Cajal course on Biosensors and actuators for cellular and systems neuroscience

Projects

Project 1: Get to know your tools

*When:* Block 1 & Block 2

*Who:* 2 students/block

*Instructor:* Jonas Wietek (Humboldt-Universität, Berlin, Germany)

**Abstract:**
Since the discovery of channelrhodopsins and their utilization as optogenetic tools, the field vastly expanded over the recent years. Nowadays the optogenetic toolbox has grown to an optogenetic “home depot” in which it is quite difficult to find your way around. More and more actuators and sensors are published. However, often these tools won’t behave as naively expected and sometimes show undesired side effects. A better understanding of the molecular machinery and the biophysical principles underlying the specific properties of a given optogenetic tool may help to circumvent such experimental limitations. Such knowledge helps to better tailor the experimental conditions to the properties of a given tool and select appropriate actuators and sensors that meet the demands and limitations of the experimental system. This project aims at characterizing both excitatory and inhibitory optogenetic actuators in a simple experimental system. A better understanding of how these tools work will provide a solid background for appropriate experimental design and troubleshooting. We will focus on various biophysical properties such as spectral sensitivity, kinetics and the photocycle of selected tools, including unpublished ones. We will further explore combined use of optogenetic actuators for dual color manipulation of cellular activity and combination with optical sensors to achieve all-optical control and read-out of cellular function.

**Methodology:**
To characterize biophysical properties of optogenetic actuators, participants will get a hands-on training on patch-clamp measurements of opsin expressing cells in-vitro, including all preparations necessary. Expression of optogenetic actuators will be studied by fluorescence imaging. The biophysical properties of various excitatory and inhibitory rhodopsins will be assessed by whole-cell patch-clamp measurements including application of various illumination protocols. In addition, data analysis training will be provided.
Project 2: Electrophysiological and optical detection of neurotransmitter interactions in the striatum

*When*: Block 1 & Block 2

*Who*: 2 students/block.

*Instructor*: Lief Fenno (Stanford, CA, USA)

**Abstract**: The basal ganglia circuit involves complex interactions between neurons releasing “classical” neurotransmitters such as GABA and glutamate, neurons releasing monoamines such as dopamine, and peptidergic neurons that release potent neuropeptides that can alter the excitability and synaptic properties of other neurons in the circuit. It is now possible to dissect the contribution of each of these neuron types by combining optogenetics, calcium imaging and pharmacological interventions. In this project, students will record from primarily cultured neurons and striatal acute slices to observe the effects of electrical and optogenetic stimulation through both electrophysiology and calcium imaging, and examine the impact of opioid receptors on the responses of striatal neurons to dopaminergic input from the ventral tegmental area (VTA).

**Goals**:

1. To familiarize students with the principles of optogenetics, including the theoretical framework of the read-in (optogenetic) and read-out (genetically-encoded calcium indicators) toolboxes.
2. To give students a hands-on, introductory experience in utilizing optogenetic tools and GECI in a lab-based setting via primary neuron electrophysiology.
3. To give students a hands-on experience in utilizing optogenetic tools and GECI in a lab-based setting via slice electrophysiology and epifluorescence imaging.

**Methodology**:

1. Primary neuron electrophysiology (whole-cell patch-clamp) of neurons expressing either the excitatory optogenetic tool ChR2 or inhibitory optogenetic tool NpHR (introduced to primary neuron cultures using transient transfection)
2. Primary neuron field stimulation of neurons expressing GECI (introduced to primary neuron cultures using transient transfection)
3. Primary neuron electrophysiology of neurons expressing “mystery” optogenetic tools to characterize their adsorption wavelength and ionic selectivity
4. The option to patch neurons expressing both excitatory and inhibitory optogenetic tools (e.g. ChR2 AND NpHR, or equivalent)
5. Slice electrophysiology of mice injected with Adeno-associated virus (AAV) expressing excitatory optogenetic tools (ChR2 or equivalent) in the VTA to examine the direct effect of optogenetic tool expression in VTA slices by patching neurons identified as expressing the optogenetic tool based on fluorophore expression
6. The indirect network effect of optogenetic tool expression in NAcc slices by patching neurons that are likely to be downstream of the VTA-expressing neurons
7. The combination of optogenetic and chemical modulation through assaying changes in...
optogenetically-modulated synaptic response in the presence of opioid receptor agonists and antagonists.

Project 3: Monitoring and manipulating intra-organelle physiology with optical tools
*When*: Block 2
*Who*: 2 students
*Instructors*: Fabrice Cordelieres and Sandrine Pouvreau (IINS, BIC, Bordeaux, France)

**Abstract**: Intracellular calcium stores play central roles in neurons by acting as sources or sinks of calcium thereby allowing a fine modulation of spatiotemporal calcium dynamics. The precise tuning of subcellular calcium signals underlies diverse neuronal functions including basal synaptic transmission presynaptic short-term plasticity long-term plasticity and activity mediated gene expression. ImpairedCa²⁺ handling by intracellular calcium stores has been proposed to play an important function in the physio-pathological mechanisms underlying several neurodegenerative disease, such as Alzheimer’s and Parkinson’s diseases. The regulation of Ca²⁺ dynamics by organelles has been studied for decades using electrophysiology and optical or electron microscopy combined with pharmacological and genetic alterations. More recently, organelles targeted genetically encoded calcium sensors have been designed to record calcium movement in live samples, at high spatial and temporal resolution. Availability of new probes might lead to the conclusion that monitoring intracellular intra organelles calcium is easy as pie. To the opposite lack of a proper probe characterization makes monitoring calcium stores quite challenging and prone to error. First, these organelles can constitute extreme environments with unusual conditions of pH, calcium concentration, redox signaling, etc, potentially affecting the sensor’s response to calcium. Secondly, due to the small size of these compartments, calcium measurement using classical imaging techniques can be spatially inaccurate leading to false interpretation of data. Super resolution microscopy might be an alternative but its combination with genetically encoded sensors is not straightforward.

**Methodology**
In this workshop, we will explore different constraints underlying intra organelles calcium imaging. Using a panel of biological samples going from isolated cell cultures to organotypic slices the students will explore intra organelles calcium signaling in relation to neuronal activity, using confocal and super resolution microscopy. Topics such as targeting of the sensors artefacts kinetics affinities and post hoc analysis will be addressed.
Project 4: Mapping synaptic activity with two-photon microscopy

*When:* Block 2

*Who:* 2 students

*Instructors:* Christine Gee and Brenna Fearey (Hamburg ZMNH, Germany)

**Abstract:**

Two-photon microscopy is a great technique to measure the brief calcium signals generated inside single active synapse. To scan a large volume of tissue with hundreds of synapses, however, takes several minutes, which means that we would miss most synaptic calcium transients. To solve this problem, we developed a genetically encoded calcium sensor that is localized to dendritic spines and switches color (from green to red) when illuminated with violet light. Importantly, this photoconversion happens only in spines with high internal [Ca^{2+}]. As photoconversion is irreversible, we have now plenty of time to find all active (= red) synapses in a large two-photon image stack. Using this new tool (SynTagMA), we will investigate synaptically driven activity and the back-propagation of action potentials into the dendrite.

**Methodology:**

We will use single-cell electroporation to transf ect individual pyramidal neurons in organotypic slice cultures with SynTagMA. To acquire large 3D two-color stacks of labeled synapses, we use a Femtonics two-photon microscope equipped with a tunable Ti:Sapphire laser (960 nm and 1040 nm excitation). We will stimulate either pharmacologically (block of inhibition), by somatic current injection (whole-cell patch clamp) or via stimulation electrode placed in stratum radiatum, which will result in different activity maps. A challenging part of the project is the analysis of imaging data. We will use Imaris, ImageJ and custom-written code (Matlab) to determine the change in red-to-green fluorescence ratio in many spines before and after stimulation. Plotting ΔR/G values back into 3D space, we will generate maps of all synapses that were active just before the violet light pulse.
**Project 5: Optogenetic interrogation of synaptic transmission at hippocampal synapses**

*When*: Block 2

*Who*: 2 students/block

*Instructors*: Simon Wiegert and Mauro Pulin (Hamburg ZMNH, Germany)

**Abstract**

In recent years numerous different types of optogenetic and chemogenetic tools for neuronal silencing were evolved. Some of them are highly suitable to suppress action potential firing, while others can be used to inhibit release of neurotransmitters at synaptic terminals or to shunt depolarization of postsynaptic dendritic compartments. The aim of this course is to explore various recently developed silencing tools with different functional properties and to assess their suitability for hippocampal circuit manipulation on different temporal and spatial scales.

**Methodology:**

We will express optogenetic or chemogenetic tools in organotypic hippocampal slice cultures, verify their expression with two-photon microscopy and use whole-cell patch-clamp electrophysiology to assess the effect of these tools on multiple biophysical neuronal parameters. With recordings from pairs of synaptically connected CA3 and CA1 neurons we will further test how these various tools affect synaptic transmission and plasticity. Moreover, we will use an in silico model neuron to simulate optogenetic manipulations of neuronal function. Finally, we will run a closed-loop experiment using the model neuron to drive optogenetic silencing of a hippocampal neuron in response to its spiking activity.
Project 6: Effect of GABA on thalamocortical projections

When: Block 1 & Block 2
Who: 2 students/block.
Instructor: Mathias Mahn (FMI, Basel, Switzerland)

Abstract
GABA is often described as the main inhibitory neurotransmitter in the mammalian nervous system. It exerts its effect on postsynaptic neurons through ionotropic and metabotropic receptors. The effect of presynaptic ionotropic GABA (A) receptors is determined by the reversal potential of the conducted ions: chloride and bicarbonate. The predominant current theory is that GABA (A) type receptors exert an inhibitory effect in mature neurons. However, evidence for an excitatory effect of GABA (A) type receptors in the axonal compartment is accumulating, for instance in hippocampal mossy fibers, cerebellar granule cells, pyramidal neurons in the basal nucleus of the amygdala and brainstem axon terminals. Moreover, GABA (A) type receptors in the axonal compartment seem to be more common than previously thought. We recently found, that the opening of light-gated anion channels leads to light onset triggered vesicle release in thalamocortical projections. However, if chloride conductance plays a role in thalamocortical projections under physiological conditions is unknown. During the project we will perform a pilot experiment to address the open question what effect GABA exerts on thalamocortical projections.

Goals
Characterize the effect of GABA on:
1. axonal chloride levels
2. axonal calcium levels
3. vesicle release

Methodology
1. Viral injections and optical window implantation in the mouse.
2. Optogenetic excitation of thalamocortical projection neurons and local inhibitory neurons
4. Analysis of the imaging data
Project 7: Mapping synaptic connectivity with 2-photon holographic photoactivation and voltage imaging

*When:* Block 1 & Block 2

*Who:* 2-3 students/block. Preferably, one student per block should have experience with whole-cell patch clamp recording.

*Instructors:* Dimitrii Tanese (Emiliani team, Paris Descartes, France), Nicolò Accanto (Emiliani team, Paris Descartes, France), Sophie Bouccara (Yizhar team, Weizmann Institute of Science, Rehovot, Israel)

**Abstract**

Deficits in neuronal connectivity patterns have been associated with psychiatric diseases such as autism, schizophrenia and depression. The prefrontal cortex (PFC) is particularly involved in these disorders, but there is little knowledge regarding the connectivity structure of this region. Studying functional synaptic connectivity of neurons requires the ability to both stimulate and record neuronal activity from multiple cells with single cell resolution and high temporal precision. In this project, we will use two-photon (2P) imaging and 2P photostimulation to characterize the synaptic inputs from pyramidal cells to parvalbumin interneurons (PVI) in the PFC on mouse brain slices.

**Methodology**

The students will use a specialized microscope capable of performing 2P-scanning imaging, 2P patterned holographic illumination [1,2,3] and electrophysiological recording. They will perform whole cell patch clamp recording on PVIs and optical stimulation of opsin expressing pyramidal cells to identify synaptic connections. By varying illumination protocols and light patterns on single or multiple presynaptic cells, connections will be mapped and characterized. Additionally, protocols for optical detection of photoinduced activity will also be investigated, by using the recently developed ASAP3 voltage indicator [4] and 2P imaging.

**Bibliography:**

Project 8: All-optical dynamic neuromodulation of the E/I balance in the prefrontal cortex

*When*: Block 1 & Block 2

*Who*: 2 students/block

*Instructors*: Damaris Holder & Matthias Prigge (LIN, Magdeburg, Germany)

**Abstract:**
Neuromodulators such as serotonin, dopamine or norepinephrine have been long recognized as an immensely important factor in adaptive and maladaptive brain function. A majority of approved psychiatric interventions targeting these neuromodulatory systems with classical pharmacology. In such approach, specific agonist or antagonist for subset of receptors or transporters, are evenly distributed through brain extracellular space. This is in vast contrast to the natural release of neuromodulators in form of an uneven distribution with local ‘hotspots’ and fluctuating levels of neuromodulators. In our project, we want to explore differences in the dynamics of neural circuits when expose to pharmacological application versus optogenetic-induced release of neuromodulators. We will therefore use an all-optical approach in which cortical dynamic is monitored either with red and blue-absorbing calcium sensors while optical-release of neuromodulators is triggered via blue and red-absorbing channelrhodopsin, respectively. Furthermore, we will look carefully into cross-activation of spectrally separated sensors and actuators.

**Methodology:**
Practically, we will prepare and image acute slices from animals expressing calcium indicators in the prefrontal cortex (AAV2/1-hSyn-GCaMP6f_dlx5/6-mScarlet-nls or AAV2/1-hSyn-jRCaMP1a_dlx5/6-mNeon-nls). Dlx promoter will enable us to identify interneurons in our preparation. Light-sensitize axonal projections from neuromodulatory nuclei such as the Dorsal Raphe (serotonin), Ventral tegmental area (dopamine) or the Locus coeruleus (norepinephrine) will carry Chrimson (red-absorbing ChR) or Chronos (blue-absorbing ChR). We will use one-photon widefield microscopy to image calcium dynamics from inhibitory and excitatory neurons in a large field of view (~2 x 2 mm). In addition, we are happy to talk to you about any ideas and scientific questions you want to study together with us.
Project 9: Large-scale electrophysiology and opto-tagging during head-fixed behavior

When: Block 1  
Who: 2 students  
Instructor: Nikolas Karalis (Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland)

Abstract
The coordinated activity of neuronal ensembles comprising hundreds or thousands of cells is believed to provide the mechanism that enables the encoding and retrieval of information in the brain. The activity of such ensembles is orchestrated by inhibitory interneurons that modulate the input or output of the cells they target, effectively controlling the information flow in the network. Recent advances in genetics and neurotechnologies enable us to specifically label and manipulate distinct cell types. Using these approaches, paired with advanced electrophysiological recording technologies, we can investigate the function of different interneuron classes in coordinating neuronal ensembles and their role in controlling the learning and expression of memories during behavior. In this project, we will perform large-scale extracellular electrophysiological recordings from multiple cortical regions during behavior, using high-density silicon probes, paired with optogenetic manipulations in head-fixed transgenic mice. These recordings will enable us to identify the genetic identity of specific interneurons, while in parallel enabling us to characterize the effect of these interneurons on the formation and expression of neuronal ensembles during a discriminative learning paradigm.

Methodology
1. Experiment planning: Participants will be involved in the process of designing an experiment, exploring the different aspects of this process.  
2. Head-fixed electrophysiology setup building: Participants will get acquainted with the basic components of building a functional setup for performing optogenetic and electrophysiological experiments with head-fixed mice.  
2. Surgeries: Participants will learn how to prepare mice for head-fixed optogenetic experiments, including the head-bar implantation, stereotaxic intracerebral virus injection, and craniotomy preparation for long-term recordings.  
3. Head-fixed behavior: Participants will learn how to habituate mice to head-fixation and run a discriminative learning paradigm involving appetitive and aversive components.  
4. Physiological monitoring: Participants will learn how to establish and perform monitoring of physiological parameters from the behaving mice, including breathing, whisking, heart rate, and pupil tracking.  
5. Silicon-probe recordings: Participants will learn how to perform acute extracellular recordings of hundreds of cells from multiple brain regions simultaneously, in head-fixed mice during behavior, using multishank, high-density silicon probes (128-512 channels).  
6. Opto-tagging: Participants will learn how to use optogenetic stimulations from optic fibers paired with silicon-probe recordings, enabling the unambiguous identification of cells based on their genetic- and projection-profile.
7. Optogenetic manipulations: Participants will learn how to perform optogenetic manipulations of specific cell-types to explore the potential role of these cells in controlling neuronal ensembles and behavior.

8. Open-source hardware and software: Participants will learn to use a range of free and open-source tools for the acquisition and processing of the data, including OpenEphys, Bonsai, PulsePal, Cyclops, Arduino, Linux, KiloSort, and MountainSort.

9. Data processing: Participants will learn how to handle the large data acquired during the experiments, involving conversion, pre-processing, organization, cleaning and spike sorting, preparing the data for later analyses.

10. Data analysis: Participants will learn how to analyze local field potential (LFP) and single-unit recordings in relation to behavior, physiology, and optogenetic stimulations using custom-tools in Matlab. They will learn basic approaches to characterize the spectral content of LFP signals and understand methods for characterizing single-unit and ensemble activity.
Project 10: Optogenetic identification of cortical parvalbumin-expressing axo-axonic interneurons

*When*: Block 2
*Who*: 2 students
*Instructor*: Cyril Herry (Magendie Institute, Bordeaux, France)

**Abstract**
Over the past decades, tremendous progresses have been made in the identification of the dedicated circuitry mediating specific fear behaviors. In particular it is well known that prefrontal parvalbumin-expressing interneurons (PVIN) control fear expression. Cortical PVIN are composed of several classes of cell targeting different cellular compartment. In particular the well-known basket cells (BC) target the soma and proximal dendrites whereas axo-axonic cells (AAC) preferentially contact the axon initiation segment. Until recently there was no genetic mouse model available to separate and identify specifically AAC from BC. New transgenic mice models allowing identifying and manipulating AAC have been recently developed (He et al 2016). In the time course of the project we will perform single unit recordings coupled to optogenetic manipulation of prefrontal AAC neurons during fear-related tasks to causally relate changes in AAC neuronal activity with specific fear behaviors.

**Goals**
1. Construction of single electrode/tetrodes for in vivo recordings
2. Viral injection
3. Optogenetic identification, manipulations and recordings during fear behavior
4. Behavioral analyses

**Methodology**
1. Electrode construction: Participant will learn how to build single electrodes and tetrodes
2. Viral injection: Participant will learn how to do intracerebral injections to deliver opsins in the target area of interest.
3. Behavior: Participant will learn how to run two way active avoidance behavior and classical fear conditioning
4. Optogenetic identification and stimulations during behavior: Here will use photo-tagging approaches to unambiguously identify AAC and perform optogenetic stimulations to change firing activity of prefrontal AAC during fear behavior.
5. Behavioral analyses: Participant will learn to analyse behavioral and optogenetic data
Project 11: In-vivo imaging in behaving mice with open-source hardware

*When:* Block 1

*Who:* 2-4 students

**Instructors:** Tristan Schuman (Icahn School of Medicine, Mount Sinai, NY, USA), Lucia Harley (Icahn School of Medicine, Mount Sinai, NY, USA) and Zachary Pennington

**Abstract**

Recent developments have made in-vivo calcium imaging possible in freely moving mice. Pioneering studies have demonstrated the feasibility of this approach in a range of neural circuits [REFS] and the miniscope technology has been widely disseminated through the work of companies such as Inscopix and Doric Lenses. Recently, a team at the University of California Los Angeles (UCLA) led by Prof. Peyman Golshani and Dr. Daniel Aharoni has developed an open-source version of this technology, which is widely applicable at a small fraction of the cost of the commercially available systems. Denise Cai and Tristan Schuman were among the first developers and users of this technology, and they will provide a complete workshop on building the UCLA miniscope, implanting it in mice and performing calcium imaging from the mouse hippocampus.

**Methodology**

In this project, students will:

1. Understand the basics of miniscope imaging
2. Build a functional miniscope system
3. Perform implantation surgeries in mice expressing GCaMP6s in the dorsal hippocampus
4. Perform calcium imaging in freely moving mice
5. Analyze the recorded data using open-source software.
Project 12: Monitoring mitochondrial calcium movements in astrocytes using genetically encoded probes
When: Block 1
Who: 2 students
Instructor: Roman Serrat (Magendie Institute, Bordeaux, France)

Abstract
In the recent years mitochondria have evolved as one important key element in the regulation of brain function. In particular, mitochondrial calcium buffering capacity makes these organelles crucial for brain cells, as neurons and astrocytes are cells highly dependent to calcium signaling. In our laboratory we focus on the study of mitochondrial calcium activity using the mitochondrial-targeted genetically encoded calcium sensor Gcamp6s that allows highly sensitive detection of calcium activity.

Methodology:
In this project, primary cultures of both, neurons and astrocytes from neonatal mice will be prepared and then transfected with the indicated mitochondrial sensor using a low damaging calcium-phosphate method. In parallel, a cytosolic sensor (Rcamp2) will be transfected, allowing us to measure also cytosolic activity for correlation purposes. After allowing gene expression, mitochondrial and cytosolic calcium levels will be modified using different drugs and recorded using live-imaging recordings in a spinning disk microscope. Finally, using Image J software the collected data will be analyzed and quantified.
Project 13: The impact of Tau pathology on hippocampal CA3 network dynamics in organotypic hippocampal slices

**When:** Block 1
**Who:** 2 students
**Instructors:** Ania Goncalves and Ruth Betterton (IINS, Bordeaux, France)

**Abstract**
The hippocampus is an area of the brain responsible for the rapid encoding of memory and degeneration of the hippocampus is associated with memory dysfunction. In conditions such as Alzheimer’s disease, neurodegeneration and cognitive decline are correlated with a pathological accumulation of the microtubule-associated protein Tau. In healthy neurons, Tau is enriched in axons and involved in microtubule stabilisation and axonal transport. However, in pathological conditions Tau detaches from the microtubules and forms aggregates affecting synaptic function. Subregions of the hippocampus have specific roles in the encoding of memory. Mossy fibres of dentate granule cells synapse onto CA3 pyramidal cells with an unusual presynaptic compartment known as the giant bouton. This unusual synapse has several properties which allow rapid presynaptic potentiation leading to proposals that it acts as a conditional detonator driving firing in the CA3 network.

**Goals-Methodology**
This project will investigate whether neuronal activity in CA3 is altered in a transgenic mouse model of Tau pathology. Using genetically encoded calcium indicators in combination with 2-photon and confocal imaging, we will investigate both spontaneous activity and the impact of mossy fibre stimulation an in vitro hippocampal model: the organotypic slice culture.

Project 14: Recording sub-threshold synaptic potentials with genetically encoded voltage sensors

- **When:** Block 2
- **Who:** 2/3 students.
- **TA:** Sophie Bouccara (Yizhar team)

**Abstract**
Recent developments in voltage sensor technology has yielded genetically-encoded voltage sensors (GEVs) that can report sub-threshold activity with high precision. These sensors could potentially revolutionize the study of synaptic connectivity as they could allow all-optical detection of synaptic connections using high-throughput systems. In this project, we will focus on two specific GEVs: ArcLight-MT [1] and ASAP2s [2]. We will use a combination of patch-clamp recordings with two-photon single-cell imaging and characterize the optical responses to single-cell stimulation (through the patch pipette) and to electrical full field stimulations triggering subthreshold events through synaptic connections.

**Methodology**
The students will learn how to use a 2-photon microscope capable of performing single-neuron 2P imaging and electrophysiology recordings. We will perform whole-cell patch clamp recording of GEVI-expressing cells and will simultaneously image the optical response induced by an electrical field stimulation. By varying the electrical stimulation amplitude we will determine the range values needed to induce subthreshold responses and then correlate the optical response ($\Delta F/F$) to the voltage depolarization. Synaptic blockers and TTX will eventually be also added for a deeper characterization. Upon successful completion of this simple experiment, students will be able to
record optical synaptic response to stimulation of single neurons in the neuronal culture, aiming to identify synaptically connected partners by optically screening multiple putative post-synaptic partners for each stimulated neuron. By the end of the project, students should be able to record electrically and optically from single neurons, characterize the signal-to-noise ratio of the two voltage sensors and demonstrate optical detection of synaptic connections between identified neurons.