BORDEAUX SCHOOL OF NEUROSCIENCE

Bordeaux Neurocampus Graduate Program

université de BORDEAUX

Workshop: Introduction to experimental Neuroscience

Bordeaux School of Neuroscience July 18th-30th

First week – July 18th-23rd

Project 1: In vivo calcium imaging in behaving mice: from experiment to data analysis
Instructor: Alice Fermigier (*University of Bordeaux, France*)

Background

In both neurons and astrocytes, cellular activity in the form of action potentials, exocytosis of neurotransmitters, changes in synaptic plasticity and gene transcription is coupled to an influx of calcium (Ca2+) ions. Fiber photometry is a calcium imaging technique that can captures 'bulk' or population-level Ca^{2+} activity (Cui et al., 2014) from specific cell-types within a brain region. It relies on the expression of genetically encoded calcium indicators, like GCaMP, which can be targeted to specific cells using cell-specific promoters. The ability to record the activity of a specific subpopulation of cells is one of the main advantages of this technique compared to in vivo electrophysiology. It also limits mobility of the animal significantly less than other methods, allowing for more freely-moving, naturalistic behaviours in rodent models. Finally, since its first use in 2014, this technique has undergone substantial development: it can now be coupled with other fluorescent indicators which enables the recording of not only cell activity but specific neurotransmitter release (like dopamine or endocannabinoids) or pH changes.

Methods

Using fiber photometry recordings in the dorsal and ventral hippocampus, we will investigate the role of the pyramidal neurons in these two structures in different types of memory. Students will first learn about stereotaxic viral injection and fiber implantation. They will then perform photometry recordings in behaving mice using several memory tests, then learn how to analyse behaviour and photometry data using dedicated softwares and custom codes in Python. Finally, they will assess the quality of the fiber implantation and fluorescent labelling with simple histology techniques.

Project 2: Introduction to ex vivo patch-clamp electrophysiology
Instructor: Agata Nowacka (*University of Bordeaux, France*)

Background

Patch clamp electrophysiology, a technique widely used in Neuroscience, allows to measure ionic currents in isolated cells and thus, characterise neuronal ion channels and cell activity. With this technique we can examine the composition and activity of receptors within synapses- connections between neurons. The strength of these connections is actively tuned through synaptic plasticity and a lot of scientific effort over the years has been dedicated to understanding how synaptic plasticity in the brain is regulated. Using pharmacological and genetic strategies and patch clamp we can search for players involved in its regulation.

Methods

During this project students will have an opportunity to get an overview of the technique and different steps that need to be fulfilled to perform a patch-clamp experiment as well as get to know different models that can be used for these experiments. Students will participate in the preparation of tissue for the experiments including both organotypic and acute slices.

Together we will prepare mice hippocampal organotypic slice cultures and induce GSG1L overexpression by either single-cell electroporation or AAV micro-injections. Then, the students will be guided through the preparation of a patch-clamp recording experiment including the preparation of recording solutions or glass pipettes. Next, we will perform patch-clamp whole-cell recordings from CA1 pyramidal neurons and measure the effect of the protein overexpression on short-term synaptic plasticity. Next, students will be guided through the analysis of electrophysiological data with Clampfit software. By the end of the training, students should understand the basis of patch-clamp electrophysiology and have performed a successful patch-clamp recording. They should also know the models commonly used to perform these types of experiments and methods used to induce exogenous protein expression in tissue. Finally, students will be able to analyse electrophysiological data.

Project 3: Differentiation of C17.2 neuroprogenitors cells and comparison with primary cultures of mouse neurons

Instructor: Deepshika Arasu (*University of Barcelona, Spain*)

Background

Cell culturing techniques 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 differences.

Methods

During this project we will analyse various differentiation protocols of C17.2 neuroprogenitor cell line using PCR and immunohistochemistry analysis and we will compare the neuronal differentiation protocols with mouse primary neurons.

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

Project 4: Quantitative analysis of cell adhesion molecules dynamics and nanoscale organization at synapses using single molecule localization microscopy
Instructor: Adèle Drouet (*University of Bordeaux, France*)

This project will provide a quantitative description of the membrane dynamics and nanoscale distribution of the adhesion molecule neuroligin-1 in neurons, by correlating imaging experiments and computer simulations.

Students will first learn the basics of fluorescence microscopy, and will be introduced to live-cell and super-resolution imaging techniques. The FluoSim software, that allows to model and predict membrane protein dynamics in 2D cellular geometries, will be used as a teaching support to understand the power and limitations of each specific method. Then, we will use cultures of dissociated hippocampal neurons, previously electroporated with tagged neuroligin-1 (NLGN1) and labelled with fluorescent probes, for observations through a custom-built microscope. We will visualize in live cells trajectories of individual NLGN1 molecules by SPT (Single Particle Tracking). In addition, the distribution of densely labelled NLGN1 molecules in fixed neurons will be visualized by dSTORM (direct STochastic Optical Reconstruction Microscopy). Finally, we will analyse the images stacks using dedicated image analysis software (PALM-Tracer) and run simulations using FluoSim to quantitatively interpret the experiments performed during the week.

Project 5: Investigating the impact of proteasome inhibition on a neuronal cell line
Instructor: Georgie Lines (*UCL, London, UK*)

Background
The ubiquitin proteasome system is the dominant protein clearance mechanism in cells. Protein clearance is essential for cell survival, and a decline in proteostasis has been implicated in neurodegenerative diseases. The proteasome is composed of a 20S core unit, bookended by one or two regulatory units, forming the 26S and 30S proteasome respectively. Proteins destined for degradation via the proteasome are tagged with ubiquitin in a process involving enzymes E1, E2 and E3. Ubiquitin tagged proteasomes are recognised by ubiquitin receptors in the regulatory subunit of the proteasome. The substrate protein is then deubiquitinated, translocated to the core of the proteasome, and digested. The discovery of proteasome inhibitors allows us to investigate the importance of proteostasis for cell survival, and the coping mechanisms of cells when degradation machinery is impaired.
Methods
This project will give students experience in cell culture, cell treatment, sample extraction and preparation, Western blotting, fluorescent imaging and data analysis. Students will culture SHSY5Y cells and treat them with a specific irreversible proteasome inhibitor (epoxomicin) for either 2, 6 or 24 hours. They will lyse cells, measure the protein concentration, and use Western blotting (SDS-PAGE) to measure differences in ubiquitinated proteins. Students will also probe against P62, a marker of autophagy, cytochrome C, an indicator of apoptosis and beta-actin or GAPDH to normalise their data. Students will quantify their Western blot data and create a panel figure (N=1). The same epoxomicin treatment will be investigated on cells grown on coverslips, and fluorescence imaging of ubiquitin and cytochrome C will be performed to investigate differences in protein levels and localisation.

In parallel to the above experiments, media will be collected from cells treated with epoxomicin, and run in a cytotoxicity assay to measure effects on proteasome inhibition on cell survival.

Project 6: Fundamentals of neuronal excitability: Measuring M-currents in hippocampal neurons

Instructor: Marina Manuela Ventura Rodrigues (*Centre for Neuroscience and Cell Biology - Coimbra, Portugal*)

Background

The M-current is a low-threshold potassium current that shapes neuronal excitability and dampens repetitive firing. A decrease in M-current leads to neuronal excitability, this being a common epileptogenic mechanism. Control of hyperexcitability through the modulation of M-channels has been the last-line treatment for pharmacoresistant epilepsy. M-channels are ubiquitously expressed, low-threshold, slowly activating Kv channels.

The hippocampus is a brain region involved in the learning process and in the formation of new memories. Alterations in neuronal excitability in the hippocampal and cortical subregions are found in patients with neurodevelopmental disorders which feature a spectrum of cognitive impairment, aberrant social behaviour, and convulsion. In hippocampal CA1 neurons, axonal M-channels regulate the inherent neuronal firing properties by controlling axonal resting membrane potential and action potential threshold, and ablation of a M-channel subunit, Kv7.2, in the neocortex leads to increased neuronal excitability of layer 2/3 pyramidal neurons. M-channels activity can be inhibited by muscarinic acetylcholine-receptor agonists and is also regulated by phosphatidylinositol 4,5-bisphosphate (PIP2), calmodulin, changes in membrane voltage and other signalling pathways that control neuronal excitability.

Methods

Electrophysiological recordings will be performed under the whole-cell patch clamp configuration, both in voltage and current clamp, with and without drug perfusion. The identification and isolation the M-type component of the potassium currents will be conducted in two different ways. In the classical way, the cells are recorded before and after the perfusion of a specific inhibitor of the M-channels and so the M-current is the subtracted current which is sensitive to the drug. Alternatively, we can take advantage of a specific activator of the M-channels at resting membrane potential (RMP) and measure the hyperpolarisation of the RMP as a direct consequence of M-current activation upon drug perfusion. Finally, the impact of M-channels' manipulation upon neuronal firing activity will also be studied.

Project 7: Seeing the mouse cortex in action

Instructor: Michael Sokoletsky (*Weizmann Institute of Science, Israel*)

Background

What does the cortex do, exactly? We know that some regions respond in a distinctive manner to certain inputs (e.g. visual gratings) or behavioural outputs (e.g. limb movements), but activity in most other regions (labelled by the vague blanket term 'associative') is quite mysterious. If we stick an electrode in one of these regions, we will find large ongoing fluctuations in activity. But activity in each individual region is only a piece of the puzzle –

ultimately, this activity comes from and is directed to many other regions in the brain. Hence, to try to make sense of what the cortex does, it is useful to simultaneously record from many regions at once.

Methods

In this project we will seek to do so using a relatively simple technique called widefield calcium imaging, where the mouse cortex is exposed – leaving the skull intact – and a camera is placed above it to image cortical activity. In addition, behaviour will be recorded using a USB camera directed at the mouse's body. We will ask: Are different brain regions soloists, acting out their own roles in different ways? Or is the cortex a monolith, turning on and off at once? Perhaps the answer is somewhere in the middle, with some activity being individual to certain regions and other activity being correlated across regions. Regarding behaviour: Are its neural correlates only found in motor areas, or could we find them in sensory and associative areas as well? We will show how all these questions can be addressed using simple techniques and open science tools.

Project 8: Visualizing protein interactions with M-channels

Instructor: Ângela Inácio (*University of Coimbra, Portugal*)

Background

In neurons, voltage-gated potassium channels (Kv) are responsible for repolarization after an action potential, having a crucial role in the regulation of neuronal excitability and network activity. The M-channels are ubiquitously expressed, low-threshold, slowly activating Kv channels that mediate the non-inactivating M-current. M-channels are activated at voltages near the threshold for action potential initiation and their pharmacological inhibition results in increased excitability. M-channels are primarily composed of Kv7.2 and Kv7.3 subunits, being the heteromeric form Kv7.2/7.3 the most relevant for the generation of M-currents. These channels are present in the axonal initial segment and Ranvier nodes and at the somatodendritic compartment. M-channels can be regulated by neurotransmitters, changes in membrane voltage and multiple regulatory pathways, moreover, protein-protein interaction plays a central role in the regulation of M-channels activity.

Methods

We will use cultured hippocampal neurons and perform pharmacological manipulations to investigate how changes in neuronal activity might affect the interaction of M-channels with auxiliary proteins that regulate their function.

Protein-M-channel interacting complexes will be visualized using the proximity ligation assay (PLA). In this assay, fixed cells are stained with primary antibodies, generated from different species, for the M-channel and the interacting protein under study. Cells are then stained with secondary antibodies linked to specific DNA primers. After a hybridization step followed by an *in situ* PCR using fluorescent probes, the number of protein-M-channel complexes will be assessed by fluorescence microscopy. The PLA assay allows the detection of putative interacting proteins which are within 40 nm of each other. To visualize more transient protein-M-channel interactions, we will image live neurons using the splitFAST, a fluorescence complementation system. This technique uses a reversible split fluorescent reporter that permits the detection of the formation and dissociation of protein complexes in real-time. We will evaluate the effects of neuronal activity on the dynamics of protein-M-channel complexes taking advantage of drugs that alter the firing rate of neurons.

Project 9 and 10: Neuroanatomy and neuropsychology hands-on

Instructors: Valentina Pacella (*University of Bordeaux, France*) and Mar Martín Signes (*University of Granada, Spain*)

Background

The study of the functioning of brain grey matter has a long tradition as many tools have been developed to measure it (for example, electroencephalography, functional magnetic resonance imaging [fMRI] or neuromodulation techniques). However, the study of the functioning of brain white matter is very recent, as previous methods (i.e. postmortem dissection) limited the field. With the development of new MRI sequences (i.e. diffusion weighted imaging) that allow an in vivo examination of white matter connections, a new field has been open in neuroscience.

These new techniques allowed the accessible anatomical identification of symptoms' profiles in case of brain lesions. In the European Union alone, 1.12 million incidents of brain damage after stroke are recorded every year, and prompt symptom identification plays a crucial role in patients' recovery. At the same time, the progress made in the neuropsychology field has led to a finer assessment of abnormal behavioural manifestation after a brain injury and the discovery of new neuropsychological syndromes.

The joint contribution of advanced neuroimaging and cutting-edge neuropsychological assessment has proven fruitful in understanding the normal functioning of the brain and improving patients' rehabilitation techniques.

Methods

This workshop will focus on the study of brain grey and white matter and its involvement in healthy cognitive functions and patients population. We will learn and apply two different methodological approaches:

a) We will use a set of fMRI data to learn the pre-processing and analysis steps and we will end up by applying the Functionnectome, a new method that will allow us to associate task-related brain activations to the white matter substrates.

b) We will learn the principles of tractography and carry out virtual dissections of some relevant tracts. Moreover, the course will give a hands-on practical introduction to modern lesion analysis techniques using softwares for the exploration of grey and white matter damage. Also, the course will address the statistical procedures to analyse single stroke patients.

This workshop aims to be a practical, interactive and collaborative space in which we will build the knowledge together.

The software used during the course will be BCBToolkit, MRIcroGL, FSL and TrackVis.

Second week – July 25th-30th

Project 11: In vivo electrophysiological recordings of prefrontal pyramidal neurons with optogenetic manipulation of axoaxonic cells activity in awake head-restrained mice

Instructor: Cloé Lheraux (*University of Bordeaux, France*)

Background

The neocortex is comprised of two neural subpopulations: glutamatergic pyramidal neurons (PNs) and GABAergic interneurons (INs). Despite their minority, INs are thought to play critical roles in shaping cortical activity. Individual INs innervate hundreds of PNs, while multiple of them can converge on the same PN. The resulting GABAergic modulation confers high flexibility of cortical network activities. Axoaxonic Cells (AACs) are among the most intriguing GABAergic INs of the cerebral cortex. Synapsing with the axon initial segment of PNs, where action potentials are initiated, they are thought to play a crucial role in the modulation of cortical network activities. However, how AACs regulate PNs firing activity remains controversial. *In vitro* studies showed that AACs activation was leading to both excitatory and inhibitory effects on PNs while recent *in vivo* studies results tend towards an exclusive inhibitory role. Testing the effect of AAC activation on PN neuronal activity *in vivo* has remained limited due to the absence of selective genetic tools. Thus, with the implementation of a novel transgenic mouse line, it has become possible to specifically manipulate AACs *in vivo* and thus understand the effect of AACs on PNs in physiological conditions.

Methods

Students will learn how to create tetrodes that will be used for acute recordings. Then, surgeries will be performed to create a cranial window in order to be able to insert our tetrode and optic fiber in the mouse's medial prefrontal cortex when it will be awake, head restrained, running on a treadmill. After recovery from the surgery, mice will be attached using head-fixed bars and *in vivo* acute electrophysiological recordings coupled to optogenetic will be performed for up to two consecutive days. The neurophysiological signal measured will be visualized online (Omniplex, Plexon). Offline, students will become familiar to basic signal processing and analysis going from raw recordings traces to visualization of isolated neuron's activity and characterization of the effect of optogenetic stimulation of AACs on PN neuronal activity.

Project 12: Voice as a window to the mind

Instructor: Vincent Martin (*University of Bordeaux, France*)

Background

the voice is a useful tool to study human behaviour, involving more than 100 muscles and numerous neuromuscular, linguistic, and cognitive processes that interact together. This medium is very sensitive to multiple pathologies (e.g. neurodegenerative diseases, pulmonary and cardiovascular diseases, but also psychiatric troubles) but also to short-term states variations (e.g. emotions, stress, sleepiness, ...).

In this project, I propose to design a machine learning system that can estimate someone's cognitive state based on voice recordings.

Methods

The students' voices will be recorded multiple times, on a fixed schedule, during the experiment week. The existing vocal tasks in the literature are very diversified (pictures description, text reading, sustained vowels, ...): the selected tasks will be defined by the trainees, depending on the neuromuscular and/or cognitive aspects they aim at exploring.

Cognitive state variations will be either induced (e.g. emotions, stress), or naturally fluctuating during the day (sleepiness). They will be measured by different psychometric questionnaires (Visual Analog Scales, Self-Assessment Manikin, Karolinska Sleepiness Scale, Cartoon Faces, ...), that will be filled in before or after the voice tasks, depending on the experimental paradigm that will be chosen.

This project will include an introduction to voice recording (in adequation with neurosciences laboratories that are usually full of ventilation noises), voice signal processing (automatic extraction of vocal biomarkers), statistics (as usual), and machine learning.

Project 13: Drawing individual social profiles in mice : the ways of analyzing and interpreting a wide behavioral repertoire

Instructor: Nastasia Mirofle (*Paris-Saclay University, France*)

Background

Even if the study of behaviour is a tool widely used for many questions, it cannot be reduced to the acquisition and accumulation of behavioural data. Indeed, it is very easy to get lost in the data interpretation especially with the significant technological advances allowing a very large collection of data, which can sometimes lead to incorrect or ambiguous conclusions. To truly understand how the animal is functioning and its motivation underlying a specific behaviour, it is necessary to carry out a real ethological work of measure, analysis and interpretation. In addition, rodents develop their own individuality (regarding emotion and risk-taking) and ways to solve problems, thus allowing to model humans' cognitive individuality.

Methods

First, students will learn the different experimental protocols existing in rodent model according to the different processes studied, the existing paradigms as the technical tools of observation and measurement. Afterwards, students will carry out social interaction (Social Interaction Task), hierarchical dominance and social motivation (3 chamber task) tasks on mice and observe various others tasks on social motivation, anhedonia, risky decision-making or gambling and anxiety. To complete their experience, students will work on pre-analysed automatically data acquired in operant conditioning (Skinner box), during ultrasonic communication (ultrasonic vocalizations) or in an automated life cage (Intellicage) in order to become familiar with other types of data obtaining. Finally, students will have to apply the knowledge seen beforehand by carrying out a research group work. In order to model individual social and cognitive profiles in mice, students will have to collaborate to analyse and interpret a set of behavioural data by taking into account inter-individual variability and the limits of interpretation.

Project 14: Studying the morphology properties of developmentally- and adult-born dentate granule neurons

Instructor: Pierre Mortessagne (*University of Bordeaux, France*)

Background
In the dentate gyrus (DG) of the hippocampus, the generation of dentate granule neurons (DGNs) starts during the embryonic period, peaks around the time of birth, and continues at low levels during adulthood. The DG is therefore a peculiar brain structure composed of DGNs of different ontogenic origins constituting sub-populations of DGNs that could play different roles in hippocampal physiology and contribute differently to DG functions. Surprisingly, this hypothesis has received little attention and although the morpho-functional properties of adult-born DGNs (Adu-DGNs) have been extensively studied, very little is known about the developmentally-generated ones.

Methods
In this project, we will analyse the morphological characteristics of these two populations at the dendritic level. For this purpose, we will use *in vivo* electroporation to label DGNs generated in mouse neonates (P0) and stereotaxic injection of retrovirus into the DG of adult mice to tag Adu-DGNs. The dendritic morphology of the two populations of DGNs will be visualised once mature using immunohistochemistry coupled to tissue clearing techniques and analised with the help of a semiautomatic neuron tracing system (Neurolucida).

Project 15: Electrophysiological characterization of distinct hippocampal neuronal cell populations
Instructor: Ana Moreira de Sá (*University of Bordeaux, France*)

Background
Whole-cell patch-clamp remains until today the gold standard tool used by researchers to study the intrinsic electrical properties, which is the ability of a neuron to generate action potentials with high spatial and temporal precision, and functional connectivity of neurons corresponding to synaptic mechanisms. These properties differ in distinct populations of neurons, they are not fixed and can be modified by neuronal modulators. Moreover, these intrinsic differences underlie response properties of given neuronal populations, leading to different outcomes which can have robust effects on overall network dynamics and plasticity phenomena. In this project, we will focus on the hippocampal CA3 network, which plays a key role in higher cognitive functions such as multimodal information integration and initial memory encoding.

Methods
To get to know better the distinct neuronal types expressed in CA3 which are contributing to information processing, we will study the intrinsic membrane properties of different populations (various inhibitory interneurons subtypes, pyramidal cells) in brain slices from young healthy mice and compare their characteristics. Moreover, we will attempt to pharmacologically modulate intrinsic excitability by applying a cholinergic agonist - cholinergic drugs are known to modulate the induction of synaptic plasticity in the hippocampus, a cellular correlate for memory processes.

Project 16: Mouse brain fractionation and synaptosome cleavage assay
Instructor: Domenico Azarnia Tehran (*FMP Berlin, Germany*)

Background
Synapses are specialized intercellular junctions between neurons. At ultrastructural level, pre- and postsynaptic specializations are precisely opposed to each other and they are

characterized by electron-dense thickening of the membranes. At synapses, neurotransmission requires the regulated fusion of synaptic vesicles (SVs) at dedicated release sites within the presynaptic active zone. To allow for fast Ca2+-dependent exocytosis, SVs carry a defined set of up to ~1400 proteins which mediate e.g. Ca2+-sensing and vesicle fusion. Upon exocytosis, at excitatory synapses, glutamate is release from SVs and it binds to post-synaptic receptors. In this project, the molecular architecture of synapses will be unravelled using two different biochemical approaches.

Methods

The aim of this project will be to isolate synaptosomes and subject them 1) to limited proteolysis to dissociate pre- and postsynaptic membrane and 2) to osmotic lysis in order to obtain a cytosolic and a membrane fraction. Synaptosomes represent isolated nerve terminals that resealed during homogenization. Thus, during synaptosome purification, the presynaptic terminal reseals into an enclosed compartment that protects presynaptic proteins from proteolysis, while postsynaptic proteins remain susceptible to trypsin digest. In the one-week workshop, we will be able to purify synaptosome, subject them to trypsin digestion and precisely assign to specific proteins a pre- or post-synaptic localization, using SDS-PAGE and immunolabelling (Western blot). Finally, we will assign a cytosolic or a membrane localization to specific synaptosomal proteins.

Project 17: Stimulation of maternal behaviour in mice using optogenetics

Instructor: Vasyl Mykytiuk (*The Francis Crick Institute/UCL, London, UK*)

Background

The hypothalamus is an evolutionarily conserved brain region that is critical for various instinctive behaviours. Over the last decade, neuroscientists identified specific cell populations and subregions of the hypothalamus that are important for such instinctive behaviours as feeding, drinking, mating, maternal behaviour and aggression. GABAergic neurons in the medial preoptic nucleus of the hypothalamus are critical for maternal behaviours in mice. Optogenetic stimulation of this population induces robust pup grooming and retrieval to the nest even in virgin females and males. This is one of the strongest behavioural effects ever observed in behavioural neuroscience. In our project we will aim to recapitulate stimulation-induced maternal behaviour in mice as well as to investigate dependency of such behaviour on various stimuli.

Methods

The learning objective of the project is for students to get familiar with all theoretical fundamentals and practical aspects of in vivo optogenetic experiments. In particular, they will learn:

- basics of rodent brain anatomy and usage of rodent brain atlases for surgery planning;
- to select appropriate optogenetic tools to address the scientific question;
- to perform stereotactic brain surgery for delivery of viral constructs into the brain and implantation of light-guiding cannulas;
- to design and perform in vivo optogenetic stimulation experiment;
- to interpret optogenetic stimulation data and potential caveats.

Project 18: The role of visual feedback for collaborative performance and affective convergence

Instructor: Maylis Saigot (*Copenhagen Business School, Denmark*)

Background

Existing literature found that viewing oneself on video conferencing software may induce distraction. This could mean that during a Zoom call, meeting participants may be distracted by self-mirroring, or experience other forms of fatigue and stress symptoms. In doing so, they would become blind to the affective cues of their teammates, thereby impeding affective convergence. However, we could also argue that viewing oneself may allow users to perceive frictions in the group or inadequate affective cues, which would redirect their attention to others and the groups' affective needs. Thus, we ask: what is the role of individual biofeedback for affective convergence and collaborative performance in online interaction?

Methods

We will recruit 4 dyads (8 participants) and give them 20 minutes to solve a murder mystery case over a video call. We note post hoc the time when their performance peaked (e.g., they came up with the solution they eventually picked after 1min, 10min, etc.). We manipulate whether the participants have self-view on or off during their interaction. We will measure: eye tracking (to see if they look at each other, themselves, or the information more in each), facial expression (to see if they self-regulate or become more expressive, and to see how closely they converge), and gsr/hrv (to see if they are more or less excitable in each condition and pre/post-peak).

Hypothesis 1: longer deliberation time increases accuracy.

Hypothesis 2: self-presentation increases deliberation time.

Project 19: Neocortical alterations in human neurons

Instructor: Yuktiben Vyas (*Neurocentre Magendie, Bordeaux, France*)

Background

Autism Spectrum Disorders (ASD) are a multifactorial neurodevelopmental disorder characterized by deficits in social communication and the presence of repetitive or restrictive behaviours. According to the Centre for Disease Control and Prevention, about 1% of the world's population has ASD. Atypical sensory experience, processed by different cortical regions, has been characterized as one of the core features of the disorder, affecting around 90% of individuals with ASD. However, a key challenge of translating preclinical findings from mice to clinical application is the difficulty in establishing experimental approaches that have direct translational relevance. Hence, we are culturing human derived neurons from induced pluripotent stem cells from ASD patients, and examining the translatability of our mechanism-based therapeutics directly on human neurons.

Methods

In this mini-project, students will gain exposure to the process of culturing induced pluripotent stem cell derived human neurons, specifically cortical layer 2/3 excitatory neurons, and examine neuronal properties at the functional level. They will perform in vitro whole-cell patch-clamp electrophysiology in voltage clamp and current clamp mode, and measure the

intrinsic excitability, action potential properties and spontaneous activity of these human neurons. They will also perform systematic analysis on their experimental data to quantify their results. By the end of the mini-project, students will be guided through, and gain a practical understanding of, the experimental preparations and procedures involved in studying the functional properties of human cortical neurons using whole-cell patch-clamp electrophysiology.

Project 20: Comparing fluorescent probes for detection and live imaging of synaptic vesicle endocytosis

Instructor: Vivek Belapurkar (*CNRS & University of Bordeaux, France*)

Background

Synaptic transmission is a fast membrane dynamic process which is the basis of neuronal communication. When synaptic vesicles fuse at the presynaptic membrane they release neurotransmitters at a location juxtaposed to post synaptic receptors. This process occurs in fraction of a millisecond in nanoscale structures of presynapse called the “active zone”. New and upcoming work has highlighted tight spatial and temporal regulation of these juxtaposed active zone – post synaptic density clusters across the synapse. There is a need for fluorophores which can label and provide a good fluorescence signal for detection and imaging of these events, without compromising the physiology of the synapses or neurons. The most successful strategy so far is to utilize pH sensitive fluorophores which do not fluoresce at intraluminal pH 5.5 but on synaptic release the instantaneous change of pH to 7.4 the fluorophore provides a strong fluorescent signal. This can be used to label synaptic vesicle (SV) proteins and using the most sensitive fluorophore for these kind of fluorescence observations.

Methods

During this project we will label and visualize SV proteins with pH sensitive fluorophores in primary neuronal culture and use electric field stimulation to trigger synaptic vesicle release. We will therefore be able to image the fluorescent signal and compare signals from different fluorophores and estimate the best strategy for the live imaging of synaptic vesicle release.