registration. I will introduce light-sheet microscopy as an ideal microscopy technique for such samples and present the mesoSPIM project ([www.mesospim.org](http://www.mesospim.org)), a global initiative aimed at making high-performance light-sheet microscopes more accessible to the neuroscience community. I will also share some tips on how to plan and conduct tissue clearing experiments.

## Deep Learning for Microscopy -- old problems and new solutions

*Florian Jug*

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The necessity to analyze scientific images is as old as the ability to acquire such data. While this analysis did initially happen by observation only, modern microscopy techniques now enable us to image at unprecedented spatial and temporal resolutions, through the 'eyes' of many and very diverse imaging modalities.

The unfathomable amounts of data acquired in the context of biomedical research endeavors cannot any longer be analyzed by observation alone. Instead, algorithmic solutions help researchers to study and quantify large image data.

In the past 5 years, our abilities to use artificial neural networks (ANNs) for the automated analysis of scientific image data gained significant traction, and many important analysis problems have now much improved solutions based on ANNs. At the same time, we start being aware of limitations that come with this new set of machine learning approaches.

In my talk I would like to update you on some of the latest algorithmic developments from our lab. More specifically, I will talk about improved but easy to use denoising and segmentation methods. Furthermore, I will show how downstream processing tasks can benefit from the properties of our new methods. Finally, I will introduce you to the BioImage.IO Model Zoo -- a much needed and useful infrastructure we are currently building together with several other labs. The Model Zoo will help to improve the useability and sharability of ANN-based analysis solutions.