École des
Neurosciences
Paris Île-de-France

CHILEAN INTERNS
ENP INTERNSHIP PROPOSALS
2014-2015

Contents

Cendra AGULHON's team

Team: Glia-Glia & Glia-Neuron Interactions Group

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Investigating the role of astrocytic signaling in sensory information processing: implications for neuropsychiatric and cognitive disorders

Supervisor: Cendra AGULHON

Severe mental disorders such as schizophrenia, bipolar disorder and autism are leading contributors to cognitive illness, imposing emotional burdens and high health care costs on families as well as individuals. Based on recent literature, we hypothesize that postnatal inflammation - and associated proinflammatory mediators - can lead to abnormal activation of astrocytic protein-coupled receptors (GPCRs), which may trigger transmitters and inflammatory mediators release from astrocytes. Both of these effects could consequently alter excitatory synaptic transmission during postnatal brain development, and contribute to abnormal long-term changes of excitatory synaptic transmission and thus to changes in sensory processing and the pathogenesis of neuropsychiatric and cognitive disorders. We propose to directly test this hypothesis using the rodent visual cortex as a model system, chemogenetics, electrophysiology and biochemistry.

Period: Anytime from September 2014 to June 2015

Maria Cecilia ANGULO's team

Team: NG2 cells physiology

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Supervisor: Maria Cecilia ANGULO

Chemical synapses are specialized cellular junctions allowing neurons to transfer the information that underlies perception and cognition. However, synapses in the brain are not seen today as a property exclusive of neurons. In the last decade, several studies have shown the presence of functional synaptic contacts onto oligodendrocyte precursor cells, also named NG2 cells, throughout the brain.
NG2 cells constitute a major source of remyelinating oligodendrocytes in demyelinating diseases. Glutamatergic synaptic transmission onto NG2 cells might regulate proliferation and differentiation of these cells, and thus possibly influence myelin repair. However, our understanding of how neurones signal to NG2 cells in the injured brain is limited. The glutamatergic pathway represents an especially attractive target for developing pharmacological strategies aiming at increasing remyelination and thus repairing effectively damaged tissue, but the underlying mechanisms demonstrating a potential role of glutamatergic transmission on remyelination is still poor. We recently showed a dys-regulation of axonal-NG2 cell synaptic properties following lysolecithin (LPC) injection in the adult mouse *corpus callosum* and in multiple sclerosis lesions in humans. Our data also suggest that an extrasynaptic transmission mode might be important after the active phase of NG2 cell proliferation following demyelination (Sahel*, Ortiz* et al., *submitted*). We will evaluate the balance of synaptic and extrasynaptic signalling mechanisms that may be involved in *neuron*-NG2 cell communication following demyelination. We will combine patch-clamp recordings with a novel optogenetic approach allowing for targeted stimulation of axons with light in acute slices of *corpus callosum*. A local demyelinating lesion will be induced in these animals in vivo by stereotaxic injection of lysolecithin within the adult *corpus callosum.* Then, NG2 cells will be recorded in lesioned *corpus callosum* slices at different time points and compared to controls. Neuronal glutamate release will be induced by photostimulation of callosal axons expressing the photosensitive protein channelrhodopsin2 (ChR2). This project should open new perspectives to manipulate NG2 cell development by using controlled neuronal stimulation in demyelinating lesions.

Three selected publications:

- Sahel A*, Ortiz FC*, Kerninon C, Maldonado PP, **Angulo MC***, Nait Oumesmar B*. Alterations of NG2 cell synaptic connectivity following demyelination of *corpus callosum. Submitted*. *Co-first and Co-last authors
- Maldonado, PP, Vélez-Fort, M, Levavasseur, F, **Angulo, MC.** (2013) Oligodendrocyte precursor cells are accurate sensors of local K+ in mature gray matter. *Journal of Neuroscience*. 33(6):2432
- Vélez-Fort M, Maldonado PP, Butt AM, Audinat E, **Angulo MC.** (2010) Postnatal switch from synaptic to extrasynaptic transmission between interneurons and NG2 cells. *Journal of Neuroscience* 30(20):6921

Period: Anytime from September 2014 to June 2015

Etienne AUDINAT's team

Team: Neuron-glia interactions

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Microglia and cortical development

Supervisor: Etienne AUDINAT

Recent evidence indicates that microglial cells, the resident macrophages of the brain, have important functions during the normal development of synaptic networks. Our team has recently shown that reciprocal interactions between microglia and neurons are necessary for the proper maturation of thalamo-cortical synapses during the first postnatal week in the mouse somatosensory "barrel" cortex. On the one hand, the neuronal cytokine fractalkine regulates the recruitment of microglia at maturating thalamo-cortical synapses. On the other hand, this recruitment of microglia controls the maturation of thalamo-cortical synapses by modulating the functional expression of glutamate receptors (Hoshiko et al., 2012; Arnoux et al., 2013).
We currently combine electrophysiological and optical recordings in acute thalamo-cortical slices of transgenic mice together with in vivo manipulation (sensory deprivation, microglia depletion, microglia immune activation) immunohistochemistry, and molecular biology to identify the signaling pathways through which microglial cells influence the maturation of cortical synapses. Our preliminary results suggest that BDNF could be one of these mediators.
During this internship, the student will be trained in electrophysiology (in vitro patch-clamp recordings) and will study development of cortical synapse in transgenic mice in which BDNF has been selectively disrupted in microglia (CX3CR1-Cre x floxed BDNF). The student must therefore be motivated to learn electrophysiology and to study synapse physiology. This project is part of a collaborating program involving several other labs in the Paris area but also in Bordeaux and in Japan.

Recent publications of the team on microglia:
Arnoux I, Hoshiko M, Sanz Diez A, **Audinat E** (2014) Paradoxical effects of minocycline in the developing mouse somatosensory cortex. Glia 62(3): 399-410
Carrillo-de Sauvage MA, Pasco M, Arnoux I, Maatouk L, Sanz Diez A, Delahaye M, Herrero MT, Newman TA, Calvo CF, **Audinat E**, Tronche F, Vyas S (2013) Potent and multiple regulatory and anti-inflammatory actions of microglial glucocorticoid receptors during inflammation. *Cell Death and Differentiation* 20(11): 1546-1557
Arnoux I, Hoshiko M, Avignone E, Mandavy L, Yamamoto M, **Audinat E** (2013) Adaptive phenotype of microglial cells during the normal postnatal of the somatosensory "barrel" cortex. *Glia* 61(10): 1582-94
Ulmann L, Levavasseur F, Avignone E, Hibec H, **Audinat E***, Rassendren f* (2013) Involvement of P2X4 receptors in hippocampal microglia activation after status epilepticus. *Glia* 61(8): 1306-19
Hoshiko M, Arnoux I, Avignone E, Yamamoto N, **Audinat E** (2012) Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamo-cortical synapses in the barrel cortex. *The Journal of Neuroscience* 32:15106-15111

Period: Anytime from October 2014 to March 2015

Gilles BONVENTO's team

Team: Cell-cell interactions in neurodegenerative diseases

Fields of research: Neurological and psychiatric diseases

Internship project:

Reactive astrocytes in neurodegenerative diseases

Supervisor: Carole ESCARTIN

Astrocytes were initially considered as mere supporting cells in the brain, but recent findings have revealed that they play central roles in multiple functions such as energy metabolism regulation, antioxidant defense and synaptic modulation. In most pathological situations including neurodegenerative diseases (ND), astrocytes become reactive. This reactivity appears early in the disease process, involves stereotypic morphological changes and multiple functional alterations[1]. As astrocytes are key partners of neurons, functional changes in reactive astrocytes could directly influence neuronal survival and disease progression. It is still unclear how astrocyte functions are modified by their reactive state. In some cases, reactive astrocytes display adaptive changes that are beneficial for neurons, such as improved metabolic support[2] or enhanced clearing of glutamate[3]. However, astrocyte reactivity is unable to fully prevent neuronal dysfunction in ND. It is thus important to better delineate the functional changes associated with astrocyte reactivity and their impact on neuronal survival.
We have developed viral vectors that can selectively modulate the status of astrocytes in the rodent brain (i.e. de-activate or activate astrocytes). By using these new molecular tools, we will characterize changes induced in reactive astrocytes in vivo using transcriptional, histological and functional analysis. We will also evaluate how reactive astrocytes influence disease progression in several mouse models of ND.

We will perform stereotactic injections of viral vectors in the mouse brain. We will then use fluorescent cell sorting and transcriptional analysis of mouse astrocytes, associated with extensive post-mortem analysis (immunohistochemistry, western blotting, ELISA...). These stat-of-the-art approaches and biosafety level 2 laboratories are available in the MIRCen center (http://www.dsv.cea.fr/MIRCen). This multidisciplinary project will contribute to the better understanding of reactive astrocytes and their potential as therapeutic targets for ND.

References :

1. Escartin C, Bonvento G. Targeted activation of astrocytes: a potential neuroprotective strategy. Mol Neurobiol 2008; **38**(3): 231-41.
2. Escartin C, Pierre K, Colin A, et al. Activation of astrocytes by CNTF induces metabolic plasticity and increases resistance to metabolic insults. J Neurosci 2007; **27**(27): 7094-104.
3. Escartin C, Brouillet E, Gubellini P, et al. Ciliary neurotrophic factor activates astrocytes, redistributes their glutamate transporters GLAST and GLT-1 to raft microdomains, and improves glutamate handling in vivo. J Neurosci 2006; **26**(22): 5978-89.

Period: Anytime from September 2014 to June 2015

Alexis BRICE's team

Team: Cortical dynamics and multisensory processing

Fields of research: Neurological and psychiatric diseases

Internship project:

Modelling And Reversing Frontotemporal Lobar Degeneration

Supervisor: Morwena LATOUCHE

Frontotemporal lobar degeneration (FTLD) is the most common cause of presenile degenerative dementia after Alzheimer's disease (AD). Degeneration of neurons in the frontal and temporal lobes causes behavioural, language and cognitive disorders and ultimately leads to the death of the patients within a decade after the onset of the disease. In FTLD, approximately 30% of cases are hereditary forms and the most common causes of familial FTLD is the loss of function mutations in the gene coding for progranulin (PGRN, 15% of hereditary cases).

Progranulin is a secreted glycoprotein with multiple functions identified in peripheral tissues including tumorigenesis, development of early embryos, inflammation and wound repair. In the CNS, PGRN is expressed in specific neuronal populations, in microglia and in reactive astrocytes. Its specific function(s) in the brain remain unclear but a role in neurotrophic activity and neuroinflammation is supported by several recent studies. Consequently, FTLD linked to PGRN deficiency is supposed to result from lack of neurotrophic support and enhanced inflammatory processes. However, further understanding of precise neuronal and glial functions of PGRN will be critical to identify the pathological mechanisms of PGRN deficiency

To understand the mechanism by which progranulin (PGRN) deficiency causes brain dysfunction and neurodegeneration we use two complementary approaches. Firstly, we selectively knock-out PGRN in microglia to assess the cell-type specific phenotype of PGRN deficient mice in relation to FTLD in terms of behaviour and/or histopathology. Secondly, because patients with FTLD linked to PGRN suffer from PGRN haploinsufficiency and not complete PGRN deficiency, reversibility of the phenotype upon restoration of the PGRN level is a key issue in the disease. Therefore, we have created a conditional and reversible transgenic PGRN knock-down mouse model. This was achieved by combining a tetracycline inducible system (Tet-Off) and miR-RNAi technology. PGRN knock-down will therefore be reversible upon administration of doxycycline. Consequently, this model will allow characterization of the PGRN deficiency phenotype and analysis of the reversibility of the phenotype after re-expression of PGRN.

The project is to carry on the phenotypic analysis of these models using behavioural tests, immunohistology and molecular biology techniques.

The A. Brice team is a world-renowned team focusing its research on 3 types of neurodegenerative diseases: Parkinson's disease-PD, spinocerebellar degenerations-SCD, and frontotemporal lobar degenerations-FTLD. It counts several sub-teams developing integrated approaches to these disorders, from their genetic bases to their physiopathology. The FTLD sub-team is composed of a neurologist, a young PI (Dr latouche), two 2nd year PhD students with one working on another mouse models with similar techniques, and 3 engineers (one to be recruited Oct 2014).

Period: Anytime from January 2015 to June 2015

Serge CHARPAK's team

Team: From sensory processing to functional hyperaemia

Fields of research: Neurophysiology/systems neuroscience

Internship project:

Two-photon imaging of resting oxygen consumption during spontaneous neuronal activity

Supervisor: Serge CHARPAK

The economical cost of brain diseases associated with hypoxia is extremely high. To understand the relationship between oxygenation, blood flow and brain disease, and in the long run how therapeutics can be optimised to reduce brain damage, it is important to determine how oxygen is delivered to and consumed by neurons in vivo. Recently, a two-photon phosphorescent probe PtP-C343 has been generated and two-photon phosphorescence lifetime microscopy (2PLM) has been used for depth-resolved micron-scale measurements of Po2 in brain vessels (Lecoq et al. 2011, Nat Med) and tissue (Parpaleix et al. 2013, Nat Med.).

The project will consist, at first, in mapping the spatial distribution of resting PO2 in the olfactory bulb superficial layers. In a second step, local applications of glutamatergic, GABAergic and other receptor antagonists will used to determine the differential role of neuronal and glial activity, as well as of vascular organization in fixing resting PO2.

Period: Anytime from September 2014 to June 2015

Alexander FLEISCHMANN's team

Team: Neural Circuits and Behaviour

Fields of research: Neurophysiology/ systems neuroscience & Neurogenetics/neurodevelopment

Internship project:

Genetic analysis of olfactory processing and function

Supervisor: Alexander FLEISCHMANN

Our laboratory is interested in the neural circuits that underlie the perception of smell. We use a combination of molecular genetic, in vivo imaging and behavioral approaches in mice to understand the logic of sensory coding in the olfactory cortex.

The processing of sensory information by cortical neural circuits is of fundamental importance for sensory perception, memory and behavior. In the olfactory system, odors are detected by sensory neurons in the nose. Odor-evoked neural activity is then processed in the olfactory bulb and transmitted to several higher olfactory centers in the cortex, which have been implicated in olfactory learning and memory. Recent experiments in mice have revealed that odors activate sparse, distributed ensembles of neurons in the olfactory cortex, and that experience can modify these neural odor representations to encode odor memories and behaviors.

We plan to dissect the functions of neural cell types and circuit elements in the olfactory cortex, and understand their contributions to sensory-driven cortical activity, memory and behavior. The olfactory cortex provides a highly attractive model to study cortical functions. Compared to visual or auditory cortex, the olfactory cortex is simple and in close proximity to the sensory neurons in the nose. Furthermore, animals rely on olfactory cues to communicate with their environment, and perturbations in olfactory processing result in robust behavioral changes. Our experiments aim to discover important general principles of sensory processing and function in the mammalian cortex.

Period: Anytime from September 2014 to June 2015

Gilles FORTIN's team

Team: Hindbrain Integrative Neurobiology

Fields of research: Neurophsysiology/systems neuroscience

Internship project:

Functional connectivity of respiratory oscillators: optogenetics using patterned-light photostimulation

Supervisor: Gilles FORTIN

Our scientific program is at the interface of Developmental and Integrative Neurosciences.

Breathing is an essential behavior that offers a unique opportu-nity to understand how the nervous system functions normally, how it balances robustness with regulated lability, how it adapts to changing conditions, and how dysfunctions result in disease. We base our research on recent advances concerning the emergence in the mouse embryo of two essential sites for respiratory rhythmogenesis: the RTN (ref1) and preBötC (ref2) oscillators, bilateral collectives of about 1000 excitatory glutamatergic neurons essential for rhythm generation and CO2 sensing. *As such, respiratory oscillators constitute unique developmental and physiological objects in the CNS whose vital importance rest on a small number of genetically and optically accessible neurons.*

The candidate will be involved in a collaborative project with Valentina Emiliani (ENP, Paris Descartes University) that aims at unraveling the structure of these oscillators. In short, we will exploit Digital Holography, to for the first time reversibly and selectively stimulate or inhibit defined numbers of Channelrhodopsin2- or Acheorhodopsin-expressing oscillator cells and optically record the activity of responding neurons, at cellular and population levels, and derive the "links" connecting the "nodes", defining the structure of the oscillators' network. This will be done comparatively in the RTN and the preBötC oscillator whose rhythmic activities respectively rely on electrical and glutamatergic synapses.

Other projects ongoing in the lab seek (i) to define the large scale connectivity of the respiratory network through monosynaptic tracing using deficient rabies viruses (Jinjin WU, ENP-PhD student) and (ii) to obtain full proof genetic evidence for the implications of the RTN: as a causal factor of the human syndrome Congenital Central Hypoventilation Syndrome (CCHS), and as a crucial site for CO2 chemoception (Pierre-Louis RUFFAULT, DIM+FRM PhD).

ref1. Thoby-Brisson M, Karlén M, Wu N, Charnay P, Champagnat J, Fortin G. (2009) Genetic identification of an embryonic parafacial oscillator coupling to the preBötzinger. **Nature Neuroscience** 12(8):1028-1035.**[*F1000* Exceptional 13]**.

ref2. Bouvier J, Thoby-Brisson M, Renier N, Dubreuil V, Ericson J, Champagnat J, Pierani A, Chédotal A, Fortin G. (2010) Hindbrain interneurons and axon guidance signaling critical for breathing. **Nature Neuroscience** 13(9):1066-74. **[*F1000* Exceptional 12]**.

Period: Anytime from September 2014 to June 2015

Bruno GASNIER's team

Team: Membrane Transport group

Fields of research: Neuropharmacology/cell signaling

Internship project:

Cellular pathophysiology of neuronal ceroid lipofuscinoses

Supervisors: Corinne SAGNÉ & Bruno GASNIER

Neuronal ceroid lipofuscinoses (NCL) are a group of lysosomal storage disorders characterized by an early neurodegeneration and the accumulation of autofluorescent material in lysosomes. The clinical course includes progressive dementia, seizures and progressive blindness. NCLs are genetically heterogeneous and result from mutations in at least 13 genes. Although lysosomal enzymatic defects have been identified in some genetic forms of NCL, the membrane protein defect underlying other forms is unknown and the common pathway(s) delineated by NCL genes remains unclear.

Our recent research suggests that enzymatic and membrane protein defects found in NCLs converge to a common cellular dysfunction. The project aims to strengthen this conclusion and to explore how this cellular defect leads to neurodegeneration and how it could be corrected.

Experiments will use biochemical, molecular and cell biology approaches applied to cellular models, cultured neurons and mouse models.

Period: Anytime from September 2014 to June 2015

Thérèse JAY & Odile KREBS' team

Team: Pathophysiology of Psychiatric disorders

Fields of research: Neurological and psychiatric diseases

Internship project:

Cortical disinhibition in 22q11 mouse model of Schizophrenia: Role of glutamatergic receptors

Supervisors: Thérèse JAY & Anushree TRIPATHI

Schizophrenia causes severe deficits in cognitive functions attributed in part to disturbance in cortical synchrony by GABAergic Parvalbumin (PV) interneurons (Cardin et al., 2009). However, the mechanisms leading to desynchronization of cortical activity via PV interneuron- mediated signaling is not yet clear. One of the popular neurochemical hypothesis posits a glutamate dysfunction via N-methyl- D- aspartate receptors (NMDARs), which are present on the synapses of PV interneurons (Coyle, 2006). Thus a hypofunction of NMDARs would in turn lead to a hypofunction of PV interneurons (Seamans, 2008).

However, research regarding the role of NMDARs in PV inerneuron dysfunctioning has produced mixed results. In fact, mice lacking *serine racemace*, an enzyme responsible for endogenous NMDA agonist D-serine show no change in the density of PV interneurons (Benneyworth et al., 2011). Furthermore electron microscopy studies have revealed a lower density of NMDARs on PV cells than on pyramidal cells in the cortex (Nyiri et al., 2003). These results suggest that NMDARs have limited potential to regulate PV interneuron functioning. Additionally, mice with selective knockout of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPARs) subunits, from PV cells led to a reduction in their functioning (Fuchs et al., 2007) indicating that PV neuron deficit may in part be attributable to AMPARs. Above mentioned research implies that the initial hypothesis: NMDAR hypofunction directly causes PV interneuron hypofunctioning, may require further revisions.

Modelling neuropsychiatric illness in animals has always proved to be challenging. However, evidence that schizophrenia may in part have a genetic causality has led to development of genetic animal models that might faithfully replicate some of its phenotypes. The established genetic risk factors for schizophrenia include a number of copy number variations (CNV) which represent deletions or duplications of a large genomic area. The CNVs associated with genomic loci 1q 21.1, 2p16.3, 3q29, 15q11.2, 15q13.3, 16p13.1 and 22q11.2 have been implicated most frequently in schizophrenia (Hosak, 2013).

In the present project, we will use a mouse model with microdeletion at gene loci 22q11.2 (Karayiorgou et al., 2010) to elucidate the role of NMDA and AMPARs on PV interneurons in the medial prefrontal cortex.

Objectives:

1. Compare the density of NMDA and AMPARs on PV interneurons: a combination of immunohistochemistry and double immunofluorescence will be used to illustrate the density of functional NR1 subunit of NMDARs and Glu A1 and GluA4 subunits of AMPARs on PV interneurons. These densities will be compared between wild type and 22q11 transgenic mice.
2. Further quantification of NMDA and AMPARs interaction with PV interneurons could be analyzed using sensitive photobleaching FRET technique on a confocal microscope. The difference in interaction of each receptor with PV interneurons will be compared between wild type and 22q11 KO mice.

Period: Anytime from September 2014 to December 2014

Pascal LEGENDRE & Jean-Marie MANGIN's team

Team: Development of Spinal Cord Organisation (DSCO)

Fields of research: Neurogenetics/neurodevelopment

Internship project:

Molecular and circuit mechanisms controlling long-term memory formation in Drosophila

Supervisors: Antonny CZARNECKI & Pascal LEGENDRE

Spontaneous patterned activity constitutes a hallmark of developing neural circuits. Mechanisms underlying this activity include the generation of giant depolarizing potentials (GDP). It is now admitted that the embryonic Spinal cord (SC) electrical activity is important for the proper development of locomotor circuits. So far it was postulated that motoneurons (MNs) were the orchestra director of SC activity at these early developmental stages. But our recent data challenges this view. We have shown that MN activity pattern at the onset of the synaptogenesis is controlled by GDP evoked mainly by GABA release. This data raises the question of the role of the first functional INs in shaping the early electrical activity of the SC in the mouse embryo. ***The goal of this project is*** **to elucidate how specific subpopulation of GABAergics INs control the generation of GDPs on MNs in the mouse embryonic SC.**

Task1 - Determining the ability of pioneer INs to participate to SC spontaneous electrical activity.
In this task we propose to characterize the intrinsic activation properties of early GABAergic INs at day 12.5 embryonic age (E12.5) using a combination of extracellular recording and patch-clamp recording. This task is based on preliminary results showing that: V1-derived Renshaw cells (RC) and V2C INs are the first INs to produce enough GABA at E12.5 as detected by immunohistochemistry.

Task2 - Mechanisms controlling the activity of pioneers INs.

In this task we will determine how the activity of the first functional INs is controlled and/or regulated (E12.5-E14.5).

Our recent studies demonstrate that GABA release on MNs can be triggered by the application of acetylcholine. This suggests that GABAergic INs receive cholinergic inputs. We will investigate whether the activity of each type of early GABAergic INs (RC and V2C) is effectively controlled by neurotransmitter inputs and how. To address this issue we will analyze INs spontaneous activity using patch clamp recording combined with pharmacological tools. The presence of synaptic contacts on identified INs will be confirmed by using immunohistochemistry, confocal microscopy and electron microscopy.

Task 3 - Role of pioneer INs in the early SC electrical activity and in the control of MN activity at the onset of the synaptogenesis in the mouse embryo.

To determine the role of these INs in the control of MN and of SC network activity in the embryo we project to analyze the impact of optogenetic activation and of optogenetic inhibition of specific classes of INs. Cre mice will be crossed with LoxP mice conditionally expressing channelrhodopsin. SC electrical activity will be analyzed in an isolated SC preparation using multiple extracellular recording. MNs activity and INs activity will be analyzed using patch clamp recording.

Period: Anytime from September 2014 to June 2015

Thierry LÉVEILLARD's team

Team: Rod-derived Cone Viability Signaling for the Treatment of Inherited Retinal Degenerations

Fields of research: Neurological and psychiatric diseases

Internship project:

Restoring cone vision by delivering Rod-derived Cone Viability Factor

Supervisor: Thierry LÉVEILLARD

In patients suffering from retinitis pigmentosa (RP), the most common form of inherited retinal degeneration, the vision loss develops in two successive steps. Early in their life, these patients get night vision due to the degeneration of rod photoreceptors. The disease then progresses through loss of function and degeneration of the second class of photoreceptors, the cones that dominate at the centre of the retina. The cones are essential for daytime vision (color vision and the whole visual acuity). This secondary non cell autonomous event, leading to complete blindness results from the loss of expression of a trophic factor secreted by rods, Rod-derived Cone Viability (RdCVF)[1].

RdCVF signaling is an entirely novel neuroprotective signaling[2]. While most of neurotrophic factors trigger a cascade of phosphorylation leading to the inhibition of cell death, RdCVF stimulates cone function by enhancing glucose entry into the cell. The daily renewal of the outer segment (OS) of photoreceptors (10% every day) is very energetically demanding because proteins and lipids are necessary to built new outer segments. The OS contains the opsin molecules necessary to capture light. We will test for the capacity of RdCVF not only to maintain the structure of the cone OS and hence their survival as previously reported[3], but also to accelerate OS renewal (OS re-growth). Shortening of OSs is a hallmark of photoreceptor degenerative diseases. Such activity, if demonstrated, could restore vision in patients suffering from RP.

The student will participate in the following experiment:
In the mouse, the intraocular injection of CNTF protein triggers OS shortening (immunohistochemistry, IHC) but, presumably does not abolish the expression of the RdCVF receptor by cones (western blotting, WB). The CNTF protein will be degraded after few days (ELISA). The simultaneous injection of adeno-associated viral vector (AAV-RdCVF, gene therapy) in the mouse lacking the RdCVF gene[4], will result in a gradual raise in the expression of recombinant RdCVF in the eye (RT-PCR, WB). RdCVF should counteract the negative effect of CNTF by stimulating OS growth (IHC), after CNTF degradation. The objective will be to document a restoration of the visual function of the cone (electrophysiology, ERG).

References:
1 Léveillard, T. et al. Identification and characterization of rod-derived cone viability factor. **Nature genetics** 36, 755 (2004).
2 Léveillard, T. & Sahel, J. A. Rod-derived cone viability factor for treating blinding diseases: from clinic to redox signaling. **Science translational medicine** 2, 26ps16, (2010).
3 Yang, Y. et al. Functional cone rescue by RdCVF protein in a dominant model of retinitis pigmentosa. **Molecular therapy** 17, 787- (2009).
4 Cronin, T. et al. The disruption of the rod-derived cone viability gene leads to photoreceptor dysfunction and susceptibility to oxidative stress. **Cell death and differentiation** 17, 1199-1210, (2010).

Period: Anytime from September 2014 to February 2015

Sophie NICOLE and Bertrand FONTAINE's team

Team: NeuroGenetics and Physiology

Fields of research: Neurological and psychiatric diseases

Internship project:

Presynaptic effect of mutant agrin at the neuromuscular junction in congenital myasthenic syndromes

Supervisor: Sophie NICOLE

Our laboratory is an international leader in the pathophysiological studies of monogenic human diseases with abnormal neuromuscular excitability. If we now have a good understanding of the main pathophysiological mechanisms, major questions remain on their periodic nature, the variation in phenotype severity, and gradual muscle impairment in some forms. Congenital myasthenic syndromes (CMS) are a heterogeneous group with defective synaptic transmission at the neuromuscular junction (NMJ), the cholinergic synapse of the peripheral nervous system consisting in one well differentiated motoneuron innervating one myofiber, both cells being recovered by terminal nonmyelinating Schwann cells. One of the most challenging question in CMS is the compensatory mechanisms taking place between the nerve-muscle-terminal Schwann cells, ie the impact a postsynaptic defect has on the presynaptic element and *vice et versa* (Hantaï et al., *Current opinion in Neurology* 2013).

The project aims to investigate the presynaptic function of agrin, a heparan sulfate proteoglycan present in the extracellular basement membrane where its neural splicing isoform plays a critical role in the formation of the NMJ. We recently identified 6 missense mutations of agrin in two distinct forms of CMS. If 3 of these 6 mutations result in an unspecific form, the other three result in a very special phenotype with distal muscle atrophy and incremental response of muscle excitability to exercise suggesting presynaptic defects (Huze et al., Am J Hum Genet, 2009; Nicole et al., submitted). These results question the genotype-phenotype correlation of agrin mutations causing CMS, which is not evident when looking at their location along the protein, and their exact physiological effects on the presynaptic, glial and muscle components composing the NMJ. This project aims to dissect the effects of the mutant isoforms of agrin on motor neuron, Schwann cell and NMJ formation. To do this, we will compare the *in vitro* effects of normal and some mutant agrins on muscle cell lines, primary rat motor neurons and primary Schwann cells. One of the major limitations in the field was the need of a mature NMJ composed of one motorneuron, one myofiber and 2-3 nonmyelinating Schwann cells, but zebrafish now emerges as a particularly suitable model (Kabashi et al., Trends in Genetics 2010).

In collaboration with the team of E Kabashi, which has all the required expertise on zebrafish, we will use this animal model to compare the *in vivo* effect of the selected missense agrin mutations on locomotion, motor axon growth, NMJ formation, and synaptic transmission (morpholinos directed against agrin followed by injection of wild type or mutant agrin RNA controls).
This second project will undoubtedly leads to important clues on NMJ formation and maintenance, which are critical knowledge not only for CMS pathophysiology but also for more devastating neurodegenerative diseases in which NMJ dysfunction is one major element of the phenotype.

Period: Anytime from January 2015 to June 2015

Serge PICAUD's team

Team: Retinal information processing: pharmacology and pathologies

Fields of research: Neurophysiology/systems neurosience

Internship project:

Estimating depth from the output of the retinas

Supervisors: Serge PICAUD & Olivier MARRE

Our brain is able to estimate precisely the depth of the different objects of a natural scene by merging the information coming from our two eyes. The purpose of this project is to understand how the visual system computes depth in a complex visual scene from the output of the two retinas. Our preliminary results in psychophysics support the hypothesis that stereoscopic matching occurs in the temporal domain by selective synchronization of retinal ganglion cells when there is a surface at a specific depth. The aim of this project is to test directly this hypothesis by measuring the responses of a large population of ganglion cells to simulated stimuli that mimic the projection of an object on each eye.

In the team of Serge Picaud (Vision Institute), Olivier Marre has recently developed a new technique to record the activity of a large number (100-200) of ganglion cells, the output of the retina. Using large multi-electrode arrays, it has been shown that most of the ganglion cells above this array can be recorded. For a retinal patch, this technique allows to watch the retinal output as it is sent to the brain (Marre et al, J Neurosci 2012).

The trainee will record the inputs received from each eye by stimulating the same retina successively with what reaches the left, then the right eyes, for a given object at a specific depth. We will determine which stimuli trigger a synchronous activation of pairs of ganglion cells. We will then attempt to decode the disparity from the activity of the recorded responses.
This project is highly interdisciplinary and will allow the trainee to be exposed to different fields: physiology, psychophysics, and modeling.

Period: Anytime from September 2014 to June 2015

Alessandra PIERANI's team

Team: Genetics and Development of the Cerebral Cortex

Fields of research: Neurogenetics/neurodevelopment

Internship project:

Developmental role of transient migratory neurons in Autism Spectrum Disorders associated with Epilepsy

Supervisor: Alessandra PIERANI

The project aims at testing how alterations in genes controlling migration of "transient neurons" and signals that these neurons release at the onset of neurogenesis is sufficient to induce a permanent defect in the production of cortical excitatory neurons leading to ASD associated with epilepsy. The student will use gain- and loss-of function approaches by in utero electroporation and mouse genetics.

Period: Anytime from September 2014 to June 2015

Jean Christophe PONCER & Sabine LEVI

Team: Plasticity in Cortical Networks and Epilepsy

Fields of research: Neuropharmacology/cell signaling & Neurophysiology/systems neuroscience

Internship project:

Role of endogenous GABA in newborn granule cell maturation

Supervisors: Jean Christophe PONCER & Emmanuel EUGENE

Although dentate gyrus (DG) granule cells (GCs) are primarily glutamatergic neurons, they express the GABA synthesizing enzyme GAD67 and other GABAergic markers both early during postnatal development[1] and in adulthood upon epileptic seizures[2]. We wish to understand the role of GABA synthesis in these cells and test whether it may represent a homeostatic or, on the contrary, a pro-epileptogenic response to hippocampal insults.

Working hypothesis: We have recently shown that GAD67 expression is not correlated with a specific ontogenetic stage but instead reflects a very specific maturation period of individual GCs throughout life[3,4]. This stage (2-4 week post-differentiation) corresponds to a critical period of GC maturation with axonal growth, synapse formation and dendritic maturation[5]. We hypothesize that GAD67 expression and GABA synthesis and release may act as an autocrine mechanism that contributes to the anatomical and functional maturation of newborn GCs. We will explore this by testing the effect of a specific ablation of GAD67 in GC progenitors in mouse hippocampus. We have generated a triple transgenic mouse strain in which the *Gad1* gene encoding GAD67 was floxed, *Gad2* encoding the other GABA synthesizing enzyme, GAD65, was knocked-out to prevent compensation of GAD67 by GAD65, and a Cre-dependent GFP reporter sequence was introduced in the Rosa26 locus. We used a retrovirus expressing Cre recombinase to specifically infect proliferating GC progenitors in the DG of young mice[6]. Cre expression is expected to recombine *Gad1* and RCE and suppress GAD67 while triggering GFP expression specifically in these cells. We now wish to explore the anatomical and functional consequences of this manipulation.

We will first compare the anatomy of individual GCs infected in $Gad1^{fl/fl}$ vs.$Gad1^{fl/+}$ littermates. In particular, we will follow morphological criteria that represent key landmarks of GC differentiation including dendritic complexity and the presence of dendritic spines, the volume of GC somata, the total length of mossy fibers, as well as the presence, size, complexity and density of specific axonal structures named mossy fiber terminals (MFTs) that appear during maturation of GC axons[7,8]. After immunohistochemical amplification of GFP signal to help resolve these fine anatomical structures, the entire dendritic and axonal arbors of individual GCs will be imaged by confocal microscopy in serial hippocampal sections. These images will be analyzed using NeuroLucida for complete reconstruction of dendritic and axonal arbors and semi-automated analysis of anatomical and morphological parameters.

References:
1 Gutierrez, R., Romo-Parra, H., Maqueda, J. et al. (2003) J Neurosci 23, 5594-5598.
2 Sloviter, R.S., Dichter, M.A., Rachinsky, T.L. et al. (1996) J Comp Neurol 373, 593-618.
3 Cabezas, C., Irinopoulou, T., Cauli, B. et al. (2013) Frontiers in neural circuits 7, 60.
4 Cabezas, C., Irinopoulou, T., Gauvain, G. et al. (2012) J Neurosci 32, 11835-11840.
5 Dayer, A.G., Ford, A.A., Cleaver, K.M. et al. (2003) J Comp Neurol 460, 563-572.
6 Jessberger, S., Zhao, C., Toni, N. et al. (2007) J Neurosci 27, 9400-9407.
7 Henze, D.A., Urban, N.N., & Barrionuevo, G. (2000) Neuroscience 98, 407-427.
8 Klein, R. (2010) Neuron 65, 580-582.

Keywords: Hippocampal neurogenesis, epilepsy, learning and memory, electrophysiology, confocal microscopy, imaging

Period: Anytime from September 2014 to June 2015

Thomas PREAT's team

Team: Genes and Dynamics of Memory Systems

Fields of research: Neurophysiology/ systems neuroscience

Internship project:

Molecular and circuit mechanisms controlling long-term memory formation in Drosophila

Supervisors: Thomas PREAT & Alice PAVLOWSKY

Despite a much smaller – and simpler– brain than mammals (~10^6 times less neurons than humans) the fruit fly *Drosophila Melanogaster* can display complex neurophysiological processes. In particular, a fly can memorize the association between the perception of an odor and a pleasant or unpleasant stimulus (typically sugar or electric shocks delivery). Depending on the conditioning protocol (i.e. the nature of the reinforcement, the number of associative trials, the satiation state of the animal…) the fly will form memory of different persistence. In the case of aversive conditioning, a single association between and odor and electric shocks will induce a rapidly decaying memory, while repeated associations separated by rest intervals (spaced conditioning) induce the formation of long-term memory (LTM), that lasts for days and requires new protein synthesis after conditioning. Repeated cycles without rest intervals (massed conditioning) results in another, less stable form of consolidated memory, called anaesthesia-resistant memory (ARM).

Our specificity is that we have developed a long-standing interest in the interaction between the different aversive memory phases. In particular, we analyse the mechanisms that control LTM formation. Research on Drosophila memory benefits from a wealth of very powerful genetic tools that enable precise and sophisticated study of the neuronal networks involved in memory processing. Our group, which includes permanent researchers from various backgrounds (genetics, neurophysiology, physics), is among the world leaders in this field.

Within the Drosophila brain, the mushroom bodies, a paired structure of about 2000 neurons per brain hemisphere, has long been identified as a major structure for olfactory memory. We have recently discovered a key mechanism by which the brain controls the formation of long-term memory upon a spaced conditioning. We identified two pairs of dopaminergic neurons that innervate mushroom bodies neurons. Slow oscillatory activity in these neurons act as a switch to engage mushroom bodies into the formation of either LTM or ARM [Plaçais et al. *Nat Neurosci* 2012]. More recently, we showed that this mechanism allows for an adaptive plasticity to prevent the costly and unnecessary formation of LTM when flies are threatened to die by starvation [Plaçais and Preat *Science* 2013].

We now have a clear and validated model of the ARM and LTM interaction, based on the gating of LTM formation by oscillating dopamine neurons. The project we propose aims to unveil the mechanisms that generates these oscillations as well as their role. A first part is to identify the molecular mechanism of the oscillations by targeting interferent RNA against various candidate molecular agents in the dopaminergic neurons. The second part is to decipher the effect of dopaminergic oscillations during memory formation if the mushroom bodies. This will involve an integrated experimental approach combining behavioral experiments, *in vivo* imaging, and *Drosophila* genetics.

Period: Anytime from January 2015 to June 2015

François ROUYER's team

Team: Molecular genetics of circadian rhythms
Fields of research: Neurophysiology/ systems neuroscience & Neurogenetics/neurodevelopment

Internship project:

Identification of the neuronal circuits that are controlled by the circadian clock in Drosophila

Supervisor: François ROUYER

Drosophila has about 150 clock neurons that control time-of-day information and orchestrate circadian behavioral rhythms such as sleep-wake cycles. Different periods of diurnal time, e.g., morning and evening, are encoded by distinct neuronal subpopulations. We are interested in (a) deciphering the pathway of information flow from these master clock neurons, (b) and in understanding how the downstream circuit decodes temporal information. To this end we artificially (using thermogenetic tools) hyperexcited as well as silenced about 40 different groups of brain neurons and assayed circadian locomotor activity rhythms in these manipulated flies. Interestingly, we identified several lines (Gal4 enhancer traps) that show impaired locomotor behavior in the evening but normal locomotion pattern in the morning. This phenotype would be the starting point for the project. First, the behavioral defects will be analyzed in more details (different environmental conditions) and precise expression pattern of these lines within the nervous system will be determined. We will use molecular-genetic tools (GRASP) to anatomically verify whether these putative downstream neurons form synapses with the master clock neurons. Second, the physiological nature of the connectivity between these putative downstream neurons and the master clock neurons will be investigated. Specifically, we will ask whether the connection is monosynaptic or not, which neurotransmitter system mediates their communication and whether the connection strength shows circadian-time dependent plasticity.
The experiments will involve Drosophila genetics, behavioral analysis, immunolabeling and microscopy analysis, functional imaging (Ca2+) of the clock neurons.

Ozkaya, O., and Rosato, E. (2012). The circadian clock of the fly: a neurogenetics journey through time. Adv Genet *77*, 79-123.

Cavanaugh, D. J., Geratowski, J. D., Wooltorton, J. R., Spaethling, J. M., Hector, C. E., Zheng, X., Johnson, E. C., Eberwine, J. H., and Sehgal, A. (2014). Identification of a circadian output circuit for rest:activity rhythms in Drosophila. Cell *157*, 689-701.

Period: Anytime from January 2015 to June 2015

Fekrije SELIMI's team

Team: Mice, Molecules and Synapse formation

Fields of research: Neurogenetics/neurodevelopment

Internship project:

Immune system related proteins and synapse specificity

Supervisor: Fekrije SELIMI

The brain is composed of many different types of neuronal populations that form functional networks by establishing specific synapses. Any disturbance in the process leading to the development of these networks can contribute to neurological diseases such as autism or schizophrenia. This complex process involves both target recognition, through a putative "molecular code", and activity-dependent stabilization/elimination of synapses.

We have developed the synaptic protein profiling approach, which enables the purification of a specific type of synapse from the mouse brain and identification of its protein content. The bacTRAP approach allows gene expression studies in specific neuronal populations in mice. Combining these two innovative strategies, our specific aims are to: 1) identify the proteins characterizing the putative "molecular synaptic code"; 2) study the function of the genes identified in our analysis and their contribution to brain diseases.

As a model, we are using the olivo-cerebellar network where Purkinje cells receive two excitatory glutamatergic inputs, one from cerebellar granule cells and one from inferior olivary neurons, which are distinct in terms of their physiology and of their innervating territory on Purkinje cells. The first step of our project was to compare the gene expression profiles of these two Purkinje cell inputs using the bacTRAP technology. We have found that there is a major difference in the functional categories of the differentially expressed genes: Inferior olivary neurons express an increased variety and level of membrane and secreted proteins with a known function related to the immune system. We are currently analyzing whether this difference underlies the fundamental differences in terms of connectivity, physiology and morphology between these two neuronal types. Our current work is aimed at testing which of these specific pathways are involved in regulating synapse specificity and competition using a RNA interference approach in vivo. Consequences of these gene expression modifications are analyzed at the morphological level and, in collaboration, at the physiological level. The results obtained will allow us to better understand the molecular mechanisms underlying neuronal network formation, and related brain diseases.

References:
Lanoue V., Usardi A., Sigoillot S., Talleur M., Iyer K., Mariani J., Isope P., Vodjdani G., Heintz N & Selimi F. The adhesion-GPCR BAI3, a gene linked to psychiatric disorders, regulates dendrite morphogenesis in neurons. ***Molecular Psychiatry*** 18(8):943-50 (2013).

Selimi F., Cristea I.M., Heller E., Chait B.T. and Heintz N. Proteomic studies of a single CNS synapse type: specific regulatory components of the parallel fiber/Purkinje cell synapse. **PloS Biology** 7(4): e83 (2009).

Period: Anytime from September 2014 to March 2015 and from January 2 to June 30 2015

German SUMBRE's team

Team: Neuroethology of the zebrafish

Fields of research: Neurophysiology/systems neuroscience

Internship project:

The neural basis of visual illusions in zebrafish larva using optogenetics

Supervisor: German SUMBRE

One of the main goals in neuroscience is to understand how cognitive functions, such as sensory perception, are encoded by the dynamics of large neuronal networks. The main stream to study sensory perception has mainly focused on sensory stimulation and neuronal recordings of the induced neural responses. An alternative approach is the use of sensory illusions, in which sensory perception could take place in the absence of physical external stimulation. This strategy enables monitoring brain activity under sensory perception without sensory detection. One example of these sensory illusions is the motion after-effect (MAE), in which exposure to coherent continuous motion for a certain period of time induces, following the end of the stimulation, motion perception in the opposite direction. Our PhD thesis had for aim to understand the neuronal mechanisms underlying MAE and shedlight on the neuronal correlate of motion perception. For this purpose, we have used a multidisciplinary approach combining the zebrafish larva as the experimental model, two-photon calcium imaging, motor behavior, optogenetics and mathematical methods for that analysis. This approach enabled us to monitor the activity of large neuronal networks from various regions of the brain, in an intact behaving vertebrate, while presenting visual stimuli able to induce motion illusions. Upon the presentation of a conditioning stimulus (CS, a coherent motion stimulus), zebrafish larvae showed OKR, a behavior in which the larva moves its eyes following the direction of motion in order to stabilize the moving external world on the retina. In contrast, in the absence of moving visual stimulation, zebrafish larvae generated stereotypic spontaneous eye movements (saccade movements followed by a fixation period and a second saccade in the opposite direction). Taking advantage of these two types of movements we found that following the presentation of CS.

Methodologies: Two-photon calcium imaging, optogenetics and behavior

References:

- Pietri T, Roman AC, Guyon N, Romano SA, Washbourne P, Moens CB, de Polavieja GG, Sumbre G. (2013) The first mecp2-null zebrafish model shows altered motor behaviors. Front Neural Circuits 7:118. doi:10.3389/fncir.2013.00118
- Panier T, Romano SA, Olive R, Pietri T, Sumbre G, Candelier R, Debrégeas G. (2013) Fast functional imaging of multiple brain regions in intact zebrafish larvae using Selective Plane Illumination Microscopy. Front Neural Circuits 7:65 doi:10.3389/fncir.2013.00065.
- Sumbre G and Poo MM. (2011) Imaging neuronal ensemble activity and motor behaviour in zebrafish. in Optical Imaging in Neuroscience: A Laboratory Manual (CSHL press). Ed. Rafael Yuste.
- Sumbre G, Muto A, Baier H and Poo MM. (2008) Entrained rhythmic activities of neuronal ensembles as perceptual memory of time interval. Nature 456(7218):102-106 (AOP).

Period: Anytime from September 2014 to June 2015

Catherine TALLON-BAUDRY's team

Team: Visual Cognition Group

Fields of research: Cognitive Neurosciences/ neuropsychology/neuroeconomy

Internship project:

The role of neural responses to bodily signals in shaping ongoing neural activity and subjective experience in humans

Supervisor: Catherine TALLON-BAUDRY

We focus on understanding how brain dynamics create subjective experience, in both its cognitive and subjective dimensions. We mostly use MEG and EEG in healthy participants, as well as intracranial EEG in epileptic patients, combined with behavioral experiments, anatomical and functional MRI. Ultimately, we aim at proposing a neural framework for consciousness encompassing both experiental, subjective aspects and cognitive abilities. The Visual Cognition group hosts international students and post-doctoral fellows from various backgrounds, from engineering to cognitive neuroscience and experimental psychology or philosophy. Applicants must have some experience of Matlab.

References:
Park, H.-D., Correia, S., Ducorps, A., Tallon-Baudry C. Spontaneous fluctuations in neural responses to heartbeats predict visual detection. Nature Neuroscience, advanced online publication, 2014

Campana, F. and Tallon-Baudry, C., Anchoring visual subjective experience in a neural model: The coarse vividness hypothesis, Neuropsychologia, 51, 1050-1060 (2013)

Tallon-Baudry, C., On the neural mechanisms subserving attention and consciousness, Frontiers in Psychology, 2, 397 (2012).

Wyart, V. and Tallon-Baudry, C., Neural Dissociation between Visual Awareness and Spatial Attention, Journal of Neuroscience, 28, 2667-2679 (2008).

Period : Anytime from September 2014 to June 2015