Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: Neural circuits and value coding in Drosophila

Laboratory: *(name and address)*

Institute for Functional Genomics, 141 rue de la Cardonille, 34094 Montpellier Cedex 5

Supervisor: *(full name and email)*

PERISSE Emmanuel

emmanuel.perisse@igf.cnrs.fr

Description of the internship: *(please provide a short summary of the project including experimental approaches) – One page –*

To survive and guide appropriate actions animals must correctly encode value associated with every experience. Remembering experiences previously encoded positively or negatively will trigger, in an internal state-dependent context, approach or avoidance behaviour respectively. Animal previous experience and internal state steer value coding during learning therefore influencing future decisions. For instance, hungry or sated animals differently assign value to a similar experience. As a consequence, these two groups of animals will behave differently regarding this experience. Furthermore, some neurological diseases also include dysfunctions of valuation which is detrimental for value coding during learning and retrieval of subjective value during decision making processes. The neural mechanisms underlying the modulation of value coding during learning by previous experience and/or internal state still remain to be elucidated. The fruit fly *Drosophila melanogaster* is capable of complex behaviours. For example, flies can encode value associated with different intensity of appetitive or aversive stimuli during learning as well as the ability to discriminate between stimuli previously paired with different subjective value during decision-making. As for most animal, fly behaviour is under the influence of experience and internal state. Using the powerful genetics tools available in *Drosophila* some labs have started to undercover the neural and molecular mechanisms underlying experience- and state-dependent modulation of naïve and learned behaviour. Still a lot remains unknown especially on the consequences of internal state alteration associated with food/water deprivation or imbalanced diet on value coding during learning. Furthermore, these alterations have been shown to be dependent on specific neuropeptide expression. Recently, we found that hungry flies are better than sated flies at discriminating between two odours previously paired with two different intensities of electric shock (unpublished data). It therefore seems that food deprivation modifies the way flies encode and/or discriminate between different intensities of negative value. The underlying mechanisms of food deprivation or other internal states on value coding during learning remain to be tested. In this project I propose to first 1) study how different internal states due to food restriction, water restriction, high fat or high sugar diet, alter the encoding and the discrimination of different negative value associated with specific olfactory stimuli. I then propose to 2) decipher the molecular basis of these putative changes in behaviour capacities. To accomplish this project, the candidate will:

1) Test the ability of flies, in different internal states, to learn and discriminate between olfactory stimuli previously paired different intensities of punishment.
2) Use genetic tools to target specific molecules (neuropeptide expression or neuropeptide receptors (chosen from the literature)) to identify necessary molecular components of state-dependent value coding.

Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: **Neuron-glia crosstalk during axon degeneration**

Laboratory:

Neurogenetics and Memory
Genetics & Development Department
Institute of Human Genetics
UMR 9002 – CNRS/UM
141, rue de la Cardonille
34396 Montpellier Cedex 5, France

Supervisors: *Ana Boulanger* *ana.boulanger@igh.cnrs.fr*

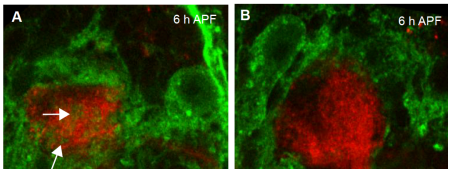
Jean-Maurice Dura *jean-maurice.dura@igh.cnrs.fr*

Description of the internship:

Axonal remodeling or pruning is a developmental mechanism of axonal degeneration essential for the formation of the nervous system. Emerging evidences suggest that axon pruning becomes reactivated in neurodegenerative diseases, as Alzheimer or ALS, leading to aberrant loss of axons. Thus, unraveling the molecular and cellular pathways involved in pruning is essential to better understand neurodegenerative diseases and has the potential to identify ways of protecting axons. To study this pruning process, we use as a model the drosophila gamma neurons of the mushroom bodies, the axons of which are pruned and eliminated after infiltration of engulfing glia during brain development *(Boulanger et al., Nat Neurosci 2011 and Boulanger and Dura, BBA, 2015).*

Using EMS mutagenesis, we have generated one drosophila line in which gamma axons were protected of degeneration and glial infiltration into axons was abolished. The generated line displays a point mutation mapped in a new gene, coding for a secreted protein: Orion, which is produced by gamma neurons. This protein is a chemokine-like protein and behaves as a neuronal “find-me/eat-me” signal leading to glial mediated axon degeneration.

The Master student will dissect the *orion* triggered molecular pathway during neuronal degeneration and will screen for potential Orion receptors present in glial cells. More precisely, receptors involved in phagocytosis. In addition, the Orion “find-me/eat-me” function will be tested in neurons that dye by apoptosis during development, as the Corazonin neurons, in synapses of the ventral cord normally removed during development and after nerve injury. He/she will use molecular biology, drosophila genetics, neurogenetics, cell culture and immunostaining techniques. This project might be continued throughout a PhD thesis.



Glial cell (green) invading the dorsal bundle of gamma axons (red) at 6 h after pupae formation in control (A). Regions of glial invasion are pointed by arrows in A. No glial invasion was observed in orion mutants (B). *(Boulanger et al., unpublished results).*

Duration of the internship: 6 months (from January to June 2020).

Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: Analgesia induced by the inactivation of a calcium channel, Cav3.2, in spinal cord interneurons of the mouse

Laboratory: Institut de Génomique Fonctionnelle, Team « Dynamique des canaux calciques et nociception », Inserm U1191 - Cnrs 5203 - Université de Montpellier. Montpellier.
http://www.igf.cnrs.fr/

Supervisor: MERY Pierre-François, pierre-francois.mery@igf.cnrs.fr

Team Leader : BOURINET Emmanuel.

Description of the internship:

We have discovered that a low-threshold activated (LVA) calcium channel, Cav3.2, was involved in the pathophysiological activity of neuronal networks within the spinal cord [1-4]. At the cellular level, the genetic inactivation of Cav3.2 changes drastically the electrical activity of spinal cord neurons, despite the fact that they still express other LVA channels (*i.e.* Cav3.1 or Cav3.3) [5]. At the behavioral level, LVA channels inhibition and notably the genetic inactivation of Cav3.2 (but not that of Cav3.1 or Cav3.3) induce strong analgesic and/or antiallodynic effects [1-4]. More recently, we have observed that all spinal cord interneurons expressing protein kinase C gamma (PKCγ) also express Cav3.2 [5]. This was striking since these neurons are responsible for the gate control of neuropathic pain, which eventually opens under pathological conditions and allows non-noxious stimuli in flowing towards nociceptive projection neurons of the lamina I of the spinal cord [6].

Thus, using multiple lines of genetically modified mice, AAV injections and anatomical and functional methods, our aim will be to understand the selective role of Cav3.2 within the spinal cord under physiological and pathological (SNI model [2]) conditions. We will concentrate our efforts on the pre- and post-synaptic mechanisms involving Cav3.2 in PKCγ interneurons using our patch-clamp techniques (whole cell recording, paired recordings, electrical stimulation [5]) and/or optogenetics (stimulation with H134R ChR2 [unpublished]). We will examine the subcellular locations of Cav3.2 in these structures using inmmufluorescence (confocal microscopy [5], clearing/ultramicroscopy [unpublished]) in order to understand if/how Cav3.2-including domains participate in the calcium homeostasis of PKCγ neurons and in their afferent and efferent neurons.

1. Fruquière, A., et al. (submitted).
2. Francois, A., et al. Cell Reports 10, 370-382 (2015).
3. Marger, F., et al. Proc Natl Acad Sci U S A 108, 11268-11273 (2011).
4. Bourinet, E., et al. Embo Journal 24, 315-324 (2005).
5. Candelas, M., et al. Scientific reports 9(1): 3112 (2019).
6. Koch, SC, et al. Ann Rev Physiol 80 :189-217 (2018).

Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: **Mode of action of novel optopharmacological ligands of GPCRs**

Laboratory: *(name and address)* ***Institut de Genomique Fonctionnelle, 141 rue de la cardonille, 34094 Montpellier 5***

Supervisor: *(full name and email)* ***Cyril Goudet,*** *cyril.goudet@igf.cnrs.fr*

Description of the internship:

In the past years, with the advent of optogenetic, optical biology has revolutionized the way to study brain circuitry. Optogenetic is a technique allowing to control a particular subset of neurons by light using exogenous expression of light-regulated proteins. Beside optogenetic, optopharmacology is a novel light-based strategy to manipulate endogenous regulatory mechanisms which offers unique opportunities for in vitro and in vivo investigation. This technique (also called photopharmacology) is based on small, diffusible, drug-like photoswitchable ligands that can be switched ON and OFF with light at the appropriate wavelengths. Combined with optical technologies, it provides precise spatial and temporal control of the drug/target biological activity. Therefore, optopharmacology offers a number of advantages, such as: 1) the modulation of endogenous targets in their native environment 2) no need for exogenous viral expression of light-activable proteins 3) improved spatial and temporal control of compound activity compared with conventional pharmacological approaches, and 4) small photoswitchable molecules with amenability to drug development. Optopharmacology therefore appears as a very promising approach to decipher the mechanisms of regulation of brain circuits. The aim of the project will be to understand the mode of action of novel optopharmacological tools. Our team is one of the pioneer in the domain of optopharmacology. Notably, we have recently generated the first light-regulated ligands of metabotropic glutamate receptors, in collaboration with the team of Dr. Llebaria (Pittolo et al., 2014, Gomez-Santacana et al. 2016) in Barcelona. We also demonstrated that photocontrollable ligands are valuable tools to explore the function of receptors in vivo (Rovira et al. 2016, Font et al. 2017, Zussy et al., 2018). The PhD student will participate to a collaborative project at the interface between neuropharmacology and medicinal chemistry to generate new photocontrolable ligands targeting GPCRs, to understand their mode of action and to validate their function in living organisms.

Bibliography

Goudet C, Rovira X, Llebaria A (2018) Shedding light on metabotropic glutamate receptors using optogenetics and photopharmacology. Current opinion in pharmacology 38: 8-15

Pittolo S, Gomez-Santacana X, Eckelt K, Rovira X, Dalton J, Goudet C, Pin JP, Llobet A, Giraldo J, Llebaria A, Gorostiza P (2014) An allosteric modulator to control endogenous G protein-coupled receptors with light. Nat Chem Biol 10:813-815.

Gomez-Santacana X, Pittolo S, Rovira X, Lopez M, Zussy C, Dalton JA, Faucherre A, Jopling C, Pin JP, Ciruela F, Goudet C, Giraldo J, Gorostiza P and Llebaria A (2017) Illuminating Phenylazopyridines To Photoswitch Metabotropic Glutamate Receptors: From the Flask to the Animals. ACS Cent Sci 3:81-91.

Font J, Lopez-Cano M, Notartomaso S, Scarselli P, Di Pietro P, Bresoli-Obach R, Battaglia G, Malhaire F, Rovira X, Catena J, Giraldo J, Pin JP, Fernandez-Duenas V, Goudet C, Nonell S, Nicoletti F, Llebaria A and Ciruela F (2017) Optical control of pain in vivo with a photoactive mGlu5 receptor negative allosteric modulator. eLife 6.

Zussy C, Gomez-Santacana X, Rovira X, De Bundel D, Ferrazzo S, Bosch D, Asede D, Malhaire F, Acher F, Giraldo J, Valjent E, Ehrlich I, Ferraguti F, Pin JP, Llebaria A, Goudet C (2018) Dynamic modulation of inflammatory pain-related affective and sensory symptoms by optical control of amygdala metabotropic glutamate receptor 4. Mol Psychiatry 23: 509-520

Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: Contribution of C-LTMRs and Insular cortex to hedonic touch

Laboratory: *Institut de Génomique Fonctionnelle, Team « Dynamique des canaux calciques et nociception », Inserm U1191 - Cnrs 5203 - Université de Montpellier. Montpellier. http://www.igf.cnrs.fr/*

Supervisor: *Amaury Francois*

amaury.francois@igf.cnrs.fr

Team Leader: *Emmanuel Bourinet*

Description of the internship:

We and other groups genetically and functionally identified a population of primary sensory neurons responsible for the detection of pleasant touch, but also contributes to pain perception in the context of chronic pain. These neurons, the C-low threshold mechanoreceptors (C-LTMRs), are at the rim of pleasure and pain, and may engage the same neuronal pathways responsible for anhedonia (the lack of pleasure) observed in chronic pain patients which often leads to depression and/or anxiety. This project aims to use C-LTMRs to reveal the organization logic of neuronal pathways linking touch, pleasure and pain in normal and pathological condition.

The master student will help Dr. Francois in his project to perform in vivo optogenetic combined with viral intersectional genetic on the spinal cord to stimulate C-LTMRs. We will use real time and conditioned place preference assays to measure if C-LTMRs is rewarding. We will also test sociability, anxiety and somatosensory thresholds. In parallel we will record neuronal activity in freely moving animal using fiberphotometry how C-LTMRs information is integrated in the insular cortex and test if the insula can support valence attribution.

Good handling practice of laboratory animals and a good understanding of animal behavior are recommended.

Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: Structural dynamics of G-protein coupled receptors – A single molecule study

Laboratory: Centre de Biochimie Structurale, Montpellier. http://www.cbs.cnrs.fr/index.php/en/home-equipeb3

Supervisor: *Emmanuel Margeat, CNRS Research Director. margeat@cbs.cnrs.fr*

Description of the internship:

Metabotropic Glutamate Receptors (mGluRs) are class C G-protein coupled receptors (GPCR), expressed throughout the central nervous system. They participate in the long term modulation of neural transmission following activation by the excitatory neurotransmitter glutamate. This critical role in the regulation of synaptic activity makes them promising targets in the development of drugs for the treatment of various neurologic and psychiatric disorders such as schizophrenia, epilepsy, anxiety and pain. This research project will focus on the elucidation of the activation mechanism of full-length mGluR using advanced single molecule fluorescence methods based on Forster Resonance Energy Transfer, specially design to investigate the structural dynamics of proteins down to the nanosecond timescale. The project involves preparative biochemistry, advanced labeling methods based on unnatural amino acids, biophysical measurements on home-made microscopes, and data analysis. It is based on a collaboration with the Team of J.P. Pin (Institut de Génomique Fonctionelle, Montpellier)

Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: FUNCTIONAL DIVERSITY AND REGULATION OF THE SODIUM LEAK CHANNEL NALCN

Laboratory: *Institut de Genomique Fonctionnelle*

Supervisor: *Arnaud Monteil (arnaud.monteil@igf.cnrs.fr)*

Description of the internship: *(please provide a short summary of the project including experimental approaches) – One page –*

The excitability of neurons is tightly dependent on their ion channel repertoire. Among these channels, the leak sodium channel NALCN plays a crucial role in the maintenance of the resting membrane potential. Importantly, NALCN mutations lead to complex neurodevelopmental syndromes, including infantile hypotonia with psychomotor retardation and characteristic facies (IHPRF) and congenital contractures of limbs and face, hypotonia and developmental delay (CLIFAHDD; *review in* Cochet-Bissuel et *al*, 2014). Our laboratory previously identified several isoforms of NALCN resulting form alternative splicing of its mRNA as well as putative binding partners (*unpublished data*). However, nothing is known about their specific properties on NALCN function. The aim of the project is (i) to investigate the consequences of alternative splicing on NALCN functional properties and (ii) to examine how identified binding partners regulate NALCN activity. For this purpose, the neuronal NG108-15 cell line will be used as a cell model in this study. Indeed, we recently demonstrated this cell line is suitable to functionally express NALCN (Bouasse et *al*, 2019). Cells will be transfected with the NALCN cDNA +/- identified binding partners then differentiated in neurone-like cells. The patch clamp technique will be then used to characterize the NALCN current properties (density, inactivation kinetics, voltage-dependence…). This project will undoubtedly demonstrate an unpreviously described diversity of NALCN currents due to alternative splicing as well as novel regulations by binding partners. This work will eventually be pursued in a thesis project.

Bibliography:

Bouasse M, Impheng H, Servant Z, Lory P, Monteil A. Sci Rep. 2019 Aug 13;9(1):11791. doi: 10.1038/s41598-019-48071-x.

Cochet-Bissuel M, Lory P, Monteil A. Front Cell Neurosci. 2014 May 20;8:132. doi: 10.3389/fncel.2014.00132. eCollection 2014. Review.

Neuroscience Internship offer

Master Biology and Health (Biologie-Santé) University of Montpellier

Head : F. Rassendren – J. Valmier

Title: Molecular mechanisms of α-synuclein neurotoxicity in Parkinson's disease

Laboratory: IGMM, CNRS, 1919 route de Mende, F-34293 Montpellier

Supervisor: Solange Desagher, solange.desagher@igmm.cnrs.fr

Description of the internship: *(please provide a short summary of the project including experimental approaches) – One page –*

Alpha-synuclein is an abundant presynaptic protein that plays a crucial role in Parkinson's disease pathogenesis. Accumulating data suggest that its deregulation and aggregation are involved in the death of dopaminergic neurons of the *substantia nigra*, which is responsible for the motor symptoms of the disease. However, the molecular mechanisms underlying the neurotoxicity of α-synuclein are mostly unknown.

Most studies conducted so far have relied on overexpressed, recombinant or exogenous α-synuclein. The goal of this project is to elucidate the neurotoxic mechanisms of α-synuclein at the endogenous level, by using a novel cellular model generated in the laboratory. These human cells carry a pathogenic mutation in the natural gene of α-synuclein and can be fully differentiated into dopaminergic neurons. The objectives of the project are : 1) determine whether the pathogenicity of the mutation is due to a higher propensity of α-synuclein to aggregate or to an increased accumulation of the protein; 2) test whether aggregated α-synuclein can induce the transcription of its own gene to form a positive feedback loop; 3) examine whether mutated α-synuclein activates pro-apoptotic proteins at the level of mitochondria or directly permeabilise the outer mitochondrial membrane by forming pores.

This project requires the use of classical and innovative techniques of biochemistry, cell and molecular biology: cell culture, knock-in and knock-out using CRISPR-Cas9, quantitative PCR, western blot, pulse chase, immunoprecipitation, immunofluorescence confocal microscopy, electronic microscopy….