List of Projects

Block 1

Project 1: "Optogenetic control of neuromodulation "
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, in particular noradrenaline. Optogenetic methods enable excitation and inhibition of molecularly defined neuronal population in the brain. This allows to probe its 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 and anesthetized 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 experiments and finishing with data analysis. In this course we aim to bidirectionally manipulate and monitor noradrenergic activity in a mouse-line dependent manner and link it to pupil-linked arousal and locomotion.

Methodology:
We will start with the implantation of optical fibers in virus-injected mice. 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 locomotion, a simple custom-made fiber photometry setup to record local calcium responses, 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 different optogenetic tools, including recent bidirectional ones (e.g., BiPOLES), and pupillometry. We will further collect data based on the ideas of participants. Finally, students will be guided through the design of a data processing analysis pipeline. The first part includes assembly of the experimental setup while the second part focuses on advanced analysis techniques of fiber photometry and pupil time courses. 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: ": Manipulating neuronal circuits with 2-photon holographic activation and fast functional imaging "
Instructor: Yoann Atlas & Christiane Grimm (Institut de la Vision, Paris, France)

Abstract :

A long-standing goal in neuroscience is to establish causal links between the behaviour of an animal and the respective active neuronal cell population. Even more, it is of high interest to functionally map this population of active neurons to identify synaptic connections and generate connectivity matrices. The low throughput nature of all-electrical approaches can be overcome by performing activity monitoring and manipulation with optical tools instead of electrodes.

Hence, in recent years invasive patch-clamp recordings could be more and more preceded by less invasive, high throughput all-optical recordings. Genetically-encoded calciums sensors like GCaMP for activity monitoring, can be combined with light-activated channelrhodopsins for the manipulation of neuronal activity[1]. This approach allows simultaneous activity interrogation for several hundred neurons and the spatiotemporally precise activation of individual neurons in the population.

In this project we focus on the use of 2-photon holographic stimulation [2] combined with different activity indicators from the GCaMP or ASAP family[3]. We will be able to manipulate neuronal activity at the single cell level, observe the activity of a larger population of neurons and investigate synaptic and functional connectivity in organotypic hippocampal brain slices. Further, students will have the possibility to experience concepts that advanced multi-cell, all-optical experiments in different ways, like using soma-targeted GCaMP, soma-targeted channerhodopsins, holographic stimulation, temporal focusing and different modalities of 2-photon functional imaging.

Methodology:

Students will operate a 2-photon microscope, capable of shaped holographic illumination[2] for optogenetic activation and 2-photon scanning imaging. Additionally, the setup will give the possibility of complementary patch-clamp electrical recordings.

Students will perform a basic characterization of the optical system and will learn to design and perform an all-optical 2-photon optogenetics experiment.

The experiments will be performed in organotypic hippocampal mouse brain slices, exploring different combinations of excitatory opsins (ChRome[4] and ChRmine[5]) and calcium indicators (GCaMP7, soma-targeted GCaMP8[6]).

The combination of 2-photon activation and calcium imaging will allow the manipulation of single cells or larger cell ensembles and the investigation of circuit response to various optogenetic stimulations. Additionally, the use voltage indicators (ASAP family[3]) and fast linescan imaging will be explored to achieve a high-speed control of cellular activity, both supra and sub-threshold.

Project 3: "Combining in vivo electrophysiology and optogenetics in freely moving mice"

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

1. Microdrive and optical fiber preparation: participants will learn how to construct microdrives, mount the silicon probes onto them, make optical fibers of custom length.
2. Surgical procedures: participants will learn how to perform the implantation of optical fibers and movable silicon probes in the same animal.
4. Electrophysiological recording with optogenetics: participants will perform electrophysiological recordings combined with optogenetic stimulation during spontaneous reward-seeking behavior.
5. 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 4: "Synaptic silencing *in vivo* using a novel switchable optogenetic tool".
Instructor: Inbar Saraf-Sinik (Weizmann Institute, Israel)

Abstract

Optogenetic silencing of synaptic transmission can allow pathway-specific targeting of inhibition[1], specifically in long-range axonal projections, and facilitate studies of their role in information processing and behavior. The Yizhar lab has been developing a novel bistable, switchable opsin called PdCO2. Activated by violet (430nm) light, PdCO2 recruits the G_o inhibitory pathway, opening GIRK (K+) conductance and directly inhibiting the vesicle transmission machinery. The inhibitory effects can be terminated with green light, thereby allowing rapid control of the inhibitory effects of this opsin. Combining optogenetics, in-vivo electrophysiology and analysis of mouse behavior, we will design and execute a comprehensive study to characterize the efficacy and on- and off-kinetics of PdCO2 in behaving mice.

Objectives

In this project, students will learn to combine in-vivo optogenetic manipulations with extracellular electrophysiology recordings and analyze the correlation of optogenetically-induced synaptic changes with neuronal firing and mouse behavior. Focusing on nigrostriatal dopaminergic neurons, we'll quantify the efficacy and kinetics of PdCO2, a novel switchable opsin for synaptic silencing, by simultaneuosly measuring induced changes in striatal single-unit and network activity, and in behavior.

Methodology

1. Electrophysiology: we will design and make chronically implantable drives that include extracellular recordings electrodes, and optic fibers for light delivery into the brain.
2. Surgical procedure: we will perform a stereotactic surgery for viral injections and implantation of the drive (1).
3. Using the Open Ephys system, we will perform in-vivo extracellular recordings with optogenetic manipulation. To examine the changes induced by activation of different excitatory (Chrimson [2]) or inhibitory (GtACR [3], eOPN3 [1]) opsins on single-unit and network activity, we'll process and analyze the data we acquire, learning: a) on-line and off-line (Plexon) Spike sorting, b) data alignment and synchronization, c) post-processing analysis (rasters, PSTH, etc.).
4. Behavior: we'll discuss different aspects of designing behavioral tests, monitoring, tracking (Deep Lab Cut), and analysis.
5. Using PdCO2 as a case study and implementing all the techniques mentioned above, we'll characterize and quantify the efficacy, on- and off-kinetics of PdCO2 and the behavioral effect of its activation and deactivation in the nigrostriatal dopaminergic pathway.

Project 5: "*In vivo* calcium imaging in freely-behaving mice using open-source miniscopes".
Instructor: J. Quinn Lee, PhD (Douglas Mental Health University Institute, USA)

Abstract

Recent technological innovations now allow for imaging of large neural populations in freely-behaving animals across protracted experience (weeks-months) using miniaturized microscopes (miniscopes). While several groups have produced such technology for use in neuroscience experiments, among the most popular versions is the open-source miniscope developed at University of California Los Angeles (UCLA). Importantly, this version of the miniscope is highly adaptable, and available to users at a fraction of the cost compared to commercially-available systems. In this course, students will learn how to construct and repair the UCLA miniscope, to perform surgical procedures for *in vivo* calcium imaging, how to carry out freely-behaving imaging experiments, and to use open-source python-based analysis methods for calcium imaging data (CaImAn) and animal behavior (Deeplabcut).

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

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: "Probing neuronal excitability with next generation two-photon voltage sensors"

Instructors: Guilherme Testa-Silva, Martina De Gennaro & Miklos Boldogkoi (Institute of Molecular and Clinical Ophthalmology Basel (IOB), Switzerland)

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 voltage and calcium imaging techniques based on next generation sensors from the **ASAP** family with the recently developed super sensitive calcium indications (**jGCaMP8s**), we aim to assess basic electrophysiological properties in the visual cortex from supragranular cortical neurons in head-fixed, awake/behaving mice.

Objectives:
Optimize *in vivo* optical measurements of intrinsic excitability in L1 interneurons. We will work with novel two-photon voltage sensors kindly provided by Michael Lin's laboratory. In this construct, expression is controlled by the Cre recombinase, and we will inject the AAV in NDNF CRE mice (https://www.jax.org/strain/030757) which 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 sensory stimulation and voltage imaging to assess baseline neural excitability without conditioning in ~30 neurons per animal. We will re-measure individual cells at 30-minute intervals for 3 hours to assess baseline variations in intrinsic excitability. We will explore different analysis methods to identify a suitable measure of excitability, e.g., optical 'rheobase,' firing rate vs. physiological background synaptic drive (*I-F* curve), subthreshold depolarization, or membrane time-constant. Additionally, we will use a similar strategy to drive the expression of jGCaMP8s, which may be used to detect single action potentials (https://www.janelia.org/jgcamp8-calcium-indicators#in_vivo). Ultimately, we expect to be able to compare side-by-side these two imaging modalities (i.e., voltage and calcium) in vivo during sensory stimulation.

Methodology:

- Stereotactic viral injections and surgeries. Implant of glass windows for voltage and calcium imaging.
- Animal handling and optical recordings during head-restrained and awake sensory stimulation paradigms.
- Design and construction of bespoke hardware for behavior and synchronization with optical electrophysiology.
- Analysis of large datasets of two-photon voltage and calcium imaging.
- Two-photon imaging and different scan modalities

Block 2

Project 2: ": Manipulating neuronal circuits with 2-photon holographic activation and fast functional imaging "
Instructor: Dimitrii Tanese & Imane Bendifallah (Institut de la Vision, Paris, France)

Abstract :

A long-standing goal in neuroscience is to establish causal links between the behaviour of an animal and the respective active neuronal cell population. Even more, it is of high interest to functionally map this population of active neurons to identify synaptic connections and generate connectivity matrices. The low throughput nature of all-electrical approaches can be overcome by performing activity monitoring and manipulation with optical tools instead of electrodes.

Hence, in recent years invasive patch-clamp recordings could be more and more preceded by less invasive, high throughput all-optical recordings. Genetically-encoded calciums sensors like GCaMP for activity monitoring, can be combined with light-activated channelrhodopsins for the manipulation of neuronal activity[1]. This approach allows simultaneous activity interrogation for several hundred neurons and the spatiotemporally precise activation of individual neurons in the population.

In this project we focus on the use of 2-photon holographic stimulation [2] combined with different activity indicators from the GCaMP or ASAP family[3]. We will be able to manipulate neuronal activity at the single cell level, observe the activity of a larger population of neurons and investigate synaptic and functional connectivity in organotypic hippocampal brain slices. Further, students will have the possibility to experience concepts that advanced multi-cell, all-optical experiments in different ways, like using soma-targeted GCaMP, soma-targeted channerhodopsins, holographic stimulation, temporal focusing and different modalities of 2-photon functional imaging.

Methodology:

Students will operate a 2-photon microscope, capable of shaped holographic illumination[2] for optogenetic activation and 2-photon scanning imaging. Additionally, the setup will give the possibility of complementary patch-clamp electrical recordings.

Students will perform a basic characterization of the optical system and will learn to design and perform an all-optical 2-photon optogenetics experiment.

The experiments will be performed in organotypic hippocampal mouse brain slices, exploring different combinations of excitatory opsins (ChRome[4] and ChRmine[5]) and calcium indicators (GCaMP7, soma-targeted GCaMP8[6]).

The combination of 2-photon activation and calcium imaging will allow the manipulation of single cells or larger cell ensembles and the investigation of circuit response to various optogenetic stimulations. Additionally, the use voltage indicators (ASAP family[3]) and fast linescan imaging will be explored to achieve a high-speed control of cellular activity, both supra and sub-threshold.

Project 5: "*In vivo* calcium imaging in freely-behaving mice using open-source miniscopes".

Instructor: J. Quinn Lee, PhD (Douglas Mental Health University Institute, USA)

Abstract

Recent technological innovations now allow for imaging of large neural populations in freely-behaving animals across protracted experience (weeks-months) using miniaturized microscopes (miniscopes). While several groups have produced such technology for use in

neuroscience experiments, among the most popular versions is the open-source miniscope developed at University of California Los Angeles (UCLA). Importantly, this version of the miniscope is highly adaptable, and available to users at a fraction of the cost compared to commercially-available systems. In this course, students will learn how to construct and repair the UCLA miniscope, to perform surgical procedures for *in vivo* calcium imaging, how to carry out freely-behaving imaging experiments, and to use open-source python-based analysis methods for calcium imaging data (CaImAn) and animal behavior (Deeplabcut).

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

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.

Project 7: "Probing neuronal excitability with Arch-derived voltage sensors "
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 8: "Optogenetic control of neuromodulation "

Instructor: Maxime Maheu (Zentrum für Molekulare Neurobiologie Hamburg, Germany)

Abstract:
Numerous brain states and functions are influenced by the spatiotemporal pattern of neuromodulatory neurotransmitter release, in particular noradrenaline. Optogenetic methods enable excitation and inhibition of molecularly defined neuronal population in the brain. This allows to probe its 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 and anesthetized 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 experiments and finishing with data analysis. In this course we aim to bidirectionally manipulate and monitor noradrenergic activity in a mouse-line dependent manner and link it to pupil-linked arousal and locomotion.

Methodology:
We will start with the implantation of optical fibers in virus-injected mice. 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 locomotion, a simple custom-made fiber photometry setup to record local calcium responses, 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 different optogenetic tools, including recent bidirectional ones (e.g., BiPOLES), and pupillometry. We will further collect data based on the ideas of participants. Finally, students will be guided through the design of a data processing analysis pipeline. The first part includes assembly of the experimental setup while the second part focuses on advanced analysis techniques of fiber photometry and pupil time courses. 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 9: "Longitudinal calcium imaging in freely behaving mice using Inscopix system"

Instructor: Nitzan Geva (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 analyse the acquired data.

Objectives
In this project, students will:

1. Learn how to prepare a mouse for chronic imaging.
2. Run an experiment using miniature head-mounted microscopes (Inscopix).
3. Perform neuronal and behavioral data processing: from raw movie to neural correlates of behavior.
4. 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 10: "All optical imaging and stimulation of neuromodulator release in freely moving mice (multiplex fiber photometry)"

Instructor: Marie Labouesse (University of Zurich)

Abstract
Until recently, methods to measure neuromodulator release in vivo were limited to analytical chemistry approaches. In the past few years, we and others have developed GPCR-based ultrafast genetically encoded sensors for neuromodulators including for dopamine (e.g. Patriarchi et al., Science 2018), acetylcholine and many others (for a summary, see Labouesse and Patriarchi, Neuropsychopharmacology, 2021). 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 using recently developed imaging methods, in particular multiplex fiber photometry, miniature endoscopy or 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.

Methodology

During this project, we will focus on multiplex fiber photometry, a highly accessible imaging method that captures the experimental versatility of neuromodulator sensors. At the practical level, students will learn how to combine dopamine imaging (dLight1 or RdLight1) with optogenetic stimulation of dopamine neurons ["all optical" setup]. Experimental techniques will include viral injections/surgical fiber implantation, setting up a photometry rig and acquiring datasets in freely-behaving mice performing reward behavioral tasks in operant boxes. Students will learn about sensor properties (eg affinity, ligand selectivity) 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 (e.g. align with videos or other behavioral equipment). They will also look into basic data analysis pipelines including pre- and post-processing (identify task-evoked dopamine transients). After this workshop, students will be able to set up *in vivo* fluorescent imaging of neuromodulators in their home labs and to multiplex this with other relevant circuit neuroscience techniques.

Project 11: "All optical characterization of eOPN3 mediated terminal inhibition in vivo"

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

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.