Projects list

Project 1: The role of CB1 in the metabolic adaptations of the hypothalamic mitochondria under fasting
Instructor: Abel Eraso-Pichot (NeuroCentre Magendie, Bordeaux, France)

Background
Among other functions, the hypothalamus is the centre of convergence and integration of different nutrient-related signals of the body. One well known challenging metabolic situation is fasting, in which many different signals converge in the hypothalamus to activate or inhibit certain neuronal populations to adapt energy expenditure and food-seeking. Among these signals, it has been described that fasting increases the hypothalamic endocannabinoids such as 2-arachidonoylglycerol (2-AG) and specially anandamide. These endocannabinoids, in turn, may modulate mitochondrial components and thus metabolism through mitochondrial CB1 (mtCB1). Previous results of the lab have shown that fasting induces a reduction in mitochondrial metabolism in the hypothalamus; however, we don’t know which mechanism may be responsible for this reduction.

Aim
In this project, we want to assess the mitochondrial adaptations occurring during fasting in the hypothalamus, as well as establish the role of CB1 in this process.

Methodology
In this project, we’ll perform 24h fasting in WT and CB1 KO mice and do hypothalamic dissection and mitochondria isolation by centrifugation. Once mitochondria are isolated, we’ll perform High Resolution Respirometry (HRR) using the O2K respirometer (Oroboros) and analyze the mitochondrial adaptations to fasting in both mouse models. We’ll perform also protein normalization to analyze the results obtained.

Project 2: From health to obesity and back: Is it too late to save the brain?
Instructor: Anna Hadjichambi (Institute of Hepatology, London, UK)

Background
Several studies have revealed the detrimental effects of obesity on the brain, and the associated increased risk for developing other neurological disorders. 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 2030.

Research performed in our laboratory at UNIL (unpublished work) has confirmed the negative effects of this relatively mild diet-induced liver disease, on cerebral function and physiology.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. However, patients with MAFLD or steatohepatitis can reverse their condition and restore their health, in most cases, by losing weight through diet and exercise.
Whether the negative cerebral alterations are also reversible or whether this chronic disease has already damaged the brain enough leaving it more susceptible to age-related cerebral decline, is unknown and yet to be investigated.

**Aim**

This project is designed to study the long-term effects of obesity and steatohepatitis on some aspects of brain function and physiology. Additionally, it will allow us to investigate whether we can rescue the brain from these long-term negative alterations if we reverse the system back to health through dieting.

**Methodology**

In order to address the above questions, a mouse model of obesity and steatohepatitis (induced by 26 weeks of high fat, high fructose, high cholesterol diet [HF/HF/HCD]), as well as animals who underwent reversal of both conditions (26 weeks of HF/HF/HCD followed by 9 weeks of normal diet for weight loss and recovery) and their analogous controls will be used. The techniques applied will include:

1) EchoMRI to accurately assess body composition
2) behavioural examination focusing on anxiety
3) immunofluorescence staining and confocal imaging assessing microglia number and morphology (neuroinflammation)
4) in vivo functional MRI during whisker stimulation assessing brain activity associated with blood flow (neurovascular coupling).

**Project 3: 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).

**Methodology**

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 4: Mitochondrial differences between Glutamatergic and GABAergic neurons  
Instructor: Antonio Pagano-Zottola (NeuroCentre Magendie, 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.

Methodology
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 5-6: Role of lactate on the synaptic transmission at the striato-pallidal synapse  
Instructor: Morgane Le Bon-Jégo (Block 1) and Jérôme Baufreton (Block 2) (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 (Block1)
Measure the presence of lactate in the GP using imaging sensors
Methodology (Block1)
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.

Aim (Block2)
Test the impact of lactate on striato-pallidal synaptic transmission
Methodology (Block2)
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 7: Microglia and mTOR signalling in the context of obesity
Instructor: Camille Allard (NeuroCentre Magendie, Bordeaux, France)

Background
Obesity is a chronic disease worldwide spread. Ingestion of a hypercaloric diet, usually rich in carbs and saturated fats, initiates brain inflammation, which in turn sustains body weight gain. In particular, the microglia present in the hypothalamus, a key brain region for energy balance regulation, are sensors of saturated fat contained in hypercaloric diet. Microglia orchestrate the physiologic responses of the hypothalamus to dietary excess and their pro-inflammatory activity favors food intake and body weight. Emerging evidence suggests that the phenotypic shift of microglia to a pro-inflammatory profile relies upon a metabolic reprogramming of the cells, from mitochondrial oxidative phosphorylation (OXPHOS) to aerobic glycolysis. The metabolic pathways that are activated in microglia in the context of obesity are not yet all characterized. The mechanistic target of rapamycin (mTOR) kinase is a critical cellular energy sensor because of its ability to couple nutrients and energy availability with the regulation of cell energy metabolism and mitochondrial function. The current evidence rules for a critical role of this kinase in the metabolic reprogramming of microglia and subsequent functional shift in various pathological conditions. Yet, the role of mTOR in microglial inflammatory response is far from being fully understood and no data are available in the context of obesity.

Aim
Decipher whether mTOR activation is linked to microglial metabolic reactivity in saturated fat and glucose-rich environment.

Methodology
We will treat primary cultures of mouse microglia with glucose and the saturated fat palmitic acid (PA) that are present at very high concentration in obesogenic diets and known as triggers of microglial inflammation in the hypothalamus. Experiments will be performed on
WT and MIG-Raptor KO (=mTOR KO) microglial cells. Using the Seahorse systems, we will measure mitochondrial respiration, as a marker of the mitochondrial metabolic activity. To investigate whether the glucose+PA affects the fuel utilization by microglia, we will examine the oxidative substrate preference of microglia by a mitochondrial fuel flex assay.

**Project 8: A cellular view of the effects of glucose and ketone-body metabolism after neurotoxic damage**

**Instructor:** Carlos Vicente-Gutierrez (*University of Salamanca, Spain*)

**Background**

Alterations in energy metabolism are a common hallmark in different neurodegenerative diseases. Despite limited clinical data, considering the failure of many therapies to treat neurological disorders, metabolic strategies may open new insights to move forward therapeutic approaches that could at least ameliorate symptoms. Inline, there is an emerging literature supporting the therapeutic potential of L-lactate and ketogenic metabolism for translational neurosciences. L-lactate is a key intermediary of glucose metabolism that plays a fundamental role both in physiology and pathology regulating neuroplasticity, memory consolidation or drug addiction. Meanwhile, ketone bodies like β-hydroxybutyrate (β-OHB) have been demonstrated neuroprotection against glutamate excitotoxicity resulting beneficial in patients with pharmaco-resistant epilepsy. Some of the neuroprotective effects of L-lactate or β-OHB suggest that both metabolites have intercellular signalling functions, still poorly explored. In the brain, astrocytes play a key role in providing glycolytic-derived lactate to neighbouring neurons to deal with their high energy needs during neurotransmission. Moreover, recent evidence shows that astrocytes are also ketogenic cells which might supply neurons with ketone bodies *in situ*, in addition to the ketone bodies derived from liver-metabolism. However, these cell type-specific metabolic differences are often barely considered in *in vivo* neuroscience studies.

**Aim**

This project proposes to address the effects of L-lactate and β-OHB against 3-nitropropionic acid (3-NP)-induced neurotoxicity studying neurons and astrocytes individually.

**Methodology**

The systemic administration (via intraperitoneal injection) of the mitochondrial complex II inhibitor 3-NP, is known to induce bilateral striatal lesions, like those seen in Huntington's disease. Students will explore the therapeutic potential of metabolic treatments, through intracerebroventricular administration of physiological concentrations of L-lactate or β-Hydroxybutyrate (β-OHB), to rescue the motor discoordination, cell death and reactive oxygen species (ROS) production induced by 3-NP. In addition to behavioural characterization (Rotarod, open-field, etc.), students will learn to explore separately how neurons and astrocytes respond after neurotoxic damage and metabolic treatment combining an adeno associated viruses (AAVs)-based strategy with flow cytometry studies.
Project 9: Hypothalamic cannabinoid type 1 receptor in the control of energy balance
Instructor: Cristina Miralpeix (NeuroCentre Magendie, Bordeaux, France)

Background
The hypothalamus includes key neuronal circuits that orchestrate whole-body energy metabolism. Among others, POMC neurons within the hypothalamic arcuate nucleus are in charge of controlling food intake, energy expenditure, glucose and lipid metabolism. Exposure to high-fat diet (HFD) critically alters POMC neurons function, and such dysregulation, in turn, favours the development of obesity. The molecular mechanisms underlying HFD-induced effects in POMC neurons are not identified yet. The endocannabinoid system and specifically the cannabinoid type 1 (CB1) receptor are involved in the control of energy balance and their activity favours food intake and fat accumulation, representing a potential target for obesity. The CB1 receptor, besides its localization at the plasmatic membrane, is also intracellularly associated to mitochondrial membranes (mtCB1) and directly modulates cellular bioenergetics processes and neuronal activity. The evidence that POMC neurons participate to the hyperphagic actions of cannabinoids, suggests that CB1 could be implicated in POMC-related changes under HFD exposure, hence pointing to novel CB1-dependent mechanisms which may favour the development of obesity.

Aim
Investigate the role of POMC CB1 receptor in the control of energy balance and peripheral metabolism under dietary conditions leading to metabolic dysregulation and obesity.

Methodology
To achieve this goal, we will use mice specifically lacking CB1 receptor in POMC expressing neurons (POMC-CB1-KO mice) and the TSE labmaster cages for the analysis of behaviour and metabolism in vivo. These cages not only allow you to monitor mice food intake and body weight daily, but also energy expenditure and locomotion activity. Therefore, we will start studying POMC CB1 mice feeding behaviour in the TSE labmaster cages by performing a food preference test. During this test, mice can choose between eating chow diet or HFD for 2h, and we will analyse the food intake and the energy expenditure. After that, mice will be exposed to HFD for 7 days in the TSE labmaster cages and we will follow up their behaviour daily. Moreover, to gain insights into the molecular mechanism driving CB1 regulation of food intake in POMC neurons, we will analyse the CB1 mRNA expression levels in POMC neurons by using the Fluorescent In Situ Hybridization (FISH) technique.

Project 10: 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.

**Methodology**
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 11: Development of a method to assess MCT2 permeability using genetically encoded FRET-sensors for pyruvate and lactate**
**Instructor:** Felipe Baeza-Lehnert (Centro de Estudios Científicos, Valdivia, Chile)

**Background**
The MCT2 (monocarboxylate transporters 2) mediates the H⁺-coupled translocation of monocarboxylates, such as lactate and pyruvate, across the plasma membrane of neurons. During brain activity, the increment in neuronal uptake of extracellular lactate has been shown; however, the acute MCT2 response upon neuronal activity has only been hypothesized, mainly due to the lack of suitable methods to study MCT2 modulation in vitro and in vivo. Previously, our group showed that during glutamatergic neurotransmission, MCT2 transport capacity matches the increment in metabolism, keeping the levels of lactate and pyruvate invariable. These results suggest that MCT2 permeability is increased during activity; however, the acute response has not been experimentally demonstrated. The recent publication of a method to study glucose permeability by means of GLUT´s trans-acceleration has paved the route for designing a novel experimental approach to study MCT2.

**Aim**
Develop a method to assess MCT2 permeability, at rest and during neuronal activity, following cytosolic lactate and pyruvate levels in real-time, in hippocampal cultures.
Methodology
We will take advantage of the accelerated exchange of MCT2 by using non-metabolizable MCT2 substrates in the extracellular face of the transporter, to induce either pyruvate or lactate drops. Cytosolic lactate and pyruvate levels will be followed in real-time thanks to the genetically-encoded FRET-based sensors Laconic and Pyronic. Electrical stimulation will be employed to mimic neurotransmission. This method has the potential to be used in vivo in combination with two-photon microscopy.

Project 12: 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.

Methodology
- 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 13: 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.

**Methodology**

Students will use mice equipped with a cannula for intracerebroventricular drug infusion coupled to systemic injection. 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 in the brain 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 14: 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.
**Methodology**

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).

**Project 15: 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 through 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 tumor cells, but also found in astrocytes. As both pathways 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 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.

**Methodology**

- 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 16: Effect of cannabinoid treatment on mitochondrial calcium in vivo - glutamatergic vs GABAergic neurons.

**Instructor:** Román Serrat (*NeuroCentre Magendie, Bordeaux, France*)

**Background**
In recent years, mitochondria have evolved as one important key element in the regulation of brain function. In particular, mitochondrial calcium buffering capacity makes these organelles crucial for brain cells, as neurons are cells highly dependent to calcium signalling to regulate its activity. In our laboratory, we study the effect of cannabinoids on mitochondrial calcium activity using the mitochondrial-targeted genetically encoded calcium sensor Gcamp6s that allows highly sensitive detection of calcium dynamics.

**Aim**
The aim of this project will be to determine if mitochondrial calcium is affected by an exogenous cannabinoid treatment in both glutamatergic and GABAergic neurons.

**Methodology**
Transgenic adult mice expressing CRE recombinase either in glutamatergic or GABAergic neurons will undergo surgery to allow the injection of Adeno-associated virus expressing a floxed version of mito-Gcamp6s sensor and fiber implantation. After 4 weeks of recovery, mitochondrial calcium will be evaluated in vivo under basal conditions and after cannabinoid treatment using fiber photometry. The results obtained will be analysed and quantified using Matlab software. In this project you will learn how to use fiber photometry system to record mitochondrial calcium including fiber preparation, implantation, recording and analysis.

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Project 17: 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.

**Methodology**
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 18: 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.

Methodology
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 19: The effect of fatty acid property on midbrain energetics and function**

**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 behaviours. Interestingly, a high-saturated fat (e.g. Lard, Palm oil) diet but not high-unsaturated fatty acid (Olive oil) diet induces these abnormal behaviours. This means that the quality of dietary fat is a key factor in the maintenance of mental health.

**Aim**
In this project, we will focus on the dopaminergic system, a major regulatory system for reward and mood behaviours, to answer 2 unsolved questions:
1- The effect of fatty acid property on dopaminergic energetics
2- The impact of dopaminergic energetics on behaviour.

**Methodology**
- AAV virus stereotaxic injection (AAV-hSyn-Dlight 1.2.)
- In vivo imaging for DA sensor using Fiber photometry
- Primary culture of midbrain neurons
- Bioenergetics using Seahorse equipment
- Lipidomic analysis (LC-MS/MS)
- Behaviour test (EPM, Open field).