PROJECTS

Introduction to confocal and super-resolution microscopy: how to image living brain slices

by Agata Idziak

Background: Fluorescence microscopy is a powerful tool widely used in neuroscience research. However, its diffraction limit does not allow us to resolve brain nanostructures, such as dendritic protrusions called spines, which are crucial in formation of a neuronal synapse and transmission of electro-chemical signal from one neuron to another. Luckily, over past few decades, progress in optical physics and microscopy lead to invention of so-called "super-resolution" imaging techniques, overcoming the diffraction limit and enabling to image smaller structures than 200 nm.
Methods: In the one-week workshop, we will first learn the basics of fluorescence/confocal microscopy and one of the super-resolution techniques, namely STED (Stimulation Emission Depletion). Using live brain slices of mouse hippocampus with fluorescently labelled neurons and a custom-built STED microscope, we will visualize synaptic contacts. In addition, we will use a labelling technique, called SUSHI (Super-Resolution Shadow Imaging, Tonnesen et al., Cell 2018) to link neuronal morphology with its surrounding environment. Finally, we will learn how to use the ImageJ software to edit and process obtained images. This one-week workshop will give you a good taste of fluorescence microscopy and super-resolution live-imaging.

Multichannel extracellular recordings in behaving rodents: from experiment to data analysis

by Fabian Stocek

Background: Much of modern understanding of the mind has been done using simple electrophysiology. Using small wires to listen to electric activity in the brain was the basis for the 2014 Nobel prize. The researchers correlated the movement of the animal to spiking in the brain and were able to discover grid cells, spatially specific units firing in a hexagonally repetitive manner.
Aim: In this course students will assemble their own electrodes made with tetrodes, learn the basics of handling animals and perform a surgery to do a chronic implantation of their own electrode. They will then acquire the signal from these freely moving animals and learn the basics of how to interpret it.
Methods: We will be using Open Ephys setup (https://open-ephys.org/) to measure the neurophysiological signal coming from the rat's brain via tetrodes.Students will learn about signal acquisition (hardware and software), animal handling, surgery with chronic implantation and basics of signal processing.

Action preparation measurement: from brain to muscles

by Mehrdad Bahadori

Background: Action and perception have a close interaction. Emotions, as considered as perceptual element, can modify the state of the brain and consequently affecting the action preparation. For example, negative emotions have been found to increase motor evoked potentials (MEPs) amplitude and decrease the time of the anticipatory changes in muscles, both of which show the facilitation in action preparation. Further to emotions, the spatial location of the external stimuli also modulates the action preparation. It is found that the stimuli reach the distance close to the body, they facilitate the action preparation by reducing the reaction time and increasing MEPs.

Conclusively, the previous discussion shows the effect of the modifications of action preparation by perception of both distance and emotion.

Aim: Measuring the state of the brain as affected by emotional and spatial information of the external stimuli for doing an action by neurophysiological measurement (anticipatory adjustments elicited by voluntary action).

Methods: In this project, we will investigate the action preparation modulations while reacting to emotion-inducing sounds stopping within different distances. The proper auditory stimuli (emotional approaching sounds) are going to be deployed to create a protocol using Matlab. Anticipatory postural adjustments of postural muscles will be measured by means of EMG. Furthermore, the subjective auditory distance perception and emotional perception (valence and arousal) are going to be further investigated to study the correlation between subjective perceptions and action preparation. To measure the emotional perception, the Self-Assessment Manikins are going to be used.

The importance of habituation to handling in animal experiments

by Lukasz Bijonch

Background: Handling laboratory animals during experiments is an important source of stress that may impair reliability of test responses. Picking up mice by the tail is especially aversive and stimulating anxiety but even gentle handling is stressful if presented occasionally. However, such stress can be reduced substantially by habituating mice to human touch. That is why it is important to handle animal by the experimenter before behavioral tests to reduce anxiety, which may affect both mouse performance and some physiological alterations in the brain driven by experimental task. A brain structure especially prone to stressful events is the Central Amygdala, as it is a part of the brain distinguishing stimuli of appetitive and aversive valence. It's Medial part processes appetitive stimuli, while the Lateral part aversive ones. Thus, the hypothesis of the project is that appetitive stimuli response and processing in Central Amygdala differ in habituated and non-habituated mice. Understanding the consequences of improper handling is crucial to improve reproducibility of different experiments in animal studies.

Aim:

1)To define differences to the appetitive stimuli of habituated and non-habituated mice

2)To define changes in the intrinsic excitability of neurons in medial and lateral parts of Central Amygdala driven by appetitive stimuli in habituated and non-habituated mice.

Methods: In this experiment habituated and non-habituated mice will be exposed to sweet water, which is a pleasant stimulus to rodents. During exposure to sweet water, number of licks will be counted with simple licometer based on microcontroller Arduino. Brains of habituated and non-habituated mice will be collected and sliced for electrophysiology experiments. The excitability of neurons in Medial and Lateral parts of Central Amygdala will be studied. The intrinsic excitability of neurons is mediated by biophysical properties of the cell membrane and determines the excitability of neuron; how easily it generates action potentials is one of the hallmarks of neuronal processing and adaptations to different events. Thus, differences in the excitability of Medial and Lateral parts of Central Amygdala neurons of habituated and non-habituated mice should be observed.

Resting-state fMRI: data visualisation and individual variability

by Liucija Vaisvilaite

Background: Since the first mention of the Default Mode Network (DMN), a term coined by Marcus E. Raichle, (Raichle et al., 2001) there has been an exponential increase in number of publications focusing on resting-state functional connectivity (rsFC) (Raichle, 2015). Approximately 20 years of research after the discovery of large-scale resting-state networks, it has been established that rsFC networks, especially the DMN, are consistent throughout healthy population (Mantini & Vanduffel, 2013). However, it is suggested that the rsFC is not stable across lifespan, i.e., it changes with increasing chronological age, and is reflective of gender and personality differences (Nyberg et al., 2010; Yang et al., 2015; Satterthwaite et al., 2014).

Methods: Given the time constraints and a lengthy fMRI procedure of image acquisition, for the purpose of this project The Midnight Scan Club (MSC) dataset will be used (Gordon et al., 2017). The aim of the project would be a partial replication of the analysis previously done by the original authors on this dataset. The idea is to model the rsFC maps on individual basis, and then correlate those to the individual neuropsychological scores. It has been previously shown that the rsFC networks can be used as predictors of self-reported five-factor personality traits, i.e., levels of Extraversion, Neuroticism, Agreeableness, Conscientiousness and Openness (Norstro et al., 2018). The authors note that the personality predictions based on the rsFC were highly gender specific, which is going to be accounted for in the present mini-project. This work is relevant in the current context, given that recently the field of psychology, as well as neuroscience has been undergoing the replication crisis (Open Science Collaboration, 2015). Therefore, it is essential, that students would be aware of group versus individual differences in fMRI studies, as well as the factors that potentially significantly affect the differences in resting-state networks.

Fast labeling of engram neurons

by Noëlle Grosjean

Background: Forming new memories after a one-time experience requires initial encoding then consolidation over time. During learning, multimodal information converges onto the hippocampus, activating sparse neuronal assemblies. Activated neurons are believed to form a memory representation through concerted activity and synaptic interconnectivity. The hippocampal CA3 region is a key structure involved in multimodal information integration and initial memory storage.

Methods: In this project, we are going to use a novel tool for fast-labeling of engram neurons (FLEN). FLEN is based on c-Fos activity-dependent transient expression of a destabilized fluorescent marker ZsGreen1 rapidly after one-trial learning (few hours). To achieve this goal, the mouse will undergo stereotactic injection with the virus expressing this FLEN tool. We will then activate the CA3 neurons by fear conditioning (constituting an engram) and compare with animals without behavior. Furthermore, we wil perform an immnunochemistry for endogen cfos and compare it to the cfosZsgreen.

Characterizing synaptic ultrastructure and connectivity in the striatum

by Paul Lapios

FASS: Synaptosome sorting using fluorescence: During this project we will use fresh mouse brain tissue to prepare synaptosomes, which are cell fragments corresponding to synapses. We will use a cell sorter to isolate a specific type of synapse based on its fluorescence (hence the name of this technique: Fluorescence Activated Synaptosome Sorting or FASS). Then, by performing immuno-labelling experiments, we can discover the expression pattern of specific proteins within the synaptic hub.

Cryo-Electron Microscopy (Cryo-EM): The synaptic hubs will also be observed thanks to cryo-electron microscopy, a technique that earned the 2017 Nobel prize in Chemistry. We will use tomography to obtain a 3D nanoscale resolution of these structures giving us the ability to even observe synaptic vesicles! We can then correlate this image with the fluorescent signal giving the signature of its specificity. This will put us on the way of achieving a 3D reconstruction of the synaptic hub.

Learning about the neuronal connectivity across the cortical layers

by Daniela Doda

Background: Cortex is responsible for brain higher functions. Brain connectivity seems to be affected in many neuropsychiatric disorders such as autism, schizophrenia and bipolar disorders. These connectivity issues can be assessed in many ways and at different magnification scales but we want to focus at the synapse level. Therefore, by looking at the neuronal connectivity, we hope to understand what happens in a pathological brain.

Aim: During this period, we will investigate the morphology of mouse cortical neurons and quantify their synaptic connections.

Methods: The project relies on the preparation of two mouse brains, one wild type and one model of a neuropsychiatric disorder (autism, schizophrenia or else). These brains will be processed for 3D electron microscopy. Once the images have been acquired, the students will proceed with the segmentation and 3D modelling of the cortical neurons. Given our limited time, we will sample out neurons from the different layers and compare them across layers and across conditions.

The students will be able to learn about tissue preparation and imaging with the serial block face electron microscopy method. They will also learn how to process electron microscopy data such as alignment, segmentation and 3D modelling.

Propagation of activity patterns in a network of locally interconnected populations of neurons

by Fjola Hyseni and Eduarda Centeno

Background: Experimental evidence shows that in several sensory and motor related parts of the brain, neurons present an activity that depends on particular features of the sensory stimuli or of the motor output. For example, specific neurons in the cat visual cortex modulate their firing rate depending on the orientation of the visual stimulus and neurons in the premotor song control nucleus HVC of singing birds fire preferentially at specific times during the song production. For the project, we focus mainly on the function of the nucleus HVC.

Aim: The aim of the project is to implement and investigate a simplified model of HVC.

Methods: The model consists of a network of interconnected neurons or populations of neurons. The neurons are modelled as integrate and fire neurons. Each neuron in the network fires at different time instants during song production. We aim to build step by step a chain of populations of neurons featuring a propagating neuronal activity. At each step we will discuss the limits of the

achieved network model and try to overcome these limits. If there will be enough time, we will build a network with populations of neurons interconnected with excitatory and inhibitory synapses.

Understanding vision
by Jonatan Malis

Background: To seeing animals, vision is an essential tool allowing them to navigate their environment. While different seeing animals have evolved based on the specificity of their environment, the basics of vision have been found to be strikingly similar across species. The eyes have photoreceptors which are specialized cells able to perform the transduction of light into biochemical signals. The resulting information is then fed into a complex circuit of neurons which in turn allows for a perception of the world. While very small, the *Drosophila Melanogaster* is a talented flying animal able to avoid obstacles, find food and mate. In order to perform such prowess, a large part of the Drosophila's brain (optic lobe) is dedicated to vision. The retina is where the phototransduction happens. It sends information to the lamina which transforms and relates this information to the medulla which will in turn process and relate visual information to higher processing centers.

Methods: In this project, we will learn via *in vitro* electrophysiology how this biochemical information is produced, manipulated and relayed between neurons allowing them to create complex information networks. We will see how neurons in the medulla are built in such a manner that they are able to receive neurotransmitter information from other cells, how they can electrically transform this information and, how they can relate it using their own neurotransmitters.

Development of new methods and tools for magnetic resonance imaging and magnetic resonance spectroscopy (MRS) for neuroscience
by Justine Deborne

Background: The structural and functional study of the brain has undergone tremendous development in recent years and advances in brain imaging and spectroscopy have been a revolution in the field of neuroscience. In order to allow the early detection of certain diseases in small animal organs research efforts always tend towards higher resolution on smaller samples. In nuclear magnetic resonance (NMR) this can be achieved by different means such as: increasing the signal-to-noise ratio or increasing the detection sensitivity of the coil used to receive the signal. The miniaturization of the coil and its implantation in an anatomical region of interest allow to improve its electromagnetic characteristics and the spatial resolution. For instance, reducing the coil dimensions leads to a reduction of the sample volume actually perceived, the noise, which comes from the whole volume, is also reduced. However, microcoils must be implanted close to the area of interest to perform images and spectroscopic studies of deep structures. Magnetic resonance spectroscopy (MRS) allows an in vivo exploration of the molecular composition of tissues such as molecular constituents and metabolites. Microcoils allow the analysis of very small volume samples and the characterization of the evolution of brain metabolites in pathological states thus leading to a better therapeutic management.

Aim: The objective of this project will be to present the different existing tools and methods for the study of neuroscience in NMR, their limitations and innovations.

Methods: A brief introduction and description of MRI and MRS techniques and their applications in the field of neuroscience will be given. The following steps of the project will include: design and conception, *in vivo* implantation of the coils by stereotactic surgery, post-treatments and results analysis.

In vivo Electrophysiology: An introduction to the study of nociceptive integration in the dorsal horn of the spinal cord

by Keri-Ann Charles

Background: The dorsal horn of the spinal cord receives the first relay of afferent inputs from different parts of the body, the skin, muscles, and viscera. These inputs include a number of different modalities including tactile, noxious, or itch. In the superficial dorsal horn, incoming information is integrated to allow the detection of these different sensory modalities and elaborate the appropriate response. Electrophysiology is well-adapted to investigate the electrical properties of neurons, and their changes during nociceptive transmission. It greatly contributes to identify and define the various pathways, receptors, neurotransmitters and neurons responsible for nociceptive modulation.

Methods: In this workshop, we will work together to identify nociceptive neurons in the lamina V of the dorsal horn of the spinal cord, that respond to electrical stimulation of the sciatic nerve. Using extracellular recordings of Wide Dynamic Range neurons (WDR), we will take a further look at the different types of responses from Aδ, β and C fibres. Electrical stimulation (1 to 20mA) of the sciatic nerve will allow studying the intensity response of WDR neurons to electrical stimulation. Together we will look at short term responses of neurons to repetitive stimulation (15 stimulations at 1Hz) at three times the C-fibre threshold, referred to as wind-up. We will finally investigate if dopamine agonist, like those given to patients with Parkinson's disease suffering from pain, alters nociceptive

System neuroscience: A Little bit of each

By Raquel Garcia-Hernandez

Background: The cholinergic system is one of the most important modulatory neurotransmitter in the brain. It is constituted by a formation of closely associated nuclei located in the basal forebrain, and their projections innervate the entire cortex and the whole hippocampal formation. Many data suggest that cholinergic projections from these basal nuclei modulate attention and memory processes, such as spatial and contextual learning and short-term processes. The cholinergic receptors can be divided into nicotinic and muscarinic receptors. This project will focus on the modulation of the muscarinic ones, in order to address in a multidisciplinary manner, their effect in memory processes.

Aim: The students will learn the basics of two major branches of neuroscience: behaviour and histology in order to be able to start working with these techniques right away and to acquire the logic and sources to go further.

Methods: In order to study the effect of muscarinic receptor modulation on short-term memory we will use Scopolamine intraperitoneal injections in mice. Scopolamine is a non-selective muscarinic receptor antagonist which crosses the blood-brain barrier and has been shown to impair cognitive functions. During this project we are going to observe and quantify its effect in two ways:
-In behaviour: we will see the cognitive output of this modulation, and whether learning is impaired compared to control animals.

-In histology: we will use cFos to see which cells have been activated to encode a memory, as it has been widely and causally associated with this process and analyse possible differences between control and injected in memory encoding organization. We will also label for astrocytes to check if significant neuroinflammation has been generated due to the scopolamine injection.

Demonstrating task-switching cost in humans
by Sze Ying Lam

Background: Task-switching cost in humans has been observed but not fully explained since the early 90s. Task-switching requires executive cognitive control, and therefore is highly associated with the activities in the prefrontal cortex. The existence of a robust, irreducible cost in performance during task-switching is a phenomenon that many cognitive neuroscientists are trying to understand because the underlying mechanism could provide important insight about the executive control pathways and their inherent limits. A task-switching cost usually takes form as either a delay in reaction time or a decrease in accuracy, or both, in the performance of a task. This cost can be observed when participants are required to switch from one task to a different one in a sequence of trials. When we compare the performance between switch vs non-switch trials, a decrease in performance in switch trial with respect to the non-switch trials is what we would call a task-switching cost. The phenomenon of task-switching cost is very relevant to our daily life and there are some surprising aspects of the task-switching cost we can easily demonstrate with simple experiments. These aspects include the fact that task-switching cost seems to be irreducible even if the switch schedule is completely predictable, and the fact that, even when given ample time for preparation for switching, the switch cost remains.

Aim: In this project we aim to demonstrate some aspects of a task switching cost through designing and conducting psychophysical experiments in humans.

Methods: The project will be composed of several stages, including brain-storming for experimental design, execution of experiment, data analysis and discussion of results for conclusion and further studies. In the brain-storming stage, we will lay out the main research questions and try to design an experiment that can put our hypothesis to test. The second stage of execution will involve the programming tasks. When the experiment is ready, we will test it by having students testing each other and collecting data. At the third stage, we will set data analysis objective and students will explore and test methods of data analysis and we will share and compare results. At the end, we will all discuss the study in terms of the obtained results and relevant conclusions and limits. Students will acquire hands-on experience in experimental design, programming experiments, data collection and analysis and hypothesis forming/testing.