Optogenetics, chemogenetics and biosensors for cellular and circuit neuroscience

Bordeaux, 8-28 March 2021

Projects

Project 1: "Optogenetic control of neuromodulation "

When: Block 1 & 2
Who: 2 students/block
Instructor: Alexander Dieter (Zentrum für Molekulare Neurobiologie Hamburg, Germany)

Abstract:
Numerous brain states and functions are influenced by the spatiotemporal pattern of neuromodulatory neurotransmitter release, such as serotonin, dopamine, or noradrenaline. Optogenetic methods enable excitation and inhibition of molecularly defined neuronal populations in the neuromodulatory brain centers of choice. This allows to probe their influence on various aspects of brain function, such as sensory perception or learning and memory formation, under different conditions (e.g. altered brain states, specific behaviors, anesthesia). In this course, we aim to establish a simple experimental workflow for all-optical interrogation of neuromodulation in awake animals.

Objectives :
The motivation of this course is to set up such a workflow from scratch, starting with animal surgery going on to the assembly of the experimental setup, designing and performing the actual experiments and finishing with data analysis. We consider the interests of the participants, offering the possibility to bidirectionally manipulate dopaminergic, serotonergic or noradrenergic activity in a mouse-line dependent manner, and read out calcium activity in the same or in different brain areas of interest (which can be communicated before the course, once the assignment is done).

Methodology:
We will start with the implantation of optical fibers in virus-injected mice of the Cre-line of choice (SERT/DAT/DBH/TH-cre). We will further offer training in surgical procedures and stereotactic injections. While animals are recovering from surgery, we will assemble an experimental setup consisting of the following components: an optogenetic stimulation pipeline, cameras to monitor pupil size (a commonly used measure for arousal) and mouse behavior, a fiber photometry setup to record local calcium responses, a reward delivery system, and a head-fixation system for mice either immobilized in a tube or running on a treadmill. After synchronization and calibration of the different components, we will use this platform to get to know the different experimental methods, such as fiber photometry, optogenetic manipulations with a new bidirectional tool BiPOLES, pupillometry and head-fixed behavior. We will further collect data based on the ideas of participants and finally students will be guided through a data processing analysis pipeline to sum up the project. The aim is to equip every student with the capability of planning and setting-up such a simple experimental workflow to be able to independently establish a similar pipeline in their own labs.

Project 2: "Optical tool exploration in culture, in slice, and in vivo"

When: Block 1 & 2
Who: 2 students/block
Instructor: Lief Fenno ((Deisseroth lab, Stanford, United States)

Abstract:

In this block, we will take three separate approaches to characterize and implement optically-modulated molecular neuroscience tools. 1) An in vitro exploration of FLARE, which requires both calcium (as a proxy for neural activity) and light to drive transcription of a reporter gene. We will build a controller to modulate a LED and field stimulator and stimulate neurons in a 24-well plate to characterize the input-output parameters for FLARE. 2) We will attempt to use FLARE to selectively express an excitatory optogenetic tool in neurons associated with water-seeking in thirsty animals and evaluate whether optically stimulating these tagged neurons initiates water-seeking behavior in the sated state. 3) (If there is time) we will attempt all-optical characterization of an unknown (to the student) optogenetic tool in slice using optical stimulation of pre-synaptic neurons with a fiber optic and post-synaptic activity read-out via a voltage indicator.

Objectives :

- Gain an operational understanding of optical molecular tools for neuroscience
- Gain hands-on experience in a controlled experimental context
- Gain familiarity and confidence with bespoke hardware for neuroscience applications

Methodology :

- Very basic soldering, easy programming
- Light math: power density
- Intersectional and dynamic gene expression systems for neuroscience
- Optical control tools for neuroscience

Project 3: "Optical tool exploration in culture, in slice, and in vivo"

When: Block 1 & 2
Who: 2 students/block
Instructor: Dimitrii Tanese & Kris Blanchard (Institut de la Vision, Paris, France)

Abstract:
The ability to identify synaptic connections and characterize their properties is essential for understanding neuronal circuits. Cell-resolved synaptic mapping typically requires whole-cell pair recordings, a low throughput approach hardly applicable in-vivo. In this project we propose to use 2-photon holographic illumination to achieve single-cell resolved optogenetic activation of presynaptic cells[1]. This technique, combined with patch-clamp recording of the post-synaptic neuron, will be used to achieve high-throughput detection of connected pairs in hippocampal slices[2,3]. A concurrent 2-photon calcium and voltage imaging will also be investigated to design an all-optical synaptic mapping experiment.

Objectives :

- Characterization of the performances of 2-photon holographic activation (spatial and temporal precision).
- Optogenetic-based mapping of synaptic connected pairs
- Characterization of identified connections (spatial distribution, synaptic strength, plasticity)
- Design of an all-optical manipulation experiments:
    1- Concurrent photostimulation and calcium imaging of the presynaptic cells
    2- Voltage imaging on the post-synaptic cell

Methodology:
Students will operate a 2-photon microscope capable of shaped holographic illumination for optogenetic activation and 2-photon scanning imaging.
The preparation will consists of hippocampal organotypic slices expressing an excitatory opsin (ChRome[4]) and/or a calcium (GcamP7) or voltage (ASAP) [5] indicator.
Patch clamp recording will allow 1) characterizing photoactivation performances 2) detecting post-synaptic currents induced by stimulation of presynaptic cells. By varying illumination protocols and light patterns on single or multiple presynaptic cells, connections will be mapped and characterized.
Protocols for optical detection of photoinduced activity will also be investigated: 1) photostimulation and 2-photon calcium imaging to assess activation of the presynaptic neurons 2) 2-photon voltage imaging to detect subthreshold depolarization of the postsynaptic cell.

Project 4: "Probing neuronal excitability with Arch-derived voltage sensors "

When: Block 1 & 2
Who: 2 students/block
Instructor: Guilherme Silva (Cohen lab, Harvard University, United States)

Abstract:
Membrane excitability controls the neuronal response to synaptic inputs. Excitability is not a static membrane property: it often displays plasticity depending upon the history of neural inputs and activity. What is the relation between changes in excitability and the I/O properties of a neuron? By combining advanced optogenetic and voltage imaging techniques based on sensors from the Arch family of transmembrane proteins with ontogenetic actuators (e.g. CheRiff) , we aim to assess basic electrophysiological properties from supragranular cortical interneurons in head-fixed, awake/behaving mice.

Objectives :
Optimize *in vivo* optical measurements of intrinsic excitability in L1 interneurons. We will work with a soma-localized Optopatch construct (Addgene 107704). In this construct, Optopatch expression is controlled by the Cre recombinase, so we will inject the AAV in NDNF CRE mice (https://www.jax.org/strain/030757) that should label interneurons in L1 with a density of expression to 5-10 neurons per field of view (important for optimizing signal-to-noise ratio of optical recordings). We will use patterned optogenetic stimulation and voltage imaging to asses baseline neural excitability without conditioning in ~30 neurons per animal. To assess baseline variations in intrinsic excitability, we will re-measure individual cells at 30-minute intervals for 3 hours. Optogenetic stimulation and imaging protocols will be adjusted to ensure that (a) the optogenetic stimulation does not induce homeostatic changes in intrinsic excitability, and (b) the voltage imaging does not induce phototoxicity or substantial photobleaching. We will explore different analysis methods to identify a suitable measure of excitability, e.g. measures of optogenetic 'rheobase', firing rate vs optogenetic drive (*I-F* curve), subthreshold depolarization or membrane time-constant.

Methodology:
The Cohen Lab's Optopatch technology combines a blue-light sensitive actuator (opsin, *CheRiff*) and a red-shifted fluorescent voltage sensor (*Archon*) (Adam et al., Nature 2019). By delivering blue light pulses of increasing intensities (and different waveforms) it is possible to optically emulate the effect

of current injection through a patch pipette, while simultaneously recording the voltage via fluorescence. Recent advances in reporter constructs, holographic structured illumination microscopy, and advanced data extraction algorithms (Xie et al., bioRxiv 2020) make it now feasible to characterize basic membrane properties (e.g. membrane excitability, spiking activity) in large numbers of neurons in behaving mice *in vivo*.

Project 5: "Longitudinal calcium imaging in freely behaving mice using Inscopix system"

When: Block 1 & 2
Who: 2 students/block
Instructor: Nitzan Geva & Meytar Zemer (Ziv lab, Weizmann Institute of Science, Israel)

Abstract:
In recent years, novel optical imaging techniques are being implemented to investigate the principles of neural coding of long-term memory and their underlying biological mechanisms. These imaging techniques utilize time-lapse Ca2+ imaging in freely behaving mice to track the activity of hundreds of individual cells over days to months in different brain areas.
In this workshop, students will learn the principles of using miniature head-mounted microscopes to record neuronal activity from large populations in the hippocampus of freely behaving mice. Students will learn surgical procedures necessary for hippocampal imaging, preform an imaging experiment, and process and analyze the acquired data.

Objectives:

- Learn how to prepare a mouse for chronic imaging
- Run an experiment using miniature head-mounted microscopes (Inscopix).
- Perform neuronal and behavioral data processing: from raw movie to neural correlates of behavior.
- Perform population level data analysis on data that was collected across multiple days.

Methodology:
Students will learn to perform all the steps required for preparing a mouse for imaging: surgery, validating tissue status using 2p imaging and setting up a micro-endoscope lens and baseplate. We will then perform a classic remapping experiment in which the mouse will explore two environments for a couple of days. If time allows, and students are interested, we might attempt combining optogenetic interference. After collecting the data, we will go over the stages of taking the raw neuronal movie and processing it into a meaningful neuronal activity matrix as well as registering the data across days. We will also track animal's behavior and perform population level analysis on the recorded data to expose neural correlates of behavior.

Project 6: ": *In vivo* imaging of divergent neural populations using dual-color fiber photometry"

When: Block 1 & 2
Who: 2 students/block
Instructor: Praneeth Namburi (Neurocentre Magendie, Bordeaux, France)

Abstract:

Most brain regions contain a large diversity of cell types that can be defined by defined genetic markers and/or by anatomical properties such as their downstream target. Simultaneous recording of the activity of different neural populations within a same brain region constitutes a leap forward in the understanding of neural coding of sensory, motor and emotional information. The development of new GECI (genetically encoded calcium sensors) emitting at different wavelengths now allows multiplexing of recordings in a single brain region. In this workshop, students will focus on two neural populations of the insular cortex defined by their downstream target: the insula neurons projecting to the basolateral amygdala (IC-BLA) and the insula neurons projecting to the central amygdala (IC-CeA). The activity of these two populations will then be recorded in different anxiety and valence-related behaviors.

Objectives:

- Learn basic principles of in vivo calcium imaging using fiber photometry
- Practice stereotaxic surgeries (viral injection and optical fiber implant)
- Run behavioral experiments while recording calcium signal of 2 neural populations
- Perform data analysis with open-source python and/or Matlab pipelines for imaging and behavioral (Bonsai) analysis
- Discuss ongoing developments and future directions of fiber photometry

Methodology:

- Stereotaxic surgeries in mice to inject 4 viral constructs in 3 regions: AAVretro-flip in BLA, AAVretro-cre in CeA, and a mix of 2 vectors expressing 2 different GECI in the insular cortex (AAV-flipDIO-GCaMP6f and AAV-creDIO-jRGECO).
- Animal handling and recordings using dual-color fiber photometry: the GCaMP6 protein emits green fluorescent when activated with blue light and in presence of calcium, while jrGECO is emitting red fluorescence when activated with yellow light and in presence of calcium.
- Animals will be recorded in different behavioral assays including the elevated plus maze, the open field test, consumption of water, sucrose, quinine and food, as well as mild foot shocks.
- Calcium global and transient signal will be correlated with behavioral parameters using computational tools in open source software (Bonsai and Python) and in Matlab.

Project 7: "All optical characterization of eOPN3 mediated terminal inhibition in vivo"

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

Abstract:

Information is carried between brain regions through neurotransmitter release from axonal presynaptic terminals. Understanding the functional roles of defined neuronal projection pathways in cognitive and behavioral processes requires temporally precise manipulation of their activity in vivo. We recently characterized a novel optogenetic tool allowing for the light-gated control of the $G_{i/o}$ pathway. This targeting-enhanced homologue of the vertebrate encephalopsin (eOPN3) can be utilized to induce minutes long lasting, reversible suppression of synaptic output, overcoming the limitations of existing optogenetic tools for minutes long axon terminal inhibition, imposed by the low efficacy and off-target effects of light-driven ion-pumps[1-3], when applied to presynaptic terminals.

Objectives :

We will characterize the effects of light gated $G_{i/o}$ pathway activation on action potential induced calcium influx and vesicle release in vivo. To measure the effect of eOPN3 activation on spike triggered calcium influx, we will co-express eOPN3 with an axon targeted genetically encoded calcium indicator (GECI)[4] in thalamocortical projection neurons. We can then image whisker-stimulation induced calcium increase, in the axon terminals of thalamocortical projection neurons, in the barrel cortex through an optical window[5]. Additionally, to estimate the resulting effect on vesicle release, we will express the GECI in postsynaptic barrel cortex neurons. Ideally, we will be able to optically characterize the kinetics and efficiency of eOPN3 mediated terminal inhibition in vivo.

Methodology:

- Viral injections and optical window implantation in the mouse.
- Optogenetic inhibition of thalamocortical projections
- In vivo two-photon imaging of genetically encoded calcium indicators
- Analysis of the imaging data

Project 8: "Large-scale electrophysiology and optogenetics during head-fixed behavior"

When: Block 1 & 2
Who: 2 students/block
Instructor: Nikolas Karalis (FMI, 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.

Objectives:

- 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.
- Identify the genetic identity of specific interneurons and characterize the effect of these interneurons on the formation and expression of neuronal ensembles during a discriminative learning paradigm.
- To teach students different Open-source software's for processing and analyzing electrophysiological and behavioral data.

Methodology:

- Head-bar implantation, stereotaxic intracerebral virus injection, and craniotomy preparation for long-term recordings.

- Head-fixed electrophysiology setup building.
- Silicon-probe recordings, in head-fixed mice during behavior, using multi-shank, high-density silicon probes (128-512 channels). Physiological monitoring.
- Opto-tagging and optogenetic manipulations of specific cell-types.
- Open-source hardware and software for the acquisition and processing of the data, including OpenEphys, Bonsai, PulsePal, Cyclops, Arduino, Linux, KiloSort, and MountainSort.

Project 9: "*In vivo* calcium imaging with open-source Miniscopes"

When: Block 1
Who: 2 students/block
Instructor: Tristan Schuman (Icahn School of Medicine at Mount Sinai, New York, United States)

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 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) developed an open-source version of this technology, which is widely applicable at a small fraction of the cost of the commercially-available systems. This workshop will provide a complete overview and direct experience building open-source Miniscopes. Participants will learn all relevant skills and will use them to perform an independent experiment. This workshop will be led by Dr. Tristan Shuman, who was among the first developers and users of this technology, and whose lab at Mount Sinai routinely uses Miniscopes to study neural circuits involved in memory processes.

Objectives :
- Learn basic principles of in vivo calcium imaging with Miniscopes
- Build an open-source Miniscope for in vivo calcium imaging
- Practice all relevant skills including soldering, lens implants, baseplating, and data analysis
- Run an experiment using Miniscopes
- Perform data analysis with open-source python pipelines for imaging (Minian) and behavioral (ezTrack) analysis
- Discuss ongoing developments and future directions of Miniscopes

Methodology:
Students will first build their Miniscopes and perform all necessary soldering and assembly for *in vivo* calcium imaging. Participants will then design and perform their own experiment using calcium imaging in CA1 of dorsal hippocampus. After collecting the data, students will analyze their data using open-source analysis pipelines and present their findings.

Project 10: "Combining in vivo electrophysiology and optogenetics in freely moving mice"

When: Block 1
Who: 2 students/block
Instructor: Vasyl Mykytiuk & Robson Scheffer Teixeira (Max Plank Institute for Metabolism Research, Germany)

Abstract:
Observing behavior and recording neuronal activity in freely moving animals is crucial for our understanding of brain functioning. In this regard, extracellular electrophysiological recordings allow to investigate neuronal correlates of behavior with single-cell, single-spike and submillisecond resolution. Combining *in vivo* recording techniques with tools for manipulation of neuronal activity in the same animal enables researchers to reveal the neurochemical identity of recorded neurons as well as to investigate a functional connectivity between brain regions.
In this project we will perform electrophysiological recordings from the ventral tegmental area (VTA) of freely moving mice, paired with optogenetic stimulation in the same animal. This will allow us to analyze the neuronal dynamics in terms of single unit activity of dopamine neurons, local field potential (LFP) and spike-LFP relations during spontaneous reward seeking behavior.

Objectives :

- To familiarize students with different methods of extracellular electrophysiological recordings in freely moving mice.
- To give students a hands-on training on all steps of combined in vivo electrophysiology – optogenetics experiment, including microdrive and fiber preparation, surgical procedures and behavioral recordings.
- To teach students different approaches of processing and analysis of electrophysiological and behavioral data.

Methodology :

- Microdrive and optical fiber preparation: participants will learn how to construct microdrives, mount the silicon probes onto them, make optical fibers of custom length.
- Surgical procedures: participants will learn how to perform the implantation of optical fibers and movable silicon probes in the same animal.
- Electrophysiological recording with optogenetics: participants will perform electrophysiological recordings combined with optogenetic stimulation during spontaneous reward-seeking behavior.
- Data processing and analysis: Participant will learn to analyse behavioral and electrophysiological data (Spike sorting, analysis of spiking activity, LFP and spike-LFP relations).

Project 11: "All optical interrogation of dopamine circuits in freely moving mice using multiplex fiber photometry and biosensors"

When: Block 2
Who: 2 students/block
Instructor : Marie LaBouesse

Abstract:
Until recently, methods to measure neuromodulator release in vivo were limited to analytical chemistry approaches. In the past two years, GPCR-based ultrafast genetically encoded sensors for neuromodulators were developed, including for dopamine (e.g. Patriarchi et al., Science 2018), noradrenaline, acetylcholine or serotonin. These GPCR sensors now provide the ability to measure release in vivo or in slice with high spatiotemporal resolution and unsurpassed molecular specificity in freely behaving animals. These sensors can be imaged with some of the most recent advances in in

vivo fluorescent imaging, in particular multiplex fiber photometry, but also miniature endoscopy and 2-photon imaging. Neuromodulator imaging can be combined with other methods such as optogenetic stimulation or multi-site/multi-color imaging for an all optical in vivo circuit dissection of behavior.

Objectives:
During this project, we will focus on multiplex fiber photometry as this will capture the experimental versatility of neuromodulator sensors. At the practical level, students will learn how to establish dopamine (dLight1) imaging by combine dLight nucleus accumbens (NAc) imaging, optogenetic stimulation (VTA dopamine optogenetic stimulation with ChrimsonR) ["all optical" setup] and pharmacological ligand manipulations. Students will also perform recordings of endogenous dopamine release (dLight1) during naturalistic behaviors such as reinforcement learning tasks.

Methods:
Experimental techniques will include viral injections/surgical fiber implantation, setting up a photometry rig and acquiring datasets in freely-behaving mice performing a simple behavioral task. Students will learn about sensor properties (eg affinity, ligand specificity) giving them practical tools to choose the best sensor for their own experiments. They will learn how to validate sensor use in vivo with optogenetic or behavioral stimuli, and how to optimize data collection (dataset alignments, troubleshoot movement artefacts, photobleaching, problems with rig etc.). We will also look into basic data analysis pipelines including preprocessing corrections and postprocessing (identify task-evoked dopamine transients). After this workshop, students will have sufficient knowledge to perform *in vivo* fluorescent imaging of neuromodulators in their home labs and to multiplex this with other relevant circuit neuroscience techniques.

Project 12: "All-optical manipulation and read-out of synaptic transmission"

When: Block 2
Who: 2 students/block
Instructor : Mauro Pulin (Zentrum für Molekulare Neurobiologie Hamburg, Germany)

Abstract:
Optogenetic tools, including actuators both for excitation and inhibition and genetically encoded sensors of neuronal activity, have become indispensable for studying brain functions in a non-invasive manner. For example, recent development and constant improvement of anion-conducting channelrhodopsins, allows shunting of neuronal spiking with high temporal precision at various spectral wavelengths and over a wide time span. Yet, reliable and direct optical inhibition of vesicles release at synaptic terminals has remained challenging with currently available silencing tools. In this course we will assess the properties of new, unpublished optogenetic tools for direct presynaptic inhibition and test their performance by multiphoton imaging of synaptic transmission and whole-cell patch clamp recordings.

Objectives:
The aim of this course is to use all-optical methods to control and read-out synaptic transmission. We will explore various novel optogenetic actuators and sensors with different properties and assess their suitability for hippocampal circuit manipulation on different temporal and spatial scales. The course will focus on exploring the potential of a new generation of unpublished optical silencing tools (opto-GPCRs) capable of blocking neurotransmitter release without affecting somatic activity.

Combined with optical activity sensors, we will probe and manipulate transmission at the synaptic level.

Methods:
We will use organotypic hippocampal slice cultures as our experimental model, focusing on the well-known Schaffer collateral synapse. Students will get theoretical insights into different transfection techniques in slice cultures (viral-based transgene delivery and single-cell electroporation) and hands-on training in optogenetic stimulation (ChR2 and ChrimsonR) and inhibition (opto-GPCRs), multiphoton imaging of calcium (GCaMP) and glutamate (iGluSnFR) indicators at individual synapses and single-cell electrophysiology.