Projects list

Block 1

Project 1: Unraveling the metabolic pathways behind mitoDREADD activation in astrocytes
Instructor: Abel Eraso-Pichot (*NeuroCentre Magendie, Bordeaux, France*)
Background
Mitochondrial functions in astrocytes have always been a puzzling phenomenon in the neuroscience field. While some authors claim that astrocytes are mainly glycolytic and they obtain their energy from aerobic oxidation, thus relegating mitochondria to a calcium buffering organelle, others claim that astrocytic mitochondria may be important for energy production in these cells. One of the few behavioral alterations related to mitochondrial functions in astrocytes has been recently described in a work from our lab, in which THC elicits an impairment of social interactions in mice by inhibiting mitochondrial soluble adenylyl cyclase and astrocytic metabolism in consequence.
Interestingly, preliminary experiments from our lab have shown that expression of a mitochondria-targeted Gs-DREADD (mitoDREADD) in astrocytes is able to counteract some of the behavioral alterations induced by THC. The cellular mechanism behind these functions, however, has not been addressed yet.
Aim
In this project we will try to shed light on the signaling pathways behind mitoDREADD activation in astrocytes, analyzing its effects in mitochondrial metabolism.
Methods
In this project, we'll perform mice astrocyte cortical cultures that will be transfected with either plasmatic DREADDs or mitoDREADDs. Mitochondrial metabolism after DREADD activation using CNO will be analyzed by High Resolution Respirometry (HRR) using the O2K respirometer (Oroboros) in both isolated mitochondria and entire astrocytes. Using this technique, we will be able to analyze basal respiration as well as different metabolic profiles and the usage of different substrates to dissect the metabolic pathways that are affected by the DREADD activation.

Project 2: Double trouble: The long term effects of obesity and liver disease on the brain
Instructor: Anna Hadjichambi (*Institute of Hepatology, London, UK*) and Christos Konstantinou (*Institute of Hepatology, London, UK*)
Background
Several studies have revealed the detrimental effects of obesity on the brain1, and the associated increased risk for developing other neurological disorders1-4, including Alzheimer's disease5. Interestingly, 70–80% of obese patients also develop metabolic (dysfunction) associated fatty liver disease (MAFLD6), which is currently the most common chronic liver disease, affecting 25% of the population, and it is predicted to become the most frequent indication for liver transplantation by 20307.
Research performed in our laboratory at UNIL (unpublished work) has confirmed the negative effects of this relatively mild diet-induced liver disease, on cerebral function and physiology. These effects range from behavioural changes (anxiety and depression) to neuropathophysiological alterations, such as low grade neuroinflammation and hypoxia. Such

cerebral alterations might persist over many years in patients, slowly and silently damaging the brain and are expected to worsen as obesity remains and the liver disease progresses from MAFLD to steatohepatitis and eventually cirrhosis.

Aim

This project is designed to study the long-term effects of obesity and steatohepatitis on some aspects of behaviour, brain function and physiology.

Methods

In order to address the above question, a mouse model of obesity and steatohepatitis (induced by 26 weeks of high fat, high fructose, high cholesterol diet [HF/HF/HCD]), and their analogous controls will be used.

The techniques applied will include:

1) Behavioural examination focusing on anxiety and memory

2) in vivo oxygen measurements and cerebrovascular reactivity assesments using an optic fiber oxygen sensor

3) in vivo brain activity evaluation using SPECT (https://bioemtech.com/%CE%B3-eye-2/).

The data obtained are expected to provide the first evidence on how obesity and associated liver disease can affect the brain in the long-term, and ultimately answer the crucial question of whether it is too late to save the brain after a lifetime of unhealthy eating.

Project 3: Short-term effect of cannabinoids on astrocytic glucose metabolism

Instructor: Ignacio Fernandez-Moncada (*NeuroCentre Magendie, Bordeaux, France*)

Background

Glucose is the obligatory brain energy source that sustains its high energy demands. Astrocytes, the largest population of glia cells, play a critical role in distributing blood-borne glucose and its metabolic end-product, lactate, to promote neuronal activity and brain function. Recently, it has been shown that exposure to cannabinoids results in decreased glucose metabolism and impaired social behaviour after 24 hrs. Interestingly, cannabinoids are known to alter brain function in a time-dependent manner, but a similar effect on astroglial energy metabolism largely remain unknown.

Aim

Determine the short-term effect of cannabinoids exposure on astroglial glucose metabolism by in-vitro and in-vivo approaches with genetically encoded fluorescent sensors for metabolites.

Methods

- Genetically encoded fluorescent sensors for Glucose and Lactate
- Preparation of Mixed glia-neuron cultures and pure astrocytes cultures
- Protocols for expression of fluorescent sensors (viral and transfection protocols)
- Fluorescent FRET microscopy (in-vitro)
- Fiber photometry in freely behaving mice.

Project 4: Role of lactate on the synaptic transmission at the striato-pallidal synapse
Instructor: Jérôme Baufreton (*University of Bordeaux, France*)

Background

The basal ganglia (BG) are a group of subcortical nuclei involved in motor control, procedural learning and habit formation. The striatum is mainly composed of spiny projection GABAergic neurons (SPN) subdivided into two functionally-distinct pathways according to their projection targets. SPN of the direct pathway (dSPN) innervate monosynaptically the output of the BG, the substantia nigra pars reticulate (SNr), while the SPN of the indirect-pathway (iSPN) connect to the SNr via a polysynaptic fashion with the globus pallidus (GP) and the subthalamic nucleus. In this microcircuit, the GP stands out as this nucleus as the highest density of astrocytes, suggesting strong demand for energy supply for its normal operation. Furthermore, in Parkinson's disease, GP astrocytes become reactive indicating their dysfunction and a potential break-down in the energy balance in the GP. Moreover, several evidence suggests that astrocytes participate to synaptic transmission in several ways, including via glio-transmitters release and metabolites transfer to neurons.

Aim

Test the impact of lactate on striato-pallidal synaptic transmission

Methods

We propose to study striato-pallidal (STR-GP) synaptic transmission using patch-clamp recordings in acute mouse brain slices and test if lactate modulates this transmission. To address the metabolic effect of lactate on STR-GP transmission, inhibitors of lactate transporters will be used. To test the signalling effect of lactate, which binds to GPCR GPR81, the action of selective antagonists of this receptor will be tested on STR-GP synaptic transmission. Training in electrophysiological data analysis will be provided to the students.

Project 5: Role of Lactate in the behavioural effects of acute cannabinoid administration
Instructor: Luigi Bellocchio (*NeuroCentre Magendie, Bordeaux, France*)

Background

l-Lactate as always been considered as merely a waste by-product of anaerobic glycolysis. Nowadays it is emerging as a crucial regulatory nexus for energy metabolism in the brain and signalling transduction in synaptic plasticity, memory processes, and drug addiction. In the brain, lactate is produced mainly by astrocytes and can act on neuronal cells through 2 different mechanisms. On one hand, it acts as a metabolic substrate, entering neurons via the MCT2 transporter, being converted into pyruvate and feeding the Krebs-cycle. On the other hand, lactate directly acts as signalling molecule, by activating a specific G-protein coupled receptor called GPR81. Indeed, perturbation of both of these mechanisms can cause several motor and cognitive deficits. Cannabinoids act in the brain trough CB1 receptor expressed in both neurons and astrocytes, where they are able to interfere with both synaptic and bioenergetics processes. In this scenario we have previously shown that long term cannabinoid receptor activation disrupts social behaviour via reducing lactate production in astrocytes. However, little is known about the role of lactate in the acute behavioural effects of cannabinoids (e.g. anxiety, motor and cognitive impairment).

Aim

Since previous data from our lab show that acute cannabinoid treatment is able to increase lactate production by astrocytes, the goal of the proposed project is to study whether this

process might be the mechanism underlying the acute deleterious effects of cannabinoid compounds.

Methods

Students will use mice that will receive systemic drug injections. They will perform 2 sets of experiments with 2 different aims:

1- Lactate production might be a protective mechanism against cannabinoids.

Students will administer lactate coupled to systemic application of cannabinoid compound. Mice will then go through some behavioural tasks which have been previously shown to be impacted by cannabinoids. Tests will include motor behaviour and coordination (locomotion, catalepsy, rotarod performance) as well as memory capacity (spatial in the object location and non-spatial in object recognition task).

2- Lactate production may be responsible for cannabinoids deleterious effects

Students will interfere with the action of lactate in the brain either by blocking the MCT2 or the GPR81. These pharmacological treatments will be coupled to systemic application of cannabinoid compound and mice will be tested as explained in the previous session.

Project 6: Impact of L-serine and Phosphorylated Pathway on astrocytic lactate

Instructor: Rodrigo Lerchundi (*MIRCen, Paris, France*)

Background

Brain metabolism is tightly linked to the production of amino acids. L-serine, the precursor of D-serine and Glycine, is poorly permeable trough the blood brain barrier and needs to be synthetized mostly by astrocytes through the Phosphorylated Pathway (pp). This pathway is coupled to glycolysis by using 3-Phosphoglycerate (3PG) as primary substrate and has been shown to be regulated by glycolysis. In addition, it has been reported that L-serine can bind and activate pyruvate kinase M2, an isoform highly expressed in proliferative and tumoral cells, but also found in astrocytes. As both pathway might influence each other, it seems reasonable that l-serine might potentiate the production and release of lactate from astrocytes. Despite these antecedents, how much the PP pathway influences brain lactate metabolism is not well understood.

Aim

This mini-project intends to give a hint to this question by evaluating changes in brain pyruvate and lactate under exposure to L-serine and manipulating pharmacologically the PP pathway.

Methods

- Imaging of astrocytic lactate and pyruvate in primary culture of astrocytes using the FRET-based sensor Laconic and Pyronic .
- Quantification of extracellular lactate in brain slices and in vivo using enzymatic microelectrodes sensitive to lactate.
- Measurement of extracellular D-serine in brain slices using enzymatic microelectrodes

Project 7: Addressing how exogenous mitochondrial importation impacts astrocytic respiration and oxidative stress

Instructor: Rubén Quintana-Cabrera (*University of Salamanca, Spain*)

Background

Metabolism and energetic demands are coupled in the nervous system by coordinated maintenance of homeostasis in neurons and astrocytes. Whilst the latter has a prominent glycolytic reliance, mitochondrial function is still critical for astrocytic metabolism and energy production, underscoring their functional coupling with neurons. For instance, the generation of reactive oxygen species (ROS) has shown necessary to fine tune glycolysis and redox balance.

Aim

Elucidate whether the recently discovered importation of exogenous isolated mitochondria can remodel mitochondrial content and function in astrocytes to understand how this process may underscore critical outcomes in neurological function.

Methods

We will address how exogenous mitochondrial acquisition by astrocytes impacts their redox status, respiration, bioenergetics, respiratory supercomplexes conformation and mitochondrial morphology, dynamics and ultrastructure.

- Mitochondrial isolation from cultured cells
- Blue Native Gel Electrophoresis (BNGE) analysis of respiratory supercomplexes native configuration and in-gel activity
- Kinetic evaluation of mitochondrial morphology, transport and function by confocal microscopy: Membrane potential (TMRM), redox status (roGFP1), ATP hydrolysis rates (mtATeam)
- Flow cytometry evaluation of mitochondrial membrane potential (TMRM) and ROS levels (MitoSOX)
- Fluorometric assessment of ROS production in whole cells (Amplex Red)
- Respirometry and extracellular acidification rates (Seahorse). Analysis of respiration and metabolism
- Quantitative image analysis of mitochondrial morphology, ultrastructure and function.

Project 8: Deciphering the role of an E3 ubiquitin ligase on the mitochondrial metabolic functions and protein composition

Instructor: Giovanni Bénard (*MRGM Laboratory, Bordeaux, France*)

Background

By regulating many fundamental activities such as energy production, calcium or ROS signaling, mitochondria are essential for the cell health. These critical activities rely on the integrity of its proteome. Notably, most of mitochondrial proteins are synthesized by the cytosolic ribosome under the form of precursors which are imported to the organelle. Recently, we have identified 4 E3 ubiquitin ligases (E3s) and based on our preliminary data, we postulate that these E3s ensure the quality control of newly synthesized mitochondrial proteins. We found that deletion of these E3 in cell lines induces the mitochondrial proteome and metabolic defects. However, the role of these E3 in vivo remains unknown. To study this physiopathological role, we have generated a mouse model deleted for one of these E3.

Aim
The aim of this project is to measure mitochondrial OXPHOS composition and bioenergetics activities in mitochondria isolated from brain of WT and KO mouse.
Methods
To measure impact of the E3 deletion on mitochondrial functions and composition, we will isolate mitochondria from brain WT and KO mouse. Mitochondrial oxidative phosphorylation will be measured by oxygraphy and enzymatic assays. Mitochondrial proteins composition will be assayed by immunoblots. Students will learn how to perform mitochondrial isolation and bioenergetics assays and how to analyze and interpret the resulting data.

Project 9: Investigating lipophagic activity in neurons and astrocytes
Instructor: Marina Garcia-Macia (*University of Salamanca, Spain*)
Background
Caloric excess and sedentary lifestyle have led to a global epidemic of obesity and metabolic syndrome. The modern nutritional profile, typically rich in saturated fats and refined sugars, is recognized as a major contributing factor. Dietary fatty acids are of particular interest, as they may play a dual role, both as a component of high-calorie obesogenic diets and as signaling molecules involved in inflammatory responses. The central nervous system (CNS) obtains 20% of its energy requirements from mitochondrial oxidation of fatty acids (FAs), and not only from glucose as traditionally assumed. In turn, the CNS is a major regulator of the systemic metabolism and lipid balance. Furthermore, deregulation of lipid metabolism in neurons causes cell death, which triggers pleiotropic secondary consequences, being a major contributor to several neurological diseases.
Autophagy is the cellular clearing and recycling program that degrades cytoplasmic content in lysosomes. Besides its crucial role as a quality control mechanism, autophagy is also an alternative energy source through lipophagy. Lipophagy is the most effective pathway to process dietary lipids.
Aim
In this project, our main goal is understanding which fatty acid from the diet is the most toxic, which one is the most potent at stimulating lipophagy and which one renders more energy in brain cells.
Methods
We will culture primary neurons and astrocytes in the presence of different fatty acids: Oleic acid, Palmitic acid, Linoleic acid, and BSA as vehicle. We will then analyse their lipophagic activity using different techniques:

- Stress oxidative evaluation: by mitoSOX (with flow cytometry) and membrane potential. AmplexRed by spectrophotometry.
- Oxygen consumption rate and Extracellular acidification (ECAR), by Seahorse technology. Analysis of lactate production by spectrophotometry for ECAR validation.
- Lipid droplet accumulation: by immunocytochemistry.
- Autophagy stimulation: by western blot and by immunocytochemistry: analysis of autophagic flux.
- Mitochondrial status: by immunochemistry (mitochondrial protein or mitoProbe).

Block 2

Project 10: Respective role of glucose and lactate as energetic substrates in neurons and astrocytes
Instructor: Anne-Karine Bouzier-Sore (*Centre RMSB, CNRS, Bordeaux, France*)
Background
Both glucose and lactate are present in vivo in the cerebral extracellular space while neurons and astrocytes are still cultured with only glucose in the medium. Moreover, the concentrations are usually very high compared to the physiological concentrations (around 2 mM for glucose and lactate in vivo).
Aim
This project will aim to perform competition between glucose and lactate (both present in the culture medium but alternatively 13C-labeled) on neuronal and astrocytic primary cultures, in different conditions (rest or activation mimic-conditions, i.e. AMPA 100µM + Cyclothiazide 100µM).
Methods
The fate of each substrate will be followed by 13C-NMR spectroscopy. Glucose and lactate consumption/production will be measured using classical biochemical essays.
All data will be compared between the different cell cultures and conditions.

Project 11: Mitochondrial differences between Glutamatergic and GABAergic neurons
Instructor: Antonio Pagano-Zottola *(Institut de Biochimie Génétique et Cellulaires, Bordeaux, France*)
Background
Glutamate and gamma-aminobutyric acid (GABA) are the most abundant neurotransmitters in the mammalian brain and a balanced interaction between their releases is necessary to preserve homeostasis. In the hippocampus, the equilibrium between the glutamatergic excitation and the GABAergic inhibition seems to be essential to maintain normal cognitive functions and when altered underlies several pathological states. Both neuronal populations are highly energy demanding for their functions since synaptic communication requires ATP, mainly produced by mitochondria. However, it is well known that mitochondria are morphologically and functionally heterogeneous according to the neuronal subtypes where they belong. To date, the possible biochemical and bio-energetic difference between mitochondria from glutamatergic versus GABAergic neurons remain still unexplored due to the lack of suitable tools.
Aim
The aim of the project is to dissect and compare the respiratory activity of mitochondria isolated from hippocampal glutamatergic versus GABAergic neurons.
Methods
To study mitochondrial activity of the two cell population, viral injection and/or transgenic mice will be used in order to express a mitochondrial localized epitope tag (Mito-Tag) in one or the other neuronal populations. Hippocampal mitochondria will be extracted and sorted by immunomagnetic isolation (MACS Technology) and the oxygen consumption rate will be analysed by high resolution respirometry (Oroboros system). The OXPHOS activity of the

isolated mitochondria will be compared between the two cellular populations, normalized by citrate synthase activity or protein concentration.

Project 12: AMPK in the regulation of the neuronal metabolism under bioenergetics stress
Instructor: Daniel Jiménez-Blasco (*University of Salamanca, Spain*)

Background

In eukaryotic cells, AMP-activated protein kinase (AMPK) plays a major role in regulating cellular energy balance. In the brain, AMPK serves as an energy stress sensor that is activated when intracellular ATP levels decrease (e.g., fasting) or ATP consumption is elevated (e.g., synapses maintenance). In addition, AMPK acts as a key sensor in the whole-body energy homeostasis by integrating nutritional and hormonal signals into the hypothalamus. These roles may be of importance from a therapeutic point of view. Thus, several agents with anti-obesity potential and/or antidiabetic effects, which are currently used clinically, such as metformin, are known to act through AMPK at the central and/or peripheral level.

Aim

To study the involvement of the AMPK signalling pathway in the modulation of neuronal metabolism under energy conditions of synapses maintenance or fasting.

Methods

This project will be developed both in vitro, with primary cultures of mouse neurons, and in vivo, with a mouse model intermittently fasted or fed with high fat diet (HFD). We will then analyze the metabolic status of neurons after starvation or sustained synaptic activation by determining lactate, hypothalamic autophagic flux with lysosome inhibitors and Lysotracker. We will determine neuronal redox status with fluorometric probes such as Amplex RedTM. We will perform Western Blot to study the phosphorylated state of the AMPK protein and one of its substrates, ACC (Acetyl-CoA carboxylase), that regulates the β-oxidation of fatty acids in neuronal cultures and brain areas. We will perform Blue-Native in gel electrophoresis in isolated mitochondria to observe the degree of assembly of the mitochondrial complexes that we will use as an indicator of the energy efficiency of the respiratory chain in neuronal cultures and brain areas such as the hypothalamus. We will determine the activity of the different mitochondrial complexes spectrophotometrically and we will use mitochondrial mass markers such as citrate synthase. We will use genetic tools such as gene silencing (siRNA against AMPK) and pharmacological approaches such as AICAR or Compound C to modulate the AMPK signalling cascade in vitro, via transfection with Lipofectamine, and in vivo, by intraperitoneal injections. Finally, we will perform different metabolic tests (glucose tolerance test, blood glucose, body weight, brown and white adipose tissue weight) in mice fasted against animals fed ad libitum with HFD, treated or not with AMPK activators or inhibitors and we will study the metabolic phenotype.

Project 13: Assessment of the neuronal pyruvate metabolism using a genetically encoded FRET-sensor for pyruvate

Instructor: Felipe Baeza-Lehnert (*Carl-Ludwig-Institute for Physiology, Leipzig University, Germany; Centro de Estudios Científicos, Valdivia, Chile*)

Background

Despite the ample work conducted to study brain energy metabolites such as glucose and lactate, pyruvate has remained elusive. Being the main substrate for the mitochondrial tricarboxylic acid (TCA) cycle, and the product of glucose and lactate oxidation, pyruvate lies at the crossing point between glycolysis and oxidative phosphorylation. Surprisingly, pyruvate metabolism is still poorly understood, mainly due to the lack of suitable tools to assess it at single-cell and with time-resolved resolution, under physiological conditions.

We developed Pyronic (Pyruvate Optical Nano Indicator from CECs) a genetically encoded FRET-based nanosensor to study pyruvate metabolism (San Martin et al., 2014). Previously, a protocol devised to isolate neuronal mitochondrial response upon synaptic activity in a cellular context, reveals a rapid 2.6-fold increment on cytosolic pyruvate consumption (Baeza-Lehnert et al., 2019), consistent with a fast mitochondrial activation. This experimental approach though informative cannot study pyruvate metabolism in conditions where neurons can engage glycolysis and lactate oxidation; two pyruvate sources that are known to be activated during neurotransmission (Bouzier-Sore et al., 2003; Diaz-Garcia et al., 2017; Baeza-Lehnert et al., 2019; Zuend et al., 2020).

Aim

We aim to unveil the physiological pyruvate response upon synaptic activity, adding novel data in the discussion and understanding of brain energy metabolism.

Methods

In this mini-project, the students will assess the intracellular pyruvate metabolism in neurons maintained in glucose, lactate, and pyruvate. Equipped with Pyronic and a transport-stop method to inhibit monocaboxylate transport through MCT2 (monocarboxylate transporter 2; San Martin et al., 2014; Baeza-Lehnert et al., 2019), pyruvate steady-states and fluxes will be followed at different extracellular pyruvate concentrations. Brain activity will be emulated by eliciting a protocol of electrical field stimulation that mimics synaptic transmission driven by action potentials (Baeza-Lehnert et al., 2019), and high extracellular lactate.

Learning objectives

1. Understand the Förster resonance energy transfer phenomena and the properties of the FRET-based sensor Pyronic
2. Set an open perfusion system up for real-time imaging experiments
3. Monitor cytosolic pyruvate dynamics in neurons
4. Understand the transacceleration mechanism of MCTs using a mathematical approach
5. Process and analyze data
6. Encourage deep discussion and the development of novel proposals

Project 14: Role of lactate on the synaptic transmission at the striato-pallidal synapse
Instructor: Morgane Le Bon-Jégo (*University of Bordeaux, France*)
Background
The basal ganglia (BG) are a group of subcortical nuclei involved in motor control, procedural learning and habit formation. The striatum is mainly composed of spiny projection GABAergic neurons (SPN) subdivided into two functionally-distinct pathways according to their projection targets. SPN of the direct pathway (dSPN) innervate monosynaptically the output of the BG, the substantia nigra pars reticulate (SNr), while the SPN of the indirect-pathway (iSPN) connect to the SNr via a polysynaptic fashion with the globus pallidus (GP) and the subthalamic nucleus. In this microcircuit, the GP stands out as this nucleus as the highest density of astrocytes, suggesting strong demand for energy supply for its normal operation. Furthermore, in Parkinson's disease, GP astrocytes become reactive indicating their dysfunction and a potential break-down in the energy balance in the GP. Moreover, several evidence suggests that astrocytes participate to synaptic transmission in several ways, including via glio-transmitters release and metabolites transfer to neurons.
Aim
Measure the presence of lactate in the GP using imaging sensors
Methods
We propose to use HEK293 cells expressing a lactate sensor as sniffer cells to detect the lactate released in the GP. Sniffer cells will be deposited on acute mouse brain slices containing the GP and imaging experiments will be conducted to detect the emission of fluorescence by the lactate sensor upon stimulation of the striato-pallidal pathway.

Project 15: Mitochondrial regulation of neuronal activity and synaptic function
Instructor: Sandrine Pouvreau (*NeuroCentre Magendie, Bordeaux, France*)
Background
The role of mitochondria in the regulation of neuronal circuit activity and synaptic function has been increasingly explored over the last decade. Mitochondrial function modulates various aspects of synaptic transmission and plasticity such as vesicle cycles, calcium signalling, ionic homeostasis to name a few. Mitochondrial function is regulated by mitochondrial interaction with presynaptic organelles, such as the endoplasmic reticulum. Mitochondria targeting to presynaptic terminals is finely tuned by trafficking and anchoring mechanisms, regulated by neuronal activity. It is thus affected by long term or homeostatic plasticity. Intersynapses variability in the role and distribution of mitochondria in presynaptic terminals has been reported at the neuromuscular junction, but is poorly documented in central synapses. Taking advantage of the two populations of presynaptic terminals on the dentate gyrus granule cell axon, we have previously shown that the distribution of mitochondria in presynaptic terminals can vary between types of boutons, with, in this case, 100 % of giant mossy fiber boutons, contacting CA3 pyramidal cells, containing mitochondria, while only 40% of *en passant* boutons, contacting interneurons, containing them. We have also shown that organotypic slices are a good model to study mitochondrial distribution and function as they keep the diversity of synapses.
Aim
The goal of this project is to study the distribution of mitochondria within the diverse presynaptic terminals of the hippocampus, their interaction with other organelles, and the

modulation of their targeting by neuronal activity. We will in turn investigate how different metabolites regulate network activity.

Methods

Students will use organotypic slices infected with AAV encoding fluorescent proteins or calcium sensors targeted to different subcellular compartments coupled with confocal or super-resolution imaging on fixed and live tissues, analysis of neuronal activity using Matlab routines, and electrophysiology. They will perform two sets of experiments with two aims:

1- To determine the synapse-specific distribution of mitochondria in presynaptic terminals, their interaction with other organelles, and how this is modulated by neuronal activity.

Students will infect organotypic slices cultures with AAV encoding fluorescent proteins targeted to mitochondria, presynaptic compartment or endoplasmic reticulum. Several pharmacological combinations will be used to stimulate or inhibit neuronal activity during 24-48 hours. Slices will then be fixed, and mitochondrial distribution will be analysed using confocal or super resolution imaging.

2- To determine how metabolites supply affect neuronal activity.

Organotypic slices will be supplied with glucose, or lactate and β-hydroxybutyrate to directly fuel the mitochondria. Neuronal activity will be analysed in CA3 and CA1 using calcium imaging and custom-made Matlab routines.

Project 16: The effect of fatty acid property on mesolimbic dopamine system

Instructor: Shingo Nakajima (*University of Montreal*)

Background

Obesity is a well-known risk factor for psychiatric diseases such as major depressive disorder and schizophrenia. High-fat diet is a golden standard for the induction of diet-induced obesity models, which causes anxiety- and depressive-like behaviors. Interestingly, a high-saturated fat (e.g. Lard, Palm oil) diet but not high-unsaturated fatty (Olive oil) acid diet induces these abnormal behaviors. This means that the quality of dietary fat is a key factor to maintenance mental health. The mesolimbic dopamine system is a major regulatory system for reward and mood behaviors.

Aim

In this project, we will focus the fatty acid property on dopamine response and lipid metabolism in medium spiny neurons (MSNs) to answer the unsolved questions as below.

1. The effect of fatty acids property on dopamine response in MSNs.
2. The effect of fatty acids on lipid metabolism in MSNs.
3. The impact of lipid profile in MSNs on anxiodepressive behaviors.

Methods

- Stereotaxic surgery
- AAV virus injection (AAV-hSyn-GCaMP.)
- in vivo Ca2+ imaging using Fiber photometry
- Primary culture of nucleus accumbens neurons
- Ca2+ imaging in primary cultured neurons
- Bioenergetics using Seahorse equipment
- Lipidomic analysis (LC-MS/MS)
- Behavior test (EPM, Open field)