BORDEAUX SCHOOL OF NEUROSCIENCE

Bordeaux Neurocampus Graduate Program

université de BORDEAUX

Workshop: Introduction to experimental Neuroscience

Bordeaux School of Neuroscience July 17th-29th

First week – July 17th-22nd

Project 1: Differentiation of C17.2 neuroprogenitors and comparison with primary cultures of mouse neurons and creation of mouse neuronal spheroids using Aggrewells

Instructor: Deepshika Arasu (*University of Barcelona*)

Background

Cell culturing techniques and 3D cellular models are widely used in neuroscientific experiments and have significantly improved our knowledge of the physiological and pathological processes of living organisms. Multiple differentiation protocols are currently available for deriving neuronal cultures. Here, we will focus on the differentiation protocols of C17.2 mouse neuroprogenitor cells. C17.2 is an immortalized mouse cell line with a shorter duration of differentiation required to obtain mature neurons. Culturing these cells in mediums with different nutrient compositions (maintenance medium, differentiation medium and a modified medium) will result in populations of cells with different characteristics and maturity that will be analysed using PCR and immunocytochemistry. These will then be compared with primary neuronal cultures to understand the maturational differences. Alongside, we will use Aggrewells to create 3D mousel neuronal spheroids and analyze their physical characteristics.

Aims:

- To analyze differentiation protocols of C17.2 neuroprogenitor cell line using PCR and immunohistochemistry analysis
- To compare the neuronal differentiation protocols with mouse primary neurons.
- To create mouse neuronal spheroids of C17.2

Techniques

- Aseptic culture methods
- Neuronal differentiation protocols
- Cell culture morphological observations
- PCR
- Live/Dead Assay
- Immunocytochemistry
- Biomaterials for neuronal culture
- Epifluorescence microscopy
- Aggrewells
- Spheroid handling techniques

Project 2: Exploring Neuronal Development: A Simulation Study on Neuron Cells using NEURON Simulator
Instructor: Eslin Ustun Karatop (*University of Ottawa*)

Background
NEURON Simulator is a powerful tool that allows for the simulation of the electrical and chemical processes that occur within neurons. It enables the modeling of individual neurons as well as networks of neurons and provides a platform for testing various interventions that can modulate neuronal function.
In this project, we will use NEURON Simulator to simulate the effect the electrical and chemical properties of neuron cells. Specifically, we will investigate how changes in modifications affect the ion channels and receptors that regulate neuronal activity.
By simulating the neuron cells, this project aim to identify potential targets for modulating neuronal function and improving communication between neurons. This could have implications for the treatment of neurological disorders, such as Alzheimer's disease, Parkinson's disease, and epilepsy.

Aims:
- To investigate the effects of electrical and chemical properties of neuron cells using NEURON Simulator.
- To identify potential targets for modulating neuronal function and improving communication between neurons based on the simulation results.
- To explore the implications of the findings for the treatment of neurological disorders, such as Alzheimer's disease, Parkinson's disease, and epilepsy, and to suggest new avenues for research in this field.

Project 3: Correlation between synaptic protein organization and dendritic spine morphology
Instructor: Tiffany Cloatre (*University of Bordeaux*)

Background
Synapses are micron scale cell-cell contacts allowing communication between neurons. Synapses exist in many different morphological and functional states. Changes between these states are known to underlie essential brain functions such as learning. The post-synapse corresponds to small protrusions called dendritic spines filled with a dense actin cytoskeleton and whose dynamic remodeling sustains morphological adaptations. A central, more restricted, compartment called the post-synaptic density, which is connected to both adhesion molecules and the actin network, serves as a platform to anchor neurotransmitter receptors mediating synaptic transmission. These protein families are involved in the morphology of synapses.
Single-Molecule Localization Microscopy (SMLM) techniques have proven to be essential for monitoring molecular organization and dynamics. However, SMLM produces clouds of protein localizations that often lack a reference to the morphology of the synapse. In contrast, the complete shape of the synapse is best obtained with STimulated Emission Depletion (STED).

Aims:
The objective of this project is to super-impose the super-resolved images obtained in different microscopy modalities to map synaptic proteins at the nanoscale in relation to the synapse morphology.

Techniques
This involves the use of different techniques. Electroporation of cells with plasmids and immunolabelling will be used to visualize the proteins of interest by fluorescence. They will then be imaged by two super-resolution microscopy techniques, Photo-activated localization microscopy (PALM) and STED. Finally, the correlation between the organization of synaptic proteins and synapse morphology will be obtained by image processing.

Project 4:
Instructor: Unis Bhat (*University of Bordeaux*)
Background

Brain is plastic, and experiences have a role in shaping brain development and functions. Experiences can have a positive or negative impact on brain functions and behavior. Understanding the molecular mechanisms mediating experience induced changes in brain are of broader interest; are envisioned to provide for alternative approaches to mental wellbeing and for development of better efficacy therapeutics for neuropsychiatric disorders. Chronic exposure to undesirable experiences, like drugs of abuse, adversely affect brain physiology and are risk factors for development of other neuropsychiatric disorders viz. anxiety, depression and addiction. For example, chronic alcohol exposure attenuates hippocampal neurogenesis and induces transcriptional changes of several genes in hippocampus residing neural stem cells (NSCs). Hippocampal neurogenesis mediates several functions related to memory formation, stress resilience. Drug induced deficits in hippocampal neurogenesis potentially contributes to cognitive deficits and other addiction associated behavioral deficits. Identifying and characterizing molecular mediators of alcohol abuse in stem cells would pave for exploring novel drug targets for developing treatments for alcohol-induced neurotoxicity.

Techniques:
Neural stem cell (NSC)-based medicine holds great promise for the treatment of various neurological disorders including drug abuse. However, further studies are required to inform evidence based safety and therapeutic potential. For this, detailed mechanistic understanding of mechanisms regulating neural stem cell behavior and functions are warranted. Alcohol exposure can have significant effects on neurosphere formation (a function of stem cell proliferation), survival, and differentiation. In addition, chronic alcohol exposure perturbs NSC transcriptional profiles that in turn might be mediating the adverse effects on NSC/NPCs via epigenetic based gene regulations. Here, plan is to establish the neurosphere culture from hippocampus derived NSc/NPCs from neonatal pups. Proliferating neurosphers would be used to evaluate impact of in-vitro alcohol exposure on proliferation. Further, transcriptional analysis using qPCR would be carried out to assess alcohol treatment induced changes in several genes required for stem cell maintenance and proliferation. The protocol can be further be adapted to evaluate effects on neural stem cell

differentiation to neurons using either transcriptomic or imaging based analysis of marker genes for early differentiation.

Project 5: Using microcontrollers to use run experiments and acquire data
Instructor: Nikhil Prabhu

The first would be to use a microcontroller board to deliver visual stimuli and record the time it takes to discern oddballs vs simple visual stimuli. This is realised by wiring LEDs and single button box to the microcontroller board. The board is then instructed to control the LEDs (1 red LED among 3 green LEDs) with a simple program in C while the button press timings in response to only green LEDs and oddballs (red LED amongst green LEDs) are recorded. A simpler experiment that could be conducted is to use a simple and low-cost EMG (electromyogram) kit such as those by Myoware. In this experiment, as a first step EMG is recorded with the help of electrodes and read on a PC with the help of a programmed microcontroller board using a simple C program. This EMG signal could then also be used to control something else such as a motor which is attached to a lever that moves up and down depending on if muscles are flexed or relaxed.

Aims

- Understand a microcontroller board setup
- Program it to control inputs and ouputs
- Run a full experiment with the above knowledge

Techniques:

Programming simple programs in C. Simple hardware understanding and control. Very little amount of soldering if time permits

Project 6: The role of dopamine in the short-term synaptic plasticity at the DG-CA3 synapse, inside the hippocampus
Instructor: Simon Lecomte (IINS, Bordeaux)

Synaptic plasticity can be modulated by neuronal excitability. Two main actors that can change neuronal excitability after activation of Gs or Gi receptors are the following: activation of D1 receptors (Gs alpha subunit) via cAMP and PKA can decrease the potassium conductance and then increase neuronal excitability (Lee, Frontiers in Pharmacology, 2015); and activation of D2 receptors (via beta-gamma subunits) can increase the calcium conductance and then increase neuronal excitability. First, by recording in current-clamp mode in the DG and CA3 with or without dopamine we will be able to know if there is change in passive membrane properties as well as the AP properties of granule cells and pyramidal cells. Finally, by recording in voltage clamp mode in CA3 and stimulate the DG by using a PTP protocol with or without dopamine we will be able to know if there is a change in the short-term plasticity at this synapse.

Aims

- Study the action potential and passive membrane properties of granule cells of the DG with or without dopamine
- Study the action potential and passive membrane properties of pyramidal cells of the CA3 with or without dopamine
- Study the short-term synaptic plasticity (PTP) of the DG-CA3 synapse with or without dopamine

Techniques:
Patch-clamp electrophysiology

Project 7: Deep brain structures, anatomy, research perspectives and skills for data analysis.
Instructor: Nana Tchantchaleishvili (RebrAIn, Bordeaux)

My MSc project was a part of clinical trial focused on essential tremor targeting with a new software created in Bordeaux (OptimDBS, RebrAIn). But I'm planning to include anatomical background and clinical correlation, as different structures can be targeted for different diseases. I have been on this summer school as a student, and 1st week of my training (group fMRI analysis, GIFT, resting state) helped me a lot in choosing a correct internship project for myself and being more prepared and confident, as up to 30 % of software used are the same for fMRIs as well as lead DBS analysis. I hope my experience will also be helpful for the students interested in neuroradiological research. Reconstructed 3D structures will also help to better memorize the structures. I will use demo mode, MNI space, as well as patient data (with permit), there are multiple highest quality 3D atlases of incorporated in this softwares and it's a perfect learning tool for anatomy.

Aims

- Anatomy of deep brain structures
- Clinical Correlations and Future Research Perspectives
- Using Neuroradiological software for data analysis

Techniques:
Lead DBS software (fmatlab version), MedInria for the final simplified 2D analysis.

Project 8: Chronic in vivo recordings in multiple brain regions using movable silicon probes
Instructors: Tiago Reis and Mariana Laranjo (University of Coimbra)

Students will learn how to design and assemble a modular printable headgear that allows for stable, long-term recordings of 2 silicon probes, while allowing for independent movement of the animals and recovery for reimplantation.
Then, surgeries will be performed to implant both the silicon probes and the headgear in the mouse's medial prefrontal cortex (mPFC) and dorsal hippocampus. After recovery, dual in

vivo electrophysiological recordings coupled to optogenetic manipulation of the mPFC will be performed in a head restrained mouse and/or in a freely behaving paradigm across 3 days, where both silicon probes will be moved to different depths of the target region in order to acquire new putative single units. Electrophysiological data will be acquired, amplified, and digitised using the Omniplex Neural Recording Data Acquisition System. Students will learn the basics of signal processing and analysis ranging from visualization of raw traces to identification of isolated neuron's activity, dependent and independent of optogenetic manipulation.

Aims:

- Assembly of the 3-D printed headgear and experimental design of the dual probe implantation.
- Minimally invasive dual-site silicon probes implementation surgery.
- In vivo electrophysiological recordings in head restrained mice, signal processing and analysis.

Techniques:
Minimally invasive recovery surgery, In vivo neurophysiological recordings, In vivo neurophysiological data analysis.

Project 9: Effects of fructose consumption during adolescence on mood disorders and neuroinflammation
Instructor: Adeline Coursan (NutriNeuro, Bordeaux)

Emotional and cognitive alterations will be evaluated in mice (male) fed with fructose 25% or water (controls) in the drinking water during the adolescent period (PND28-46). When mice will reached adulthood, anxiety- and depressive-like behaviors will be studied using appropriate tests routinely performed in our institute (open field, elevated plus maze (EPM), object localisation memory). Neuroinflammation will be studied by RT-qPCR on isolated microglia from brain's mice.

Aims:

Evaluate the effects of fructose consumption on mood and cognition
Study the neuroinflammation induces by fructose consumption during aldolescence
Investigate the relevance of the fructose exposure window

Techniques:
Behavioral tests (EPM, OF, OLM), Microglia extraction, RT-qPCR

Second week – July 25th-30th

Project 10: Assessing the effects of neurodevelopmental disorders by high confocal imaging

Instructor: Jawdat Sandakly (*University of Milan*)

Background

Neurodevelopmental disorders (NDDs) are a heterogeneous group of neuropsychiatric conditions that are characterized by a pronounced deficiency in cognitive function as well as mental retardation. Intellectual disability, epilepsy and sleep disturbances are some of the common symptoms shared by these disorders. They are known to manifest very early in life as their pathogenesis is associated with the early onset development of the brain during this period, and more specifically with how they are initially constructed during development, and operate when they are prone to various cognitive and motor functions. Although they cannot fully recapitulate the human clinical and/or molecular and cellular phenotypes, mouse models have been extensively used to study the aetiology of these disorders. The combination of in vitro models and imaging techniques offers the advantage to visualize how these disorders affect the brain structure and morphology, and to investigate the functional abnormalities in these models which will lead to a better understanding of the disorders.

Techniques:

The focus of this mini-project will be on performing immunostaining on mouse brain sections, in combination with in vitro immunostaining on hippocampal neuronal cultures. We will be working with two experimental conditions: wild type samples, and samples exhibiting symptoms of a neurodevelopmental disorder. We will be checking for the expression of known brain and neuronal markers (MAP2, NEUN, GFAP, SHANK3, SYNAPSIN) that are known to be affected by these types of disorders. The samples will be subjected to confocal microscopy, and the images acquired will be further analyzed by (ImageJ) to assess the morphology and further check for defects in the expression pattern.

Project 11: Introduction to central control of energy homeostasis: “spoonful of sugar”

Instructor: Marialetizia Rastrelli (*University of Lille*)

Background

Energy homeostasis, defined as the accurate balance between energy income (food intake) and energy expenditure, is in part regulated by the brain.

Appetite and energy expenditure are orchestrated by two neuronal populations located in the arcuate nucleus of the hypothalamus (ARH) that release peptides stimulating or inhibiting food intake (orexigenic and anorexigenic peptides, respectively). In order to maintain energy homeostasis, hypothalamic neurons in the ARH must rapidly sense and integrate a multitude of peripheral signals, including hormones and nutrients (i.e. circulating glucose).

Aims:

This project aims to introduce the students to the basis of the central control of energy homeostasis.

Techniques:

Using glucose stimulation (spoonful of sugar), the students will recapitulate in a mouse model a physiological nutritional challenge and will monitor the variation of peripheral blood concentration (glycaemia) in real-time. Combining immunohistochemical labeling and fluorescent microscopy on coronal brain sections, they will also learn to highlight and identify the neuronal population(s) activated by the changes in circulating glucose.

The objective of this project is for student to get familiar with theoretical fundamental and practical aspects of central control of energy homeostasis. In particular, they will learn:

- to handle free moving mice and monitor the evolution of glycaemia after glucose stimulation
- to harvest mouse brain, to recognize basic brain anatomy and to use brain atlas for collection of coronal brain sections
- to perform immunofluorescence on brain slices
- learn the basis of fluorescence microscopy and acquisition of images.

Project 12: Mitochondrial physiology in developing neurons

Instructor: Martijn Kerkhofs (*Université Claude Bernard Lyon 1*)

Background

The development of the brain is a tightly orchestrated process that consists of different sequential steps. Neurodevelopment is highly energy consuming and thus cellular metabolism is crucial to underpin this process. Given their role in sustaining metabolism and ATP production, mitochondria are important player in the development of neurons. However, due to their extended spatial dimensions, neurons are presented with a challenge; diffusion is not sufficient to get metabolites and other mitochondrial output products from the cell body to the distal axons or dendrites. Therefore, mitochondria need to move to these distant parts of the neurons to perform their function locally. Moreover, given the different functions of dendrites and axons, mitochondria behave differently, for example in terms of morphology.

Aims:

In this project, we aim to study mitochondrial function and movement in developing neurons under various conditions.

Techniques:

We will extract primary cortical embryonic neurons and electroporate them with markers which will allow us to study mitochondrial functions in real time. In addition, we will look at mitochondrial morphology in various subcellular neuronal compartments (axon, soma,

dendrites) at different stages of neuronal development. Lastly, we will study the morphology of developing cortical neurons through time and characterize the changes they undergo during this process. To fulfil these aims, we will dissect and electroporate embryonic cortices and put them in culture. We will be doing immunofluorescence assays, confocal microscopy and live imaging microscopy as downstream applications. Acquired images will be analysed using imaging analysis software.

Project 13: Exploring the Effects of Communication Medium on Team Dynamics, Performance, and Emotion in Online Collaborations

Instructor: Maylis Saigot (*Copenhagen Business School & University of Southern California*)

With the increasing trend of remote work and online collaboration, understanding how different tools and modes of communication influence team dynamics, performance, and emotional contagion is of paramount importance. In this project, we will explore how engaging in collaborative work via instant messaging versus audio calls impact teamwork outcomes such as social connectedness and performance in small teams. Using recent technology to conduct mobile laboratory experiments, we will quantify emotional contagion as a moderator in our research model. While the scope of the projects will not allow us to measure statistically significant effects, it will provide students with an opportunity to learn about psychophysiological experimental design, opportunities for conducting remote experiments, and experimental and behavioral data analysis. Our preliminary findings will be discussed and put into perspective such that students can start explaining some of the affective processes at play during various collaboration modes. For this project, we will recruit participants who will take part in collaborative activities in pairs. They will interact either via instant messaging or an audio call. Their conversation, facial expressions, and eye gaze will be recorded. Students will be able to choose their preferred method to quantify emotional contagion from any of these channels (natural language processing, facial expression analysis, shared gaze analysis, etc.). This project will expose students to real-life dilemmas when in the lab, such as making important decisions regarding sampling methods vs. budget, scientific merit vs. feasibilities, etc. They will also learn to navigate common challenges such as protocol design, participants' recruitment, etc.

Project 14: Exploring the neural mechanisms of altered sensory perception in autism

Instructor: Ourania Semelidou (Neurocentre Magendie, Bordeaux)

In this project, we aim to explore the neurobiological mechanisms that underlie altered sensory responses in autism. To this end, we will use a sophisticated toolset, combining a novel behavioral task with in vivo two photon microscopy in a genetic mouse model of autism. This genetic mouse model exhibits sensory alterations and changes in a wide range of behavioral phenotypes related to other ASD core symptoms. To study sensory responses we will use a perceptual decision-making task. In this task, the animal will report whether it perceived a tactile stimulus to obtain a water reward. This task will allow us to study stimulus perception and to define the detection threshold of each mouse – the stimulus that the mouse perceives and reports 50% of the time. In parallel, we will study neuronal activity in the somatosensory cortex, to probe alterations in the activity of excitatory and inhibitory

neurons during the detection of the tactile stimuli. Overall, in this project we will determine how tactile responses are changed in ASD, we will measure neuronal activity at cellular resolution during behavior, and finally, we will explore how altered neuronal activity correlates with atypical sensory responses.

Aims:

- Learn about genetic mouse models of disease
- Learn about behavior in mice; observe a decision-making task and analyze the results to probe for sensory alterations in a mouse model of autism
- Learn about calcium imaging; observe a recording of neuronal activity during a behavioral task and examine if activity is altered in a mouse model of autism.

Techniques:

Behavioral experiments (reward-based decision-making task) and in vivo calcium imaging.

Project 15: fMRI data analysis
Instructor: Nikhil Prabhu

The project would be to download a publicly available dataset on any task of interest to the majority of the students in the group, organise the dataset, and learn simple steps to analyse this data. There could be two kinds of fMRI data – data that has already been pre-processed for analysis or raw data. Depending on the students interest, I could go through the pre-processing steps for the raw data and then process to analysis or start with analysis straight away with the pre-processed data. The project would be run on MATLAB with an SPM toolbox. Exposure to programming in MATLAB will make the job faster and easier to run but is not necessary since both pre-processing and analysis on fMRI data with SPM's GUI can be performed without the need for any programming knowledge.

Aims:

- Search through publicly available task fMRI datasets
- Download and organise these datasets to make them ready for analysis
- Analyse the dataset and come up with concrete results for questions that can be asked with the dataset

Techniques:

fMRI data pre-processing and analysis with the SPM toolbox that can be installed on MATLAB.

Project 16: Neuronal signature of instrumental learning in the medial prefrontal cortex
Instructor: Julien Courtin (Magendie, Bordeaux)

To reveal the encoding principles of the learning of action-outcome contingency, we will monitor the activity of CaMK2-expressing principal neurons in the PL, while naïve mice have to learn that an action (lever press) lead to the delivery of an outcome (sucrose reward). We will use a miniature microscope equipped with a chronically implanted gradient index (GRIN)

lens or prim probes, which enable the monitoring of neuronal activity of large populations of neurons with cellular resolution in freely moving mice using genetically encoded Ca2+ sensors. To express the Ca2+ sensor, GCaMP6f, we will inject AAV expression vectors at the targeted stereotaxic coordinates. To target glutamatergic CaMK2-expressing principal neurons (PNs), we will use AAV expression vector containing CaMK2 promoter. This approach will allow us to characterize, at single-cell resolution, the temporal dynamics of neuronal ensembles encoding the task. More specifically, we will examine the activity of PL neurons associated with behavioral read-out like action and reward consumption at early stage of learning.

Aims:

To reveal the encoding principles of the learning of action-outcome contingency in the PL

Techniques:

Calcium imaging in freely behaving mice

Project 17: Chronic in vivo recordings in multiple brain regions using movable silicon probes

Instructors: Tiago Reis and Mariana Laranjo (University of Coimbra)

Students will learn how to design and assemble a modular printable headgear that allows for stable, long-term recordings of 2 silicon probes, while allowing for independent movement of the animals and recovery for reimplantation.
Then, surgeries will be performed to implant both the silicon probes and the headgear in the mouse's medial prefrontal cortex (mPFC) and dorsal hippocampus. After recovery, dual in vivo electrophysiological recordings coupled to optogenetic manipulation of the mPFC will be performed in a head restrained mouse and/or in a freely behaving paradigm across 3 days, where both silicon probes will be moved to different depths of the target region in order to acquire new putative single units.
Electrophysiological data will be acquired, amplified, and digitised using the Omniplex Neural Recording Data Acquisition System. Students will learn the basics of signal processing and analysis ranging from visualization of raw traces to identification of isolated neuron's activity, dependent and independent of optogenetic manipulation.

Aims:

- Assembly of the 3-D printed headgear and experimental design of the dual probe implantation.
- Minimally invasive dual-site silicon probes implementation surgery.
- In vivo electrophysiological recordings in head restrained mice, signal processing and analysis.

Techniques:

Minimally invasive recovery surgery, In vivo neurophysiological recordings, In vivo neurophysiological data analysis.