Projects

Project 1: ***"Monitoring neuronal activity in the song-control circuits during singing in freely behaving zebra finches."***

When: Block 1
Who: 2 students
Instructor: Arthur Leblois & Roman Ursu (Neurodegenerative Diseases Institute, Bordeaux, France)

Abstract:
Thanks to the development of motorized microdrives in the 2000', it is possible today to record neuronal activity in deep brain structures in behaving animals without interfering with their behavior. This technique is particularly helpful in songbirds, a well recognized model to study the neural mechanisms of vocal learning, and more generally sensorimotor learning. Indeed, songbirds can sing spontaneously hundreds to thousands of renditions of their courtship song (likely rehearsing to improve their singing skills) if left unperturbed. We are currently extracellular recordigs through microelectrodes moved with a motorized drive to record from the BG-cortical song-related circuit and the cerebellum of singing zebra finches (young and adults) to better understand the function of these circuits during song learning and maintenance.

Methodology:
During this project, we will teach all basics requirements to perform extracellular recordings in singing birds using motorized micro-drive. We will first guide participants through the process of building a microdrive with 3-4 single electrodes to target one or 2 brain structures. The student will also participate in on-going recordings in singing birds (depending on the status of current recordings in the labs, as these challenging experiments cannot be planned in advance with 100% certainty), and we will teach how to collect, sort and analyze song-related spiking activity from these deep brain regions. In particular, we will show some examples of in-depth analysis of behavioral (song) data and its relation to the simmultaneously recorded neuronal activity.

Project 2: ***"MesoSPIM lightsheet imaging of anatomical projections in the cleared mouse brain"***

When: Block 1
Who: 2 students
Instructor: Nikita Vladimirov & Philipp Bethge (Helmchen Lab, University of Zurich, Zurich, Switzerland)

Abstract:
The 'mesoscale selective plane illumination microscopy' (mesoSPIM.org) initiative aims to provide the imaging community with open-source light-sheet microscopes for large cleared samples. On the one hand, it is aimed at biologists seeking high-quality anatomical data from cleared samples, on the other hand, it strives to provide instrumentation developers with imaging platforms that can be tailored towards specific needs – i.e. to accommodate uncommonly large samples or different illumination schemes.

Methodology :
During this project we will learn to operate a laser scanning lightsheet microscope by disassembling and reassembling a benchtop version of the mesoSPIM before preparing, acquiring and analysing various cleared neuronal samples. We will try to clear tissue during the course but will rely on prepared samples and course participants may bring their own samples.

Project 3: ***"Disentangling Functional Representations of Tactile Stimuli in Dendrites and Soma Across the Cortical Column Using In Vivo Two-Photon Calcium Imaging"***

When: Block 1
Who: 2 students
Instructor: Gwendolin Schoenfeld (Helmchen Lab, Brain Research Institute, UZH, Zurich, Switzerland)

Abstract:
Functional compartmentalization is one important feature that makes cortical computations so effective. Pyramidal neurons (PN) of a certain cortical layer in primary cortices are highly specialized in their unique computational tasks (e.g. sensory processing in L2/3 PN and integration with higher-order inputs in L5 PN). Additional, single PN also exhibit functional compartmentalization between their soma and apical dendrites, for example via nonlinear soma-independent spikes. In this project we will record calcium activity of L2/3 and L5 apical dendrites as well as of L2/3 soma in mouse barrel cortex using two-photon imaging during the presentation of tactile stimuli. Using our own functional calcium data set we will explore various analysis methods for feature extraction, data visualization, clustering and classification.

Methodology:
During this project the participants will learn how to operate a two-photon microscope and how to do basic trouble-shooting. After that, we will image neuronal calcium activity in L2/3 and L5 apical trunk cross-section as well as L2/3 soma in mouse barrel cortex using SNAP25-GCaMP6f mice while various tactile stimuli are applied to the whiskers. With our own acquired data set, we will cover the whole range of data analysis from dFF extraction to advanced data mining approaches for large and complex data sets. Depending on time, skills and interest of the participants we might explore principle component analysis, tensor component analysis, t-sne embeddings, various clustering approaches, random forest regression/classification and general linear models.

Project 4: ***"Combination of large scale multi-electrode recordings with optogenetic manipulations for identification/manipulation of neuronal subtypes in freely moving animals."***

When: Block 1
Who: 2 students
Instructor: Lisa Roux (Interdisciplinary Institute for Neuroscience CNRS-University of Bordeaux, Bordeaux, France) & Gabrielle Girardeau (Institut du Fer-à-Moulin, Sorbonne Université, Paris, France)

Abstract:
Understanding neural network functions requires studying the computational role of specific neuronal subtypes in intact circuits. Towards this goal, we will combine optogenetic approaches with large-scale extracellular recordings in behaving animals to identify and manipulate specific neuronal classes in freely behaving rodents.

Methodology:
During this project, students will learn: (1) how to assemble a so-called "diode-probe" (silicon probe with light guides) with a microdrive for chronic in vivo recordings, (2) how to perform stereotaxic surgeries in mice and/or rats for electrode implant, (3) how to conduct recordings and "opto-tagging" experiments in freely moving rodents and (4) basic spike data analysis (spike sorting, detection of tagged units…).

Project 5: ***"In vivo multimodal, multiscale physiology"***

When: Block 1
Who: 2 students
Instructor: Christopher Lewis (Helmchen Lab, University of Zurich, Zurich, Switzerland)

Abstract:
Brain activity exhibits precise coordination across spatial and temporal scales using a rich variety of cellular signals. Understanding the principles of these multi-scale dynamics requires simultaneous monitoring of multiple signals from distributed nodes of brain-wide networks. The combination of electrical recording techniques with optical methods is particularly promising for collecting multi-modal data sets at multiple scales. The recent development of flexible and stretchable electronics provides a means to construct conformal brain-machine interfaces that are chronically stable and optically transparent. We will integrate diverse multi-channel electrode arrays with widefield calcium imaging to investigate brain activity patterns across spatial and temporal scales. Multimodal measurements allow complementary techniques to contribute to a richer understanding of the brain's multi-scale dynamics.

Methodology:
During this project, participants will be exposed to the basics of electrode design, construction and instrumentation. We will cover diverse preparations, from acute placement of surface and intracortical arrays, to considerations for stable chronic recordings. The strengths and limitations of diverse approaches and measurement modalities will be described and experiments to tackle fundamental questions in multiscale cortical dynamics will be discussed. Participants will be introduced to basic analysis of electrophysiology and imaging data sets and methods to assess coordinated dynamics between diverse signals. Upon completion, students should have sufficient knowledge to begin *in vivo* multimodal experiments in their home labs.

Project 6: "*Multi-site electrophysiological activities in a working memory task and related consolidation sleeping phases.*"

When: Block 1
Who: 2 students
Instructor: Yann Humeau, Frederic Lanore & Cecilia Castelli (Interdisciplinary Institute for Neuroscience CNRS-University of Bordeaux, Bordeaux, France)

Abstract:
Actual questioning about the role of long range communication between brain regions during behavioral adaptation and memory encoding requires to record simultaneously distant cortical and sub-cortical structures. In order to minimize artefacts due to manipulation during the behavioral testing, reward-based learning tasks can be fully automatized, allowing the collection of unbiased correlative information from animal tracking and electrophysiological recordings. This can be achieved all along the learning process, including the sleeping phases important for memory consolidation.

Methodology:
During this project, we will teach all basic requirements to perform *in vivo* multisite electrophysiological experiments in freely moving mice. We will first guide participants through the process of designing and building electrophysiological implants. Then working-memory based spatial learning will be conducted, and recordings will be performed within the maze and in a resting box. We will further look into a basic analysis pipeline for animal tracking, electrophysiological single units and LFP analysis. After this workshop, students should have sufficient knowledge about the required techniques to start *in vivo* electrophysiological experiments in their home labs.

Project 7: ''Birth of a memory: *ex vivo* optogenetic approach to study hippocampal engrams''

When: Block 1
Who: 2 students
Instructor: Catherine Marneffe & Noelle Grosjean ((Interdisciplinary Institute for Neuroscience CNRS-University of Bordeaux, Bordeaux, France)

Abstract:
Memory formation is a key process to normal brain functioning and to elicit adapted behaviour. The most important region for episodic and contextual memory encoding is the hippocampus. Therefore, in this project we are going to use a mouse model to investigate how the neurons in the hippocampal circuit can be activated by fear conditioning. In detail, we will monitor the connectivity between hippocampal granules cells and CA 3 pyramidal neurons by using optogenetic tools to control the circuit. Additionally, we will compare the connectivity in CA3 neurons that were activated by fear conditioning (constituting an engram) to non-activated neurons.

Methodology:
In this project, we will teach the technique of patch-clamp in CA3 pyramidal cells and how perform stereotaxic injections. In practical, the participants will learn how to inject a combination of viruses (lentivirus and AAV): one expressing a fluorescent marker protein under a specific promotor that is active in recently triggered neurons in CA3 (RAM+), and a second expressing channel rhodopsin in transfected granule cells. The students will also learn how to conduce a fear-conditioning experiment in best conditions. Finally, we will operate electrophysiological recordings in acute brain slices prepared by the student. Thanks to the optogenetic manipulation, we will be able to trigger photoactivation of granule cells in the dentate gyrus and record the downstream response of the post synaptic cell in CA3. This project is conceived to investigate the hippocampal circuit from a single unitary connection between two neurons to the behavioural level. It will lead the participant to master the cutting-edge technologies hosted at the Bordeaux Neurocampus, together with a strong up-to date theoretical course.

Project 8: *"Imaging neural population activity along the gut-brain axis in adult Drosophila"*

When: Block 2
Who: 2 students
Instructor: Sandra Soukup (Neurodegenerative Diseases Institute, Bordeaux, France), Pavan Ramdya & Matthias Durrieu (The Ecole polytechnique fédérale de Lausanne, Switzerland)

Abstract:
The Gut-Brain-Axis is defined by bidirectional communication between the central nervous system and the intestinal tract. Dysfunction of gut-brain-axis communication is implicated in psychiatric disorders and the two most common neurodegenerative diseases, Alzheimer's and Parkinson's Disease. As well, the ability of the gut to respond to cellular damage and invading pathogens requires regeneration by intestinal stem cell proliferation: a process that is regulated by enteric neurons projecting from the CNS to the gut. Remarkably, very little is known about how gut-brain circuits function to regulate homeostasis and dysregulate in neurodegenerative diseases.
In this mini project, we will open up a new understanding of gut-brain communication by recording enteric neurons in adult, *Drosophila*, during the ingestion of different foods. To do this, we will leverage a newly devised live imaging approach for visualizing enteric neurons and synapses one the intestines of intact animals.

Methodology:
- 2-photon and confocal microscopy
- Dissection, microimplantation, and specimen preparation for live imaging
- Calcium imaging data acquisition and computational data analysis
- *Drosophila* genetics

Project 9: ***"Combining two-photon targeted patch-clamp recordings with calcium population imaging to monitor neuronal activation in mouse neocortex during tactile stimulation."***

When: Block 2
Who: 2 students
Instructor: Jean-Sebastien Jouhanneau (MDC, Berlin), Ourania Semelidou & Yuktiben Vyas (Frick lab, Neurocentre Magendie, Bordeaux)

Abstract:
Recent technical advances have enabled us to visualize and monitor the activation of individual neurons *in vivo*. While functional calcium imaging provides information about single cell and population level of activity, whole-cell patch-clamp recordings enable the investigation of sensory integration at the synaptic level. Sparse level of activity is the hallmark of cortical sensory neurons, and combining these techniques allows targeting electrophysiological recordings to sensory activated neurons.

Methodology:
The aim of this project is to perform functional calcium imaging and two-photon targeted patch-clamp recordings of layer 2/3 excitatory neurons of forepaw primary somatosensory cortex in anesthetized mice. Using GCaMP6f mice, we will first detect responding neurons to tactile stimulation and subsequently investigate their electrophysiological profile, using two-photon targeted patch-clamp recording. Applicants will able to perform calcium imaging experiments as well as 2-photon targeted patch-clamp recording. Successful applicants should have experience in patch-clamp recording and Python/Matlab coding.

Project 10: ***"Ex vivo optogenetic manipulations of basal ganglia circuits"***

When: Block 2
Who: 2 students/block
Instructor: Jerôme Baufretton & Lorena Delgado (Neurodegenerative Diseases Institute, Bordeaux, France)

Abstract:
Since it development in early 2000, optogenetic has been proven an excellent tool to manipulate neuronal circuit in a selective and timely controlled manner. This tool is especially powerful to interrogate functional connectivity of neuronal subpopulations ex vivo or in vivo. Combined with patch-clamp recordings in acute brain slices, optogenetic allow the characterization of synaptic pathways which cannot be studied selectively with conventional electrical stimulation. During the workshop, we will interrogate the properties of GABAergic synapses within the basal ganglia circuitry.

Methodology:
During this project, we will teach all basics requirements to perform ex vivo optogenetic and patch-clamp recordings in acute brain slices. We will first guide participants through acute brain slice processing. Then we will teach them to perform patch-clamp recording of channelrhodopsin-2 expressing basal ganglia neurons and manipulate their activity with light. Finally, we will record output structure of these neurons and characterize synaptic transmission using light-activation of synaptic terminals. After this workshop, students should have sufficient knowledge about the required techniques to start ex vivo patch-clamp experiments combined with optogenetics in their home labs.

Project 11: "*In vivo* calcium imaging of hippocampal CA1 population activity in the freely moving mouse using miniaturized microscopes"

When: Block 2
Who: 2 students
Instructor: Roman Boehringer (Institute for Neuroinformatics, ETH, Zurich, Switzerland)

Abstract:
Recent improvements in calcium imaging and the development of miniaturized microscopy now allow for recording the activity of large populations of neurons over an extended time course in the freely moving and behaving animal. While a few years ago, calcium imaging was mainly head fixed and restricted to superficial cortical areas, new mobile and micro-endoscopic imaging approaches now allow researchers to image neuronal network activity in deeper brain regions. These include the amygdala, the hippocampus, the thalamus, the hypo-thalamus, the striatum, and the deeper cortical areas opening new areas of research.

Methodology:
During this project, we will teach all basics requirements to perform *in vivo* calcium imaging experiments in freely moving mice using miniaturized microscopes. We will first guide participants through the process of building a miniscope. Further experimental techniques we teach include implanting gradient index lenses (GRIN) to target area CA1 of the hippocampus, mounting of the mini-scope head-plate, and acquiring a first data set of an animal running in a linear track. We will further look into a basic analysis pipeline and distract place-cell activity from the recorded dataset. After this workshop, students should have sufficient knowledge about the required techniques to start *in vivo* calcium imaging experiments in their home labs.

Project 12: ***"Prefrontal large scale multi-electrode recordings during defensive behaviors."***

When: Block 2
Who: 2 students
Instructor: Daniel Jercog, Nancy Winke & Cyril Herry (Neurocenter Magendie, Bordeaux, France)

Abstract:
Over the past decades, it has become clear that the medial prefrontal networks have a central role in the regulation of associative learning and defensive behaviors. Previous tone fear conditioning studies suggest that, while the prelimbic area promotes passive fear responses (freezing), infralimbic areas attenuate them. Moreover, published work suggests that extinction of tone fear conditioning "move" neuronal representations of threat-predicting cues from prelimbic towards infralimbic areas. Those experiments were performed by recordings in either prelimbic or infralimbic cortices, where data collected in both regions simultaneously during such processes is missing. During this project, we will perform electrophysiological recording along the dorso-ventral axis of the mPFC using silicon electrodes in mice during the habituation, acquisition and extinction of defensive behaviors.

Methodology:
Students will be trained on: (1) stereotaxic electrode implantations, (2) electrophysiological single-unit and local field potential recordings in freely moving animals during defensive behaviors, (3) basics of data analyses and neuronal decoding approaches.

Project 13: ***"Two-photon calcium imaging of cortical dendrites in awake head-fixed mice.***

When: Block 2
Who: 2 students
Instructor: Naoya Takahashi (Institute for Interdisciplinary Neuroscience, Bordeaux, France)

Abstract:
The role of sensory cortices is to analyze incoming environmental stimuli based on one's internal belief to make decisions or guide motor actions. Previous in vitro studies demonstrated that dendrites of cortical pyramidal neurons can exhibit a range of linear and nonlinear mechanisms that allow them to implement elementary computations in the cortical circuits. However, it has remained largely unknown how the dendrites operate and process sensory information in the living brain. The development of two-photon microcopy and genetically-encoded calcium indicators (GECIs) has transformed our ability to investigate the structure and function of small neuronal processes such as dendrites, spines, axons in living animals. Over the past 10 years, two-photon calcium imaging has been applied to visualize dynamic activity patterns of neuronal dendrites across sensory cortices (e.g., somatosensory, visual, auditory) in awake rodents performing a sensory task. There is emerging evidence that dendrites of cortical pyramidal neurons play casual roles in sensory processing and sensory-guided decision making.

Methodology:
During this project, participants will go through the basic procedures to image the activity of individual dendrites of cortical pyramidal neurons in awake mice. We will use mice in which pyramidal neurons in the primary somatosensory cortex are sparsely labeled with a GECI, GCaMP8, via virus injection. Participants will first learn how to implant a chronic imaging window over the cortex, as well as a head-post for head-fixation. After habituating mice for head-fixation, we will image GCaMP8 signals from apical and basal dendrites of pyramidal neurons using a two-photon microscope. We will deliver tactile stimuli to the whiskers of the mice to see sensory responses in dendrites. This project will also look into basic analysis to quantify dendritic calcium signals from the collected dataset.

Project 14: ***"In vivo recordings in freely moving mice: fiber photometry vs. miniscopes"***

When: Block 2
Who: 2 students
Instructor: Marie Labouesse, Simon d'Aquin (University of Zurich) & Jonathan Zapata (Inscopix).

Abstract: An increasing number of techniques now allow to image in vivo functional activity of specific cell types in behaving animals. With the advent of fiber photometry and miniscopes, we can now do this in freely behaving mice who perform behaviors of interest in home cages or in complex behavioral setups. Fiber photometry is a technique allowing to measure bulk fluorescent dynamics in brain regions of interest, either to record calcium transients as a proxy for neural activity or more recently to detect release of neurotransmitters like dopamine in real time with biosensors (see eg Labouesse and Patriarchi, Neuropsychopharmacology, 2021). This technique has several advantages, including being relatively quick to learn, high throughput, affordable, and able to be used in multiplex mode including multicolor imaging, multi-site imaging or in combination with optogenetics. Miniscope imaging is a technique allowing to measure calcium activity at the cellular resolution, allowing to dissect neural encoding at the single-cell level. This technique is more labor-intensive and less high-throughput, but in turn allows to measure calcium dynamics at the cellular resolution, what is not achievable with photometry. Miniscope imaging can be multiplexed with 2-color imaging or with optogenetics, and more recently has been tested to allow recording of biosensors as well.
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Methodology: During this project, students will learn how to do fiber photometry and miniscopes in freely behaving mice. In the first few days, students will learn multiplexed fiber photometry combined with optogenetics, where we will stimulate dopamine neurons and record dopamine release. Students will also learn about biosensor properties (eg affinity, ligand selectivity) helping them choose which sensor to use in their homelab experiments. In the next following days, students will then learn miniscope imaging where neural activity will be imaged with a GCaMP indicator. In both cases, techniques students will learn and perform will include viral injections/surgical fiber implantation, setting up the recording equipment, optimizing data collection for behavior and dataset alignments, acquiring datasets in mice performing simple behavioral tasks and finally, running basic data analysis pipelines to analyze collected data. After this workshop, students should be able to set up *in vivo* fluorescent imaging techniques of interest in their home labs and to multiplex this with other relevant circuit neuroscience techniques.
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